

SINGLE-KERNEL ANALYSIS OF GLUTENIN: USE IN WHEAT GENETICS AND BREEDING¹

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ABSTRACT

Because of the importance of glutenin proteins in determining wheat flour quality and dough properties, new methods were developed for examining glutenin's subunit composition in hexaploid and tetraploid varieties with known genetic differences. Glutenin was quantitatively extracted from single kernels, and its subunit composition observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In the variety Chinese Spring, the five highest molecular weight (mol wt) subunits (1: 133000 mol wt; 2: 104000 mol wt; 3: 93000 mol wt; 4: 86000 mol wt; and 5: 68000 mol wt) are coded by the long arms of chromosomes 1B (subunits

2 and 3), 1D (subunits 1 and 4), and 4D (subunit 5). Analysis of genetic stocks which contain these markers for chromosomes related to quality may permit selection or development of improved varieties; half-kernels may be examined, and lines maintained by growing germ ends. Hexaploid wheats were also compared with durum and derived tetraploid wheats, which lack the entire D genome. Most tetraploid wheats lack glutenin subunits 1, 4, and 5, but only subunit 4 is consistently absent, and in many varieties considerable variability occurs. Hexaploid varieties, however, are more uniform in glutenin subunit composition.

Glutenin is the class of wheat flour proteins having high-molecular-weight (mol wt) and formed by interchain disulfide crosslinking of several distinct polypeptides. Glutenin seems to be the most important determinant of structure and elasticity in bread dough and also of wheat flour quality (for review see ref. 1). Differences in performance between wheat varieties may be due largely to variations in glutenin.

Glutenins from wheat varieties of different baking quality differ in size (2-6), such that long-mixing varieties generally have high mol wt, highly aggregated glutenins. The number and amounts of glutenin subunits also differ among wheats of different classes and baking qualities (5,7,8), but specific subunits have not been related to quality.

Bread wheat (*Triticum aestivum*, AABBDD) is hexaploid, containing three genomes or sets of seven-paired chromosomes; durum wheats (*Triticum durum*, AABB), however, are tetraploid and lack D genome chromosomes. These types of wheat have been compared in attempts to relate protein compositional differences to quality. Both durum wheat and AABB tetraploids extracted from hexaploid wheat (9) generally have poor breadmaking quality (10,11), suggesting that D genome chromosomes govern good quality characteristics. Albumin, globulin, and gliadin proteins from hexaploid and tetraploid wheats have been compared by electrophoresis (12-17), and several polypeptides have been associated with the D genome; much less is known about the influence of the D genome on glutenin, however. Durum wheats lack a high mol wt glutenin

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subunit characteristic of most hexaploids (7), and high mol wt glutenin subunits may be absent in extracted tetraploids (18); such subunits could be related to quality.

Wheat aneuploids, which lack all or part of a chromosome, have also been examined to locate sites that influence wheat quality. A gene or genes for breadmaking quality are located on the long arm of chromosome 1D (10,19); other chromosomes, however, have also been linked to wheat quality characteristics (for review see ref. 20). The chromosomes governing synthesis of most wheat gliadin proteins have also been identified through the use of aneuploids (21-23).

Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7) and newly developed extraction procedures (24), Bietz and Wall detected differences in glutenin subunit composition between hexaploid wheats and their derived tetraploids, and between the hexaploid variety Chinese Spring and certain of its aneuploids (25). Following completion of these initial studies, however, we found that a labeling error⁴ necessitated reinterpretation of some data. Also, since Orth and Bushuk (18,26) had simultaneously been performing some similar studies, we expanded our investigation in scope and objective. Through a newly developed method of single-kernel analysis, we have now examined subunit compositions of glutenins from many hexaploid and tetraploid wheats with normal or modified chromosomal contents. The results establish the chromosomal and genomic location of genes coding specific glutenin subunits.

MATERIALS AND METHODS

Wheat Varieties

E. R. Sears (University of Missouri, Columbia 65201) provided the hexaploid wheat variety Chinese Spring and all compensating nullisomic-tetrasomic stocks (which lack one chromosome pair but have an additional pair of related chromosomes) except N2AT2B, N2AT2D, N4AT4D, N7DT7A, and N7DT7B. Nullisomic-trisomic (41 chromosome) lines of 2A-2B, 7D-7A, and 7D-7B were also supplied. In addition, Sears supplied 31 ditelocentric (ditelo) lines in which both of a pair of chromosomes lack one arm, so that genes coding protein synthesis could be located on specific chromosome arms; these included ditelo 1A_L, 1B_L, 1B_S, 1D_L, 2A_S, 2B_L, 2D_α, 2D_β, 3A_L, 3A_S, 3B_L, 3B_S, 3D_L, 3D_S, 4A_α, 4B_L, 4D_L, 4D_S, 5A_L, 5B_L, 5D_L, 6A_α, 6B_L, 6B_S, 6D_α, 6D_β, 7A_L, 7A_S, 7B_L, 7B_S, and 7D_S. For the ditelos, the subscript following the chromosome designation indicates the arm that is present.

W. Bushuk (Department of Plant Science, University of Manitoba, Winnipeg, Canada) furnished the hexaploid wheat varieties Prelude, Canthatch, Thatcher, and Rescue, and their derived AABB tetraploid strains (9,10). Paul J. Mattern (Department of Agronomy, University of Nebraska, Lincoln 68503) provided Cheyenne-Chinese Spring substitution lines (except 2B, which is not available),

⁴A sample thought to be the compensating nullisomic 1D-tetrasomic 1B (N1DT1B) line of Chinese Spring was shown by starch-gel electrophoresis (21) to be the N1BT1D aneuploid. Consequently, the 93000 and 104000 mol wt subunits of glutenin are coded by chromosome 1B, rather than 1D as previously reported (25).

in which individual chromosome pairs of the strong-quality hard red winter wheat variety Cheyenne replace the corresponding chromosome pair in the soft red spring variety Chinese Spring. All substitution lines had been backcrossed six times to Chinese Spring. Mattern also furnished 80 hexaploid varieties (referred to in the text) from the U.S. Department of Agriculture World Wheat Collection, specially selected to cover a wide range of geographical origins and quality types.

Leonard R. Joppa (Department of Agronomy, State University Station, Fargo, ND 58102) furnished 55 AABB tetraploid wheats; most were *Triticum*

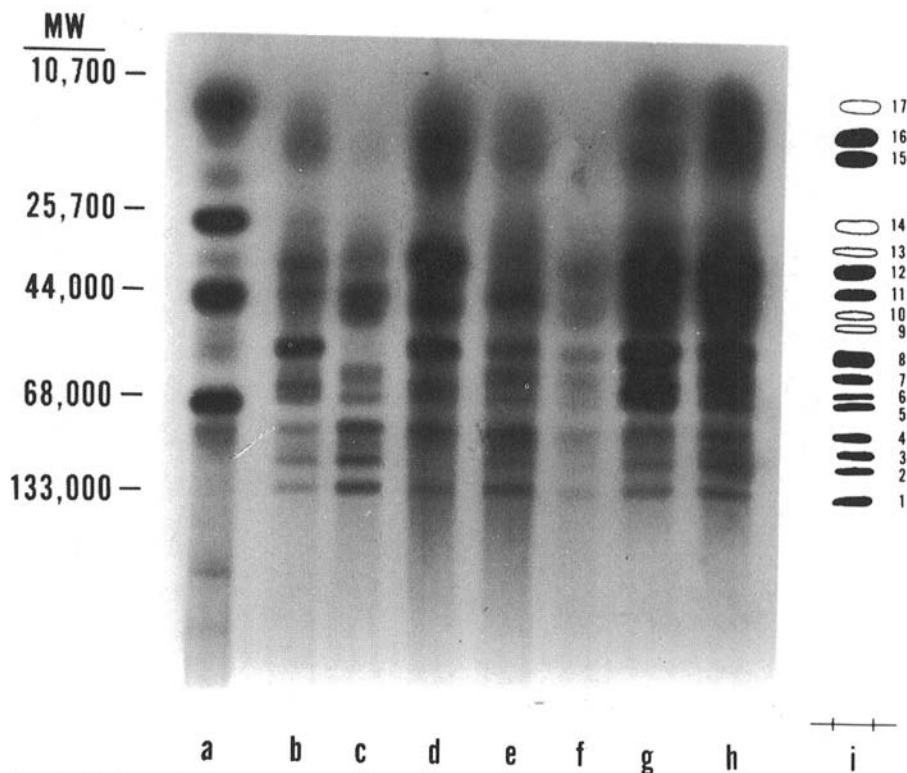


Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at pH 8.9 of single kernel and bulk extracts of glutenin and total protein from the wheat variety Chinese Spring: **a**) molecular weight (mol wt) standards – cytochrome c (mol wt 11700), chymotrypsinogen A (mol wt 25700), ovalbumin (mol wt 43000), and serum albumin (mol wt 68000); **b**) glutenin, single kernel; **c**) glutenin from gluten ball; **d**) precipitated glutenin extracted with acetic acid from flour; **e**) precipitated glutenin extracted with HgCl_2 from flour residue remaining after acetic acid extraction; **f**) glutenin extracted by 2-mercaptoethanol from flour residue remaining after acetic acid and HgCl_2 extractions; **g**) total protein from single kernel; **h**) total protein extracted from milled flour; and **i**) diagram of glutenin subunit bands. Mol wt values at left of figure are approximations based both on standard proteins (**a**) and previously determined glutenin subunit mol wt values (7). Mol wt standards (**a**) are not depicted in most other figures since the variety Chinese Spring serves as a basis for comparison.

durum, but some wild emmer wheats were included. These varieties (referred to in the text) were selected from diverse sources to represent a random sample of the world population. Joppa also supplied a sample of the disomic addition line of Chinese Spring chromosome 1D to Langdon durum (27).

Gordon Kimber (Department of Agronomy, University of Missouri, Columbia 65201) provided nine AA diploid wheats (*Triticum monococcum*, *T. aegilopoides*, and *T. boeoticum*). One of the authors (KWS) had available *Aegilops squarrosa* (var. *strangulata*). In addition, various common and durum wheat varieties previously examined at the Northern Regional Laboratory were used in our studies.

Methods for Single-Kernel Analysis

Based on Shepherd's analysis of gliadin proteins from single kernels of wheat (21) and recent sequential extraction studies of glutenin (24), the following

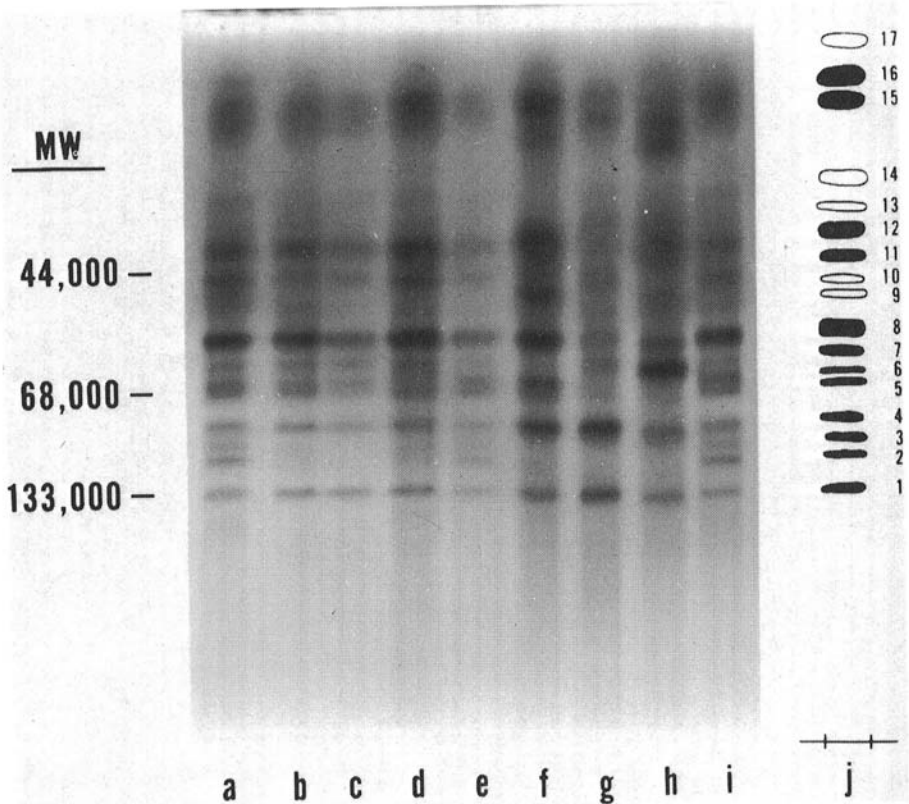


Fig. 2. Single-kernel glutenin analysis by SDS-PAGE at pH 8.9 of 1B aneuploids of Chinese Spring: **a**) Chinese Spring; **b**) N1BT1A; **c**) N1BT1D; **d**) ditelo 1B_S; **e**) ditelo 1B_L; **f**) acetic acid extract of N1BT1D; **g**) HgCl₂ extract of N1BT1D flour after acetic acid extraction; **h**) 2-mercaptoethanol extract of N1BT1D flour after acetic acid and HgCl₂ extractions; **i**) Chinese Spring; and **j**) diagram of subunits in Chinese Spring.

procedure was developed for isolation of glutenin from single kernels of wheat:

The embryo ends of single wheat kernels (15–40 mg) were removed with a razor blade and discarded (or saved to be grown); the endosperm plus bran was then ground thoroughly with a mortar and pestle and transferred to 16 × 150-mm tubes. Alternatively, 15–20 mg of milled flour was used. The material was first suspended in 5 ml 0.04M NaCl and shaken for 30 min with sufficient vigor to keep it suspended (a vortex mixer or a reciprocating shaker operating at about 300 rpm). The tube was then centrifuged (2–3 min on a bench-top centrifuge), and the supernatant (containing albumins and globulins) discarded. The pellet was resuspended in *ca.* 5 ml 0.04M NaCl using a vortex mixer and the extraction was repeated. Similarly, the flour was extracted twice with 70% ethanol to remove gliadins. The pellet was then suspended in 2.0 ml 0.7% acetic acid and

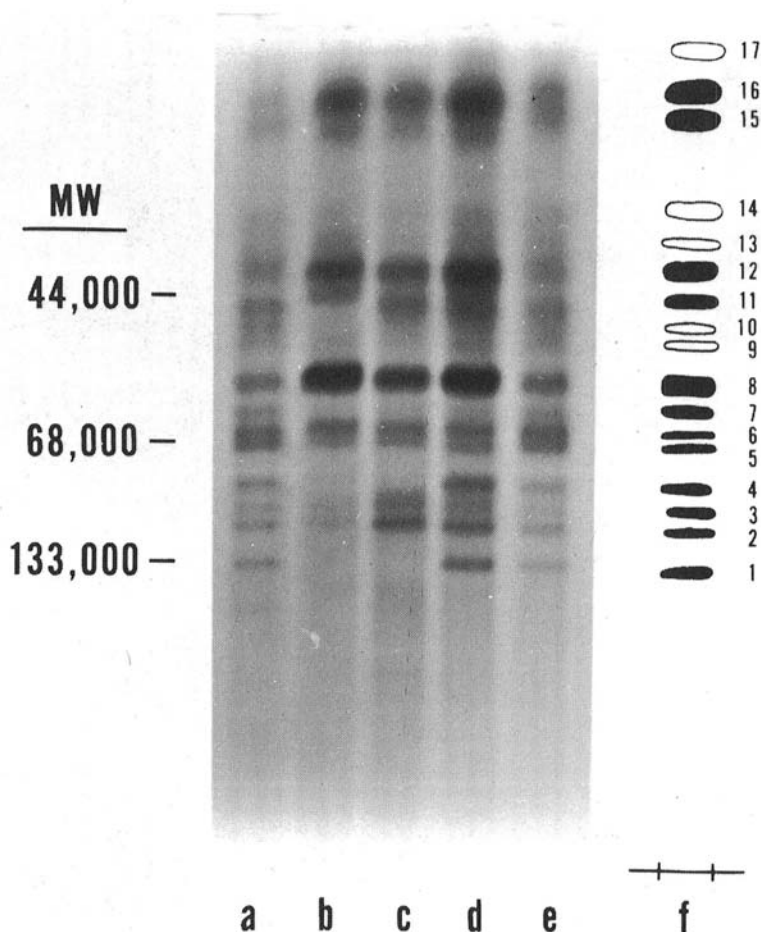


Fig. 3. SDS-PAGE at pH 8.9 of glutenins from single kernels of Chinese Spring's 1D aneuploids: a) Chinese Spring; b) N1DT1A; c) N1DT1B; d) ditelo 1DL₁; e) Chinese Spring; and f) subunit diagram for Chinese Spring.

shaken 30 min; 5.6 ml 95% ethanol was added (to bring the ethanol concentration to 70%), and the tube was again shaken for 30 min. The pH was then adjusted to 6.6–8.0 with three drops (*ca.* 0.1 ml) 2*N* NaOH, and the tube was cooled to 3–4°C (overnight in refrigerator, 1–2 hr in freezer, or a few minutes with shaking in an ice bath) to reprecipitate that glutenin which was previously solubilized. This procedure leaves soluble the protein (primarily gliadin) which associates tenaciously with glutenin (24), thereby purifying the glutenin. The mixture was again centrifuged, the supernatant decanted, and the pellet lyophilized (about 1 hr). To the dry residue was added 0.1 ml 0.125*M* Tris-borate, pH 8.9, containing 1% 2-mercaptoethanol and 0.1% SDS, along with 4–5 mg added SDS (at least twice the weight of protein present). The residue was dispersed, incubated at 37°C for *ca.* 16 hr, and centrifuged. The supernatant was then analyzed directly by SDS-PAGE. Replicate single kernels of each genetic stock were examined.

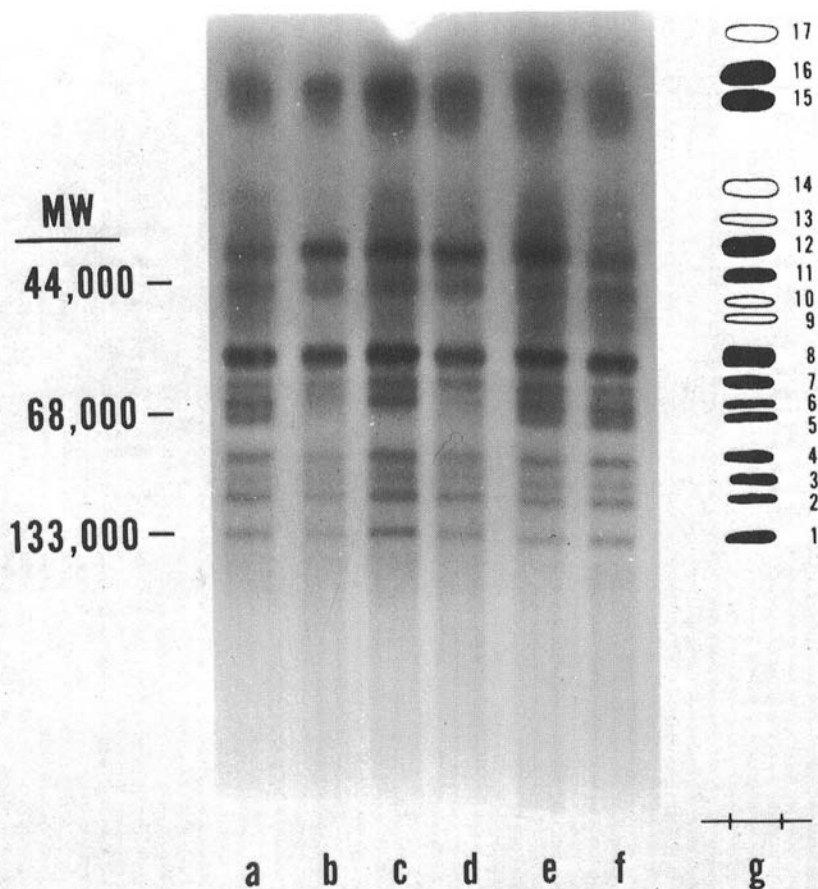


Fig. 4. SDS-PAGE analysis at pH 8.9 of glutenins isolated from single kernels of a) Chinese Spring; b) N4DT4A; c) N4DT4B; d) ditelo 4D_s; e) ditelo 4D_L; and f) Chinese Spring; and g) diagram of Chinese Spring glutenin subunits.

Using this procedure, one person can prepare purified glutenin from more than 100 samples in a single day.

For some experiments, when isolation of glutenin was unnecessary, the total protein contents of single kernels were examined by incubating the ground endosperm or flour directly with pH 8.9 Tris-borate buffer containing 2-mercaptoethanol and SDS as described. Similarly, residue protein not extracted by conventional solvents may be examined in this manner.

Other Analytical and Preparative Methods

Bulk samples of flour were milled and defatted, and glutenins were sequentially extracted (with 0.1*N* acetic acid, 0.01*N* acetic acid-0.2*mM* HgCl₂, and 0.1*N* acetic acid-0.1% 2-mercaptoethanol) and purified as described previously (24). Glutenin was prepared from gluten balls by dispersion in 70% ethanol-0.1*N* acetic acid and repeated precipitation at pH 6.6 (28). Glutenin was also extracted from ground endosperm with AUC (0.1*M* acetic acid-3*M* urea-0.01*M* cetyltrimethylammonium bromide) and purified by pH precipitation (29).

Yields of fractions were determined gravimetrically or by micro-Kjeldahl

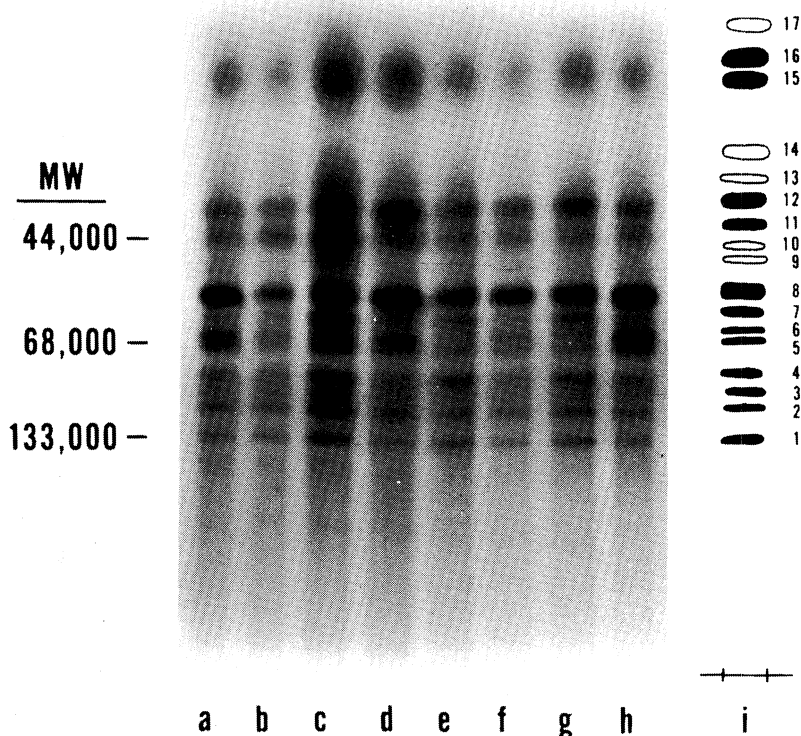


Fig. 5. SDS-PAGE at pH 8.9 of glutenin isolated from single kernels of a) Chinese Spring; b) N2DT2B; c) N2DT2A; d) N3DT3B; e) N3DT3A; f) N6DT6B; g) N6DT6A; h) Chinese Spring; and i) diagram of Chinese Spring glutenin subunits.

analysis using an improved automated ammonia procedure after manual digestion (30).

SDS-PAGE at pH 8.9 was performed on 5% gels in 0.125 M Tris-borate buffer containing 0.1% SDS (7); mol wt standards used were cytochrome c, chymotrypsinogen A, ovalbumin, and serum albumin (7). Staining intensity and contrast of protein bands on gels were improved greatly by double staining and destaining; surface blemishes introduced during handling were easily removed by brief immersion in dilute sodium hypochlorite.

Attempts to duplicate Orth and Bushuk's conditions for SDS-PAGE at pH 7.3 (8) in our horizontal electrophoresis apparatus were unsuccessful. Their method was changed as follows: a) 0.33 ml N,N,N',N'-tetramethylethylenediamine was replaced by 0.55 ml dimethylaminopropionitrile; b) ammonium persulfate concentration was decreased by half; c) 2.83mM Na₂SO₃ was included in the gel; d) electrode buffer was recirculated during the run; and e) cooling the gel before electrophoresis, which caused a precipitate to form, was avoided. Starch-gel electrophoresis (31) was used to assess purity of glutenin fractions. Reagent-grade chemicals and deionized water were used throughout.

RESULTS AND DISCUSSION

Single-Kernel Analysis

Glutenin prepared from a single kernel of Chinese Spring wheat (Fig. 1, pattern b) contains all subunits which are found in glutenin prepared by bulk extraction techniques (Fig. 1, patterns d-f). This suggests that grinding of the endosperm with a mortar and pestle is adequate for protein extraction, and shows that the bran (not present in milled flour) does not contain additional contaminating proteins in significant amounts and does not interfere with the extraction of endosperm proteins. SDS-PAGE patterns were identical in subunit composition for glutenins from replicate single kernels of each genetic stock used.

The glutenin isolated from a gluten ball (Fig. 1c) is deficient in subunit band 8 (see Fig. 1i) compared to the extracted glutenins (Fig. 1: b, d-f). Two explanations for this observation are possible: One, the subunit may be part of a native glutenin species insoluble in 0.7% acetic acid-70% ethanol during preparation of glutenin and gliadin from a gluten ball. Initial experiments do not support this possibility. A second, and more likely, possibility is that this polypeptide (subunit 8) represents a labile glutenin constituent which is released during dough formation and washed out of the gluten ball along with albumins, globulins, and starch. When proteins are extracted directly from flour, however, and when dough formation, which presumably involves disulfide interchange, does not occur, the linkage of this polypeptide to native glutenin may remain intact.

SDS-PAGE patterns observed for small-scale total protein extracts (Fig. 1, g-h) are also identical in the high mol wt region to glutenin prepared from a single kernel (Fig. 1b); thus it is often sufficient to examine total protein extracts when it is desired to compare only high mol wt glutenin subunits from different varieties.

The purity of glutenin isolated by the single-kernel technique is evident from its similarity upon SDS-PAGE to other preparations of known purity (Fig. 1).

Also, when starch-glutenin residues from single kernels are incubated with 2*M* urea and analyzed by starch-gel electrophoresis (21), no albumin, globulin, or gliadin proteins enter the gel.

The amount of protein extracted during single-kernel analysis was estimated by incubating several portions of a bulk sample of defatted Chinese Spring flour with 0.125*M* Tris-borate buffer, pH 8.9, containing SDS and 2-mercaptoethanol under the same conditions used during complexing of protein and SDS before SDS-PAGE. The bulk flour sample is similar in particle size to the manually ground single-kernel samples and both lack germ components. Following incubation, the mixtures were centrifuged and supernatants were analyzed for N (30). In three separate experiments, 97.3%, 100.3%, and 101.2% of the N in flour was extracted. Therefore, since all glutenin is solubilized, SDS-PAGE patterns of glutenin isolated from single kernels are expected to include subunits of all types of glutenin molecules (24).

Chromosomal Location of Genes Coding Glutenin Subunits

Glutenins were isolated from individual kernels of all nullisomic-tetrasomic, nullisomic-trisomic, and ditelocentric lines of Chinese Spring and examined by SDS-PAGE; those lines whose glutenins differ significantly from the parent variety in subunit composition are discussed in the following paragraphs.

Glutenin subunit bands 2 (mol wt 104000) and 3 (mol wt 93000) are either absent or present in small amounts in N1BT1A, N1BT1D, and ditelo 1B_s (Fig. 2, patterns b-d), but present in ditelo 1B_L (Fig. 2e). Therefore, the genes coding these subunits are on the long arm of chromosome 1B. Subunits 2 and 3 were previously assigned to chromosome 1B when a bulk sample of N1BT1D was examined [(25); see also footnote 4]. We therefore compared the single-kernel results (Fig. 2c) to those obtained for glutenins sequentially extracted from N1BT1D flour with solvents containing acetic acid, HgCl₂, and 2-mercaptoethanol (Fig. 2, f-h). Subunits 2 and 3 are absent in all three bulk extracts, but subunit 7 is also absent in the acetic acid extract, and subunit 6 is absent in the HgCl₂ and 2-mercaptoethanol extracts. Similar results were obtained for the parent variety, Chinese Spring (Fig. 1, d-f; see also ref. 24). Since glutenins extracted with different solvents differ in subunit composition, less than complete extraction could have erroneously indicated that chromosome 1B codes glutenin subunits 6 and 7.

Chromosome 1D also codes two glutenin polypeptides (Fig. 3); subunits 1 (mol wt 133000) and 4 (mol wt 86000) are absent in N1DT1A and N1DT1B, but present in ditelo 1D_L, and are, therefore, coded by genes on the long arm of 1D. Band 7 is less intense in the nullisomic 1D lines (Fig. 3, b-c) than in Chinese Spring, so it is likely that chromosome 1D also codes one glutenin subunit in this band. These results basically agree with those of Orth and Bushuk (26) who concluded by SDS-PAGE at pH 7.3 that four subunits, apparently including subunits 1 and 4 and two lower mol wt ones, were coded by 1D.

Analysis of the Chinese Spring aneuploids also revealed that chromosome 4D codes a major high mol wt glutenin subunit. Subunit 5 (mol wt 68000) is absent in N4DT4A (Fig. 4b), N4DT4B (Fig. 4c), and ditelo 4D_s (Fig. 4d), but present in ditelo 4D_L (Fig. 4e), and is, therefore, coded by a gene on the long arm of chromosome 4D. Orth and Bushuk (26) failed to observe a deletion corresponding to this subunit in N4DT4A or N4DT4B by SDS-PAGE at pH 7.3.

Glutenin subunit bands 1-5 are, therefore, coded by chromosome arms 1B_L, 1D_L, and 4D_L. Since no other aneuploid stocks of Chinese Spring exhibited band deletions, the remaining bands must contain more than one subunit coded by more than one chromosome. Indeed, differences in intensity were occasionally apparent, particularly for bands 6 and 7. Glutenin's 44000 mol wt gliadin-like subunit (band 10) has previously been shown to be heterogeneous (32).

Some of our results differed markedly from those reported by Orth and Bushuk (26) using similar genotypes. For example, they found by SDS-PAGE at pH 7.3 that the N2DT2B, N3DT3B, and N6DT6B lines of Chinese Spring apparently lacked subunits 2 and 3, but that the corresponding nulli-D tetra-A lines contained them. They attributed this difference to a repression of normal gene expression due to doubling the number of certain B genome chromosomes. We found, however, that N2DT2B, N2DT2A, N3DT3B, N3DT3A, N6DT6B, and N6DT6A (Fig. 5, b-g) are all identical to Chinese Spring in glutenin subunit composition. These conflicting results may possibly be explained in part by native glutenin's heterogeneity: if some, but not all, glutenin molecules are deficient in subunits 2 and 3 (24), and if glutenin extraction is incomplete (29), results may be similar to those of Orth and Bushuk (26). For example, acetic acid extracts of Chinese Spring (Fig. 1d) and N1BT1D (Fig. 2f) are deficient in subunits 2 and 3, while other glutenin preparations from these lines contain these subunits (Fig. 1, e-f; Fig. 2, g-h; see also ref. 24). Also, since we observed similar quantities of subunits 2 and 3 in N2DT2B, N3DT3B, N6DT6B, and Chinese Spring (Fig. 5), our results provide no evidence of interference with normal gene expression for glutenin subunits by other chromosomes. The subunit omissions noted by Orth and Bushuk (26) may possibly be explainable by effects on protein solubility or degree of aggregation due to doubling of the number of 2B, 3B, or 6B chromosomes.

The results obtained with Chinese Spring were further tested by analyzing additional stocks of wheat and some related species. The effect of chromosome 1D on Chinese Spring glutenin was confirmed with the disomic addition line of this chromosome to Langdon durum (27); the parent durum lacked subunits 1 and 4, but these bands were present in the 1D addition line.

In another study, the chromosomal control of glutenin subunits in the soft red spring variety Chinese Spring was compared with that in the hard red winter variety Cheyenne by analyzing the lines in which individual chromosome pairs of Cheyenne substitute for their homologs in Chinese Spring. The subunit pattern of Cheyenne was identical with that of Chinese Spring except that Cheyenne's highest mol wt subunit had slightly greater electrophoretic mobility than subunit 1 of Chinese Spring (Fig. 6, a-b). All substitution lines tested gave the Chinese Spring subunit pattern except for line 1D (Fig. 6c), which resembled Cheyenne. Thus it may be concluded that chromosomes 1B, 1D, and 4D have the same effect on glutenin composition in both varieties, except that subunit 1 of Cheyenne, coded by chromosome 1D, is slightly more mobile than subunit 1 of Chinese Spring. It is also of interest to note that the improved dough-mixing and baking characteristics noted for Cheyenne-Chinese Spring substitution lines 1B, 4B, 7B, and 5D (33) are not associated with any changes in glutenin subunit composition.

Since we failed to find any high mol wt glutenin subunits coded for by the A genome in Chinese Spring, glutenins of some contemporary AA diploid species

were examined. Surprisingly, each of the accessions examined (representative patterns are shown in Fig. 6, e-g) possessed high mol wt glutenin subunits including a prominent band between subunits 1 and 2, and faint bands in the region of subunits 2 and 3 of Chinese Spring. These faint bands may be similar to those observed in nullisomic 1B lines of Chinese Spring; since they are generally more pronounced in NIBT1A than in NIBT1D (see Fig. 2, b-c), they are possibly controlled by chromosome 1A and overlap the stronger bands controlled by chromosome 1B in Chinese Spring. The prominent high mol wt subunit present in all AA diploids examined, however, is not present in Chinese Spring; if the gene(s) coding for it were present in the original diploid donor of the A genome to polyploid wheat, they must have been lost or suppressed during the evolution of the Chinese Spring genotype. In this respect, it will be of interest to learn whether other hexaploid and tetraploid wheats possess such a high mol wt glutenin subunit coded for by the A genome.

One accession of *Ae. squarrosa*, the accepted donor of the D genome to hexaploid wheat, was also examined (Fig. 6h). It contains glutenin subunits with mol wt similar to but not identical with subunits 1, 4, and 5 of Chinese Spring,

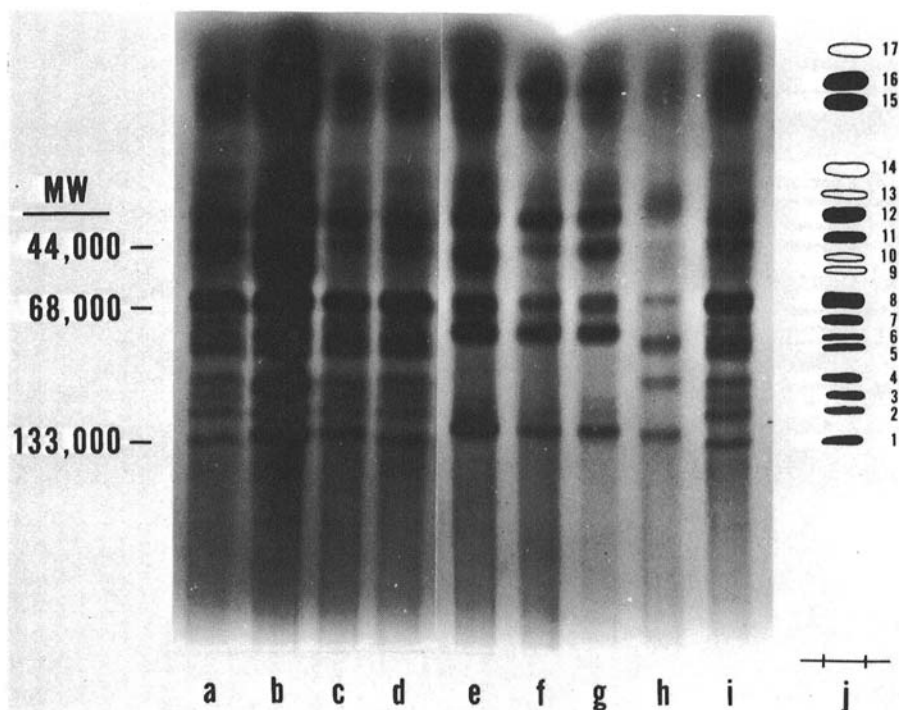


Fig. 6. SDS-PAGE at pH 8.9 of glutenin subunits isolated from single kernels of a) Chinese Spring; b) Cheyenne; c) Chinese Spring (Cheyenne 1D) substitution line; d) Chinese Spring; e) *Triticum monococcum* (Sweden); f) *T. aegilopoides* (Russia); g) *T. boeoticum* (Turkey); h) *Aegilops squarrosa* (var. *strangulata*); i) Chinese Spring; and j) glutenin subunit diagram for Chinese Spring.

known to be controlled by D genome chromosomes. In addition, both *Ae. squarrosa* and AA diploids possess a prominent band at position 8, possibly accounting for the apparent heterogeneity of this band in Chinese Spring.

Comparison of Hexaploid Wheats with Derived Tetraploids

The preceding data suggest that if coding of glutenin subunits is similar among hexaploid wheats, AABB tetraploid wheats, which have no D genome chromosomes, should lack subunits 1, 4, and 5 since they are coded by chromosomes 1D and 4D. Indeed, many commonly grown durum wheats lack these subunits (Fig. 7). Due to varietal differences, however, a more rigorous study of hexaploid and tetraploid wheats should compare materials with identical A and B genomes.

We, therefore, compared glutenins from the hexaploid wheat varieties Prelude, Canthatch, Thatcher, and Rescue with their extracted AABB

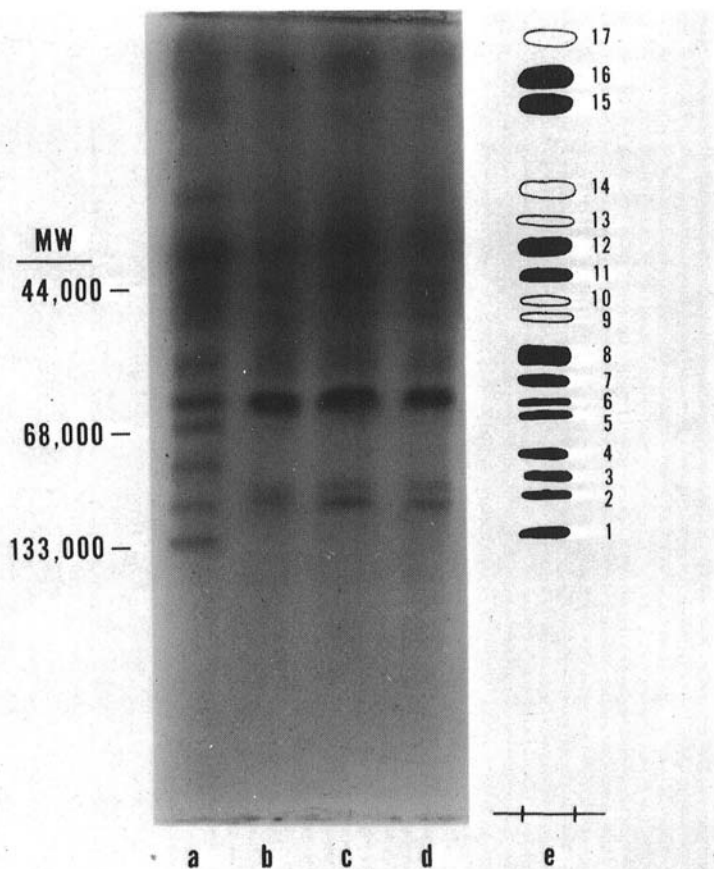


Fig. 7. SDS-PAGE at pH 8.9 of total protein extracts of a) Chinese Spring wheat; b) Wells durum; c) Stewart durum; and d) Mindum durum; e) diagram of Chinese Spring glutenin subunits.

tetraploids (9,10) by SDS-PAGE at pH 8.9 (Fig. 8). Compared to Prelude (Fig. 8b), glutenin subunits of Tetraprelude corresponding to subunits 4 and 5 of Chinese Spring were deleted or greatly diluted and subunit 1 is replaced by a subunit of slightly greater mobility upon removal of the D genome (Fig. 8c). The difference in mobility of subunit 1, as well as all other observed differences, is significant and reproducible. In Tetracanthatch (Fig. 8e), subunit 5 of Canthatch (Fig. 8d) is deleted, and subunit 4, which is very close in mol wt to subunit 3, is deleted or diluted. In Thatcher (Fig. 8f) and Rescue (Fig. 8h), subunits 4 and 5 are also deleted or diluted in the extracted tetraploids. Also in Rescue, two high mol wt subunits replace subunit 1 of Chinese Spring; one of these is deleted or greatly diluted in the extracted tetraploid. Longer gel separations do not separate these subunits better, nor reveal any further heterogeneity.

We attempted to determine why our results for the extracted tetraploids (Fig. 8) differed significantly from those of Orth and Bushuk (18). To determine if different extraction methods give different results, glutenins from Prelude, Thatcher, and their derived tetraploids were extracted with AUC, purified by pH

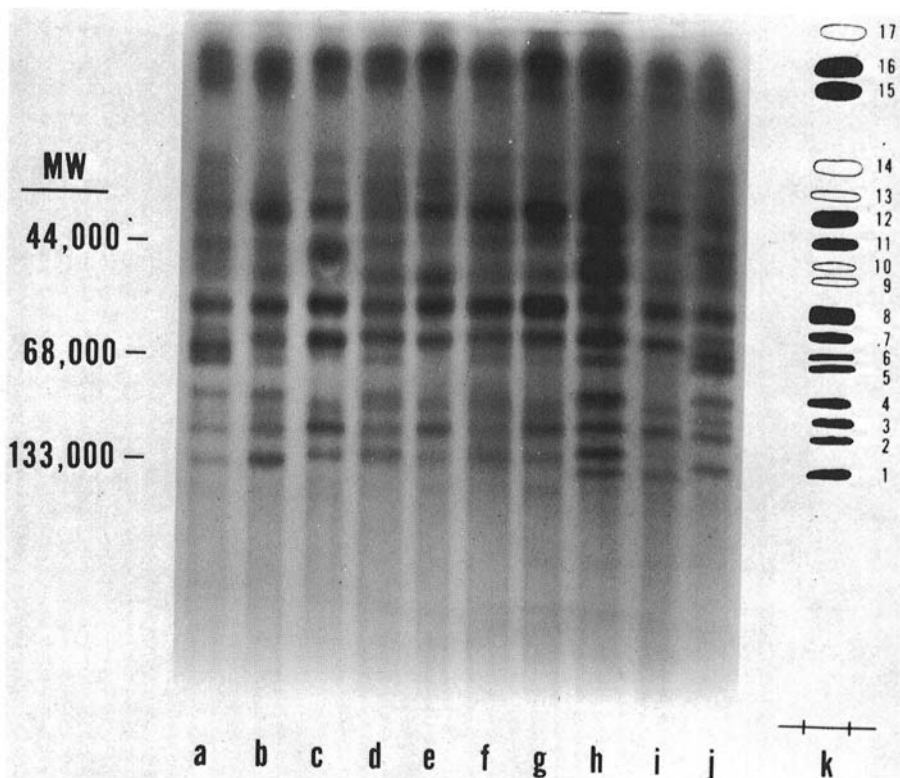


Fig. 8. SDS-PAGE at pH 8.9 of glutenins from single kernels of hexaploid wheats and their derived tetraploids: a) Chinese Spring; b) Prelude; c) Tetraprelude; d) Canthatch; e) Tetracanthatch; f) Thatcher; g) Tetrathatcher; h) Rescue; i) Tetrarescue; and j) Chinese Spring; k) diagram of glutenin subunit composition in Chinese Spring.

precipitation (29), and compared by SDS-PAGE at pH 8.9 to the AUC-insoluble protein remaining in the residue. For each variety, gel patterns for AUC-soluble and -insoluble glutenin were similar to each other and to the total glutenin extract (Fig. 8). Therefore, the difference between results does not seem to be explainable on the basis of solubility or insolubility of glutenins with different subunit compositions.

We also compared glutenins from the hexaploids and extracted tetraploids by SDS-PAGE at pH 7.3 (Fig. 9A). The results support the pH 8.9 data in that subunits 4 and 5 are deleted or greatly diluted in the derived tetraploids. Subunit 1 is unusually faint, however, and we were uncertain whether it differed between the hexaploid and derived tetraploid varieties. We, therefore, examined total protein extracts of these varieties (Fig. 9B). Proteins were reduced and complexed to SDS at pH 7.3 or 8.9 and analyzed by SDS-PAGE at pH 7.3. Since only glutenin contains subunits 1-5, these bands in total protein extracts should be equivalent to those in isolated glutenin. Gel patterns for Chinese Spring reduced at pH 7.3 and 8.9 (Fig. 9, L-m) are identical and also correspond closely to the pattern obtained at pH 8.9 (Fig. 1g). Subunits 3 and 4, however, for unknown reasons, migrate with almost equal mobilities. The standard proteins (Fig. 9k) further show that SDS-PAGE separations at the two pH values are otherwise identical.

The pH 7.3 gel patterns for total protein extracts of Prelude, Thatcher, and their derived tetraploids (which were representative of all four hexaploid-derived tetraploid pairs) are shown in Fig. 9(n-q). The derived tetraploids always contain a subunit equal in mol wt to subunit 1 of their parent hexaploid varieties. Subunit 4 appears to be decreased in intensity in the derived tetraploids, apparently due to the presence of subunit 3.

Therefore, glutenin subunit 1 is evident in all four extracted tetraploids upon

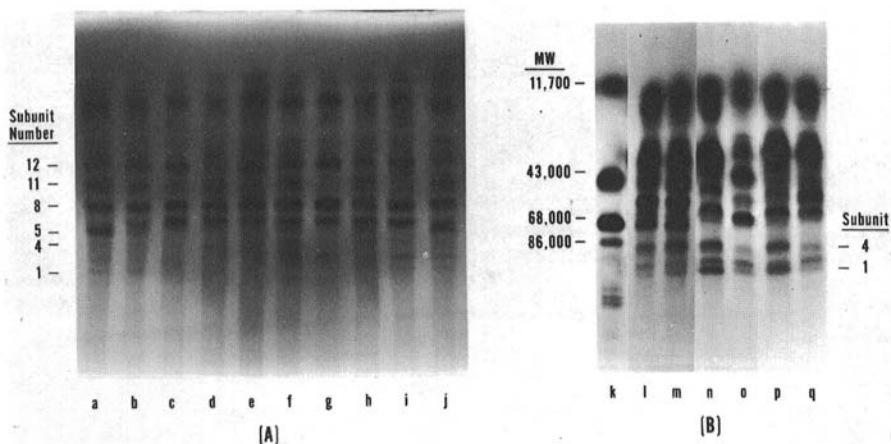


Fig. 9. (A) SDS-PAGE at pH 7.3 of glutenins from single kernels of: a) Chinese Spring; b) Prelude; c) Tetraprelude; d) Canthatch; e) Tetracanthatch; f) Thatcher; g) Tetrathatcher; h) Rescue; i) Tetrarescue; and j) Chinese Spring. (B) Standard proteins: k); total protein extracts from single kernels of l) Chinese Spring extracted at pH 7.3; m) Chinese Spring extracted at pH 8.9; n) Prelude; o) Tetraprelude; p) Thatcher; and q) Tetrathatcher.

SDS-PAGE at pH 8.9 and 7.3. If glutenin subunit coding for all varieties is similar to that in Chinese Spring, glutenin subunits 1, 4, and 5 should always be coded by D genome chromosomes. Since subunit 1 was not deleted in the extracted tetraploids, it may be heterogeneous in these four varieties but not in Chinese Spring, so that deletion of a 1D-coded subunit appears only as a decrease in band intensity. Alternatively, translocation of part of the 1D_L chromosome with an A or B genome chromosome, as observed for Prelude (10), may also have occurred during preparation of the other extracted tetraploid wheats. We are unable to choose between these possibilities on the basis of our data.

Glutenin Subunit Variability Among Hexaploid Wheats

Since previous studies (7,8) had failed to relate the glutenin subunit composition of bread wheats with their properties, we were interested in examining a wider range of hexaploid wheat samples, using single-kernel methodology, to seek such differences that could be related to properties or

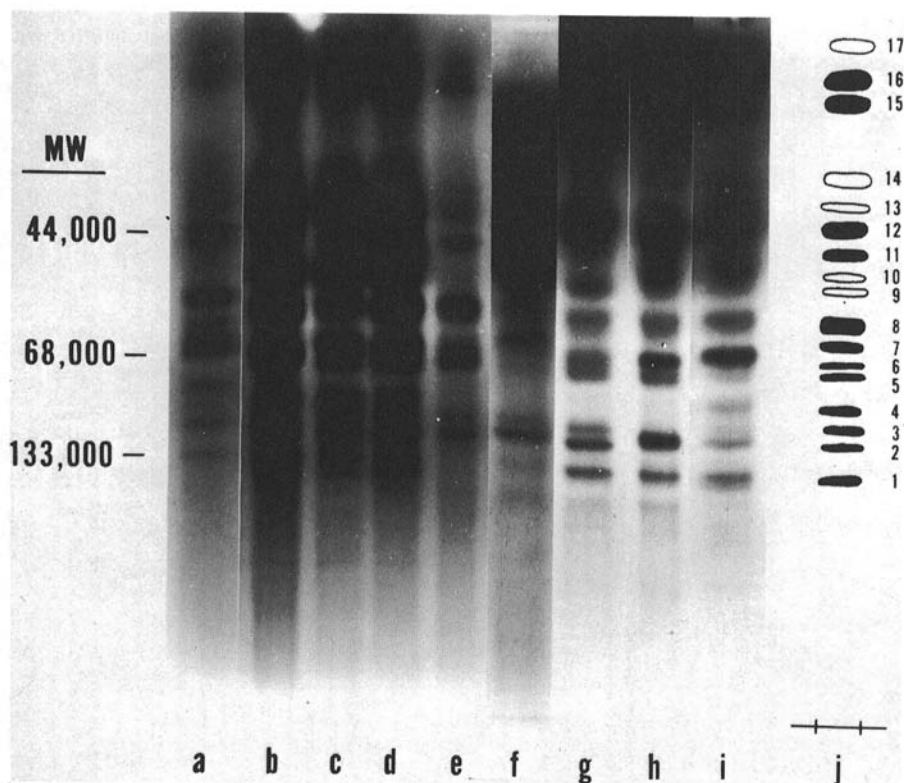


Fig. 10. SDS-PAGE at pH 8.9 of glutenins isolated from single kernels of various hexaploid wheat varieties: a) Chinese Spring; b) Vulcano; c) MV-72-16; d) Hokuei; e) Nap Hal; f-i) unusual genotypes selected from a bulk sample of Nap Hal; and j) diagram of glutenin subunit composition in Chinese Spring.

qualities. We, therefore, examined 80 hexaploid varieties selected to cover a wide range of origins; some results are shown in Fig. 10.

Approximately 75 of the 80 varieties examined had glutenin subunit patterns identical or quite similar to that of Chinese Spring (Fig. 10a). Four other varieties [Zlatna Dolina (Yugoslavia); Vulcano (Fig. 10b) (Italy); MV-72-16 (Fig. 10c) (Hungary); and Hokuei (Fig. 10d) (Japan)], like Rescue (Fig. 8h), had two high mol wt bands in place of band 1 of Chinese Spring (Fig. 10, b-d). One variety, Nap Hal (Fig. 10e), was most unusual because glutenin subunits 1 and 4 were absent. Preliminary cytological analysis revealed an apparently normal chromosome number, but it is conceivable that a fragment of 1D_L, which carries genes for these subunits in Chinese Spring, has been lost. Interestingly, Nap Hal has both high-protein and high-lysine contents (34), but has bad baking properties.⁵ We suggest that these poor properties may be related to the absence of these two high mol wt glutenin subunits. The unusual glutenin subunit pattern observed for Nap Hal may, however, prove useful in further genetic or breeding studies.

A bulk sample of Nap Hal seed contained a few kernels which were larger, had a deeper crease, or were more wrinkled in appearance. Some of these kernels (Fig. 10, f-i) were found to contain glutenins with very unusual high mol wt subunit compositions. The germ ends of these kernels are currently being grown,

⁵P. J. Mattern, personal communication.

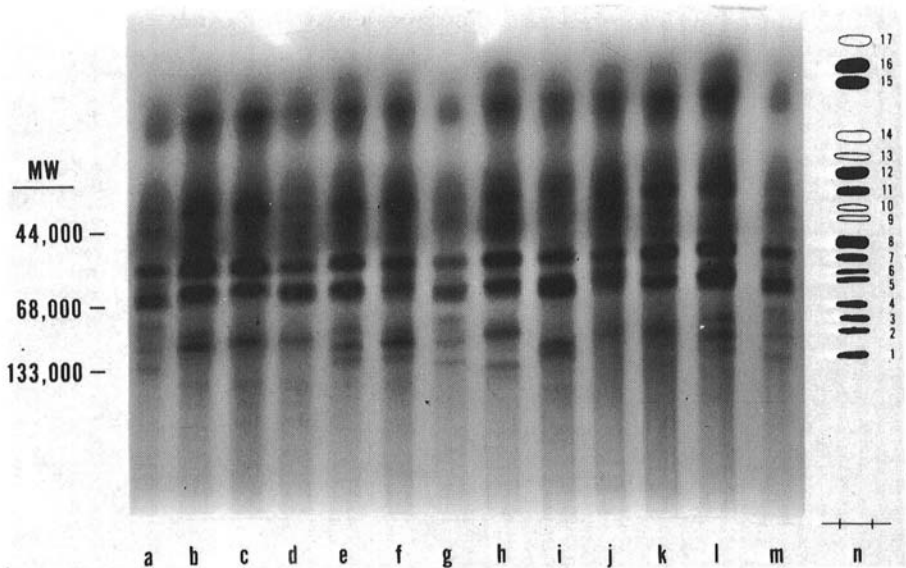


Fig. 11. SDS-PAGE at pH 8.9 of glutenin subunits isolated from single kernels of various AABB tetraploid wheat varieties: a) Chinese Spring (standard hexaploid variety); b) Sivousska-3; c) Leeds; d) PI225167; e) *Triticum turgidum*; f) St 464; g) Chinese Spring; h) Bagudo 10150 V56A; i) *Tr. dicoccum*?; j) Cudesnaja Blagodat; k) Tremes Preto 3687; l) Argelino; m) Chinese Spring; and n) diagram of glutenin subunit composition of Chinese Spring.

and plants and seed from the next generation will be examined for unusual properties. This study demonstrates how bulk samples can be examined by the single kernel technique, and shows how kernels with unusual genotypes can be selected on the basis of differing gross kernel morphology.

Glutenin Subunit Variability Among AABB Tetraploid Wheats

Most durum wheats previously examined were commonly grown varieties of limited geographical origin and had similar glutenin subunit compositions (Fig. 7, b-d). A diverse sample of the world AABB tetraploid population, however, exhibits considerably more variability (Fig. 11).

Of the 55 lines examined, 18 had "normal" glutenin subunit compositions (as in Fig. 7) in that subunits 1, 4, and 5 are absent and 2 and 3 are present; a typical example is Sivousska-3 (No. 478445) (Fig. 11b). In another 18 varieties, subunits 2 and 3 were present in greatly different amounts, only one was present, or these two subunits were replaced by a different subunit of slightly different mol wt; typical examples are the varieties Leeds (No. 13768) (Fig. 11c), PI225167 (Fig. 11d), Cudesnaja Blagodat (PI349056) (Fig. 11j), and Tremes Preto 3687 (PI192851) (Fig. 11k). In six varieties, subunit 5 seems to be present; examples are St 464 (Fig. 11f) and Argelino (PI266906) (Fig. 11l). The nondurum tetraploid *Triticum dicoccum* (?) (CI15590) (Fig. 11i) is uniquely different: two major high mol wt subunits are present; one equal in mol wt to subunit 2; and one intermediate in mol wt between subunits 1 and 2. Finally, 12 varieties contain glutenin subunits of mol wt equal or similar to subunit 1; examples are *Triticum turgidum* (Fig. 11e) and the durums St 464 (Fig. 11f), Bagudo 10150 V56A (No. 192823) (Fig. 11h), and Argelino (PI266906) (Fig. 11l). Cytological analysis of root tips of these varieties revealed 28 chromosomes, precluding the possibility that hexaploid materials were inadvertently examined.⁶ Thus, considerable variability in glutenin subunit composition exists among AABB tetraploid species and durum varieties; also, A and B genome chromosomes frequently code polypeptides of mol wt similar to those of the high mol wt glutenin subunits 1 and 5, which are coded by chromosomes 1D and 4D in Chinese Spring and, presumably, in most hexaploid wheats.

GENERAL DISCUSSION

The single-kernel method for analyzing wheat glutenin was extremely valuable in determining chromosomal coding of the five highest mol wt subunits of Chinese Spring since small amounts of seed of most lines were available. Since all protein, including glutenin, is extracted from flour by the SDS- and 2-mercaptoethanol-containing solvent, atypical results due to incomplete extraction of glutenin are avoided. Since the single-kernel technique is rapid, large numbers of samples can be analyzed, as in screening seed populations for unusual genotypes. Single-kernel analysis should also be valuable to the wheat breeder when specific glutenin bands indicate the presence or absence of chromosomes carrying desirable or undesirable traits. The technique is essentially nondestructive because brush halves of kernels may be analyzed and

⁶L. R. Joppa, personal communication.

germ ends grown; the method is, therefore, well suited for selection during early crosses.

The long arms of chromosomes 1B, 1D, and 4D contain genes coding the five largest glutenin subunits in Chinese Spring; presumably these same chromosomes contribute to the glutenin composition of other hexaploid wheats also, as indeed was indicated for the results obtained with Cheyenne in the present study. Since glutenin's high mol wt subunits must be major determinants of its properties, the chromosome arms that code these subunits may be essential for good-quality characteristics in wheat, as previously observed for chromosomes 1D (10,19) and 1B (33). Since single chromosomes almost entirely code these subunits, each band probably represents an individual subunit. Similarly, Huebner and Wall (35) noted that high mol wt glutenin subunits giving single bands upon SDS-PAGE were also homogeneous by starch-gel electrophoresis, indicating that these bands are single polypeptides. Glutenin's 44000 mol wt subunits (band 11) (32), however, and presumably the remaining bands that do not show single-chromosome control, are heterogeneous and contain more than one polypeptide of similar mol wt.

The D-genome codings observed for Chinese Spring agree with the glutenin subunit compositions of many varieties of *T. durum* and of *Ae. squarrosa*, but not entirely with those of four extracted AABB tetraploids. This difference could conceivably be due to a 1D translocation as observed in Tetraprelude (10). Alternatively, however, many AABB tetraploids exhibit considerable variability in glutenin subunit composition, so high mol wt glutenin subunits may be heterogeneous in some hexaploid varieties, and removal of the D genome may still leave subunits similar in mol wt to subunits 1 and 5. Subunit 4, however, is absent or greatly diluted in all naturally occurring and derived AABB tetraploids examined to date, and may, therefore, be the best differentiator (by SDS-PAGE at pH 8.9) of hexaploid and AABB tetraploid wheats.

Hexaploid wheat varieties seem to have much more uniform glutenin subunit compositions than do AABB tetraploids, as expected if only certain AABB tetraploids hybridized with *Ae. squarrosa* (DD) to produce hexaploid bread wheats with desirable functional properties. Since all AA diploids (Fig. 4) and *Ae. squarrosa* (DD) lines (see Fig. 3 in ref. 18) examined to date also have similar glutenin subunit compositions, it seems possible that glutenin subunits 2 and 3, synthesized by chromosome 1B_L, may be indicators of which diploid species is the origin and donor of the B genome of bread wheat. The major high mol wt glutenin subunit in three diploid (AA) *Triticum* species, however, does not appear to correspond to any of the bands in varieties of *T. aestivum* examined so far, suggesting either that considerable differentiation with respect to glutenin may have occurred in *T. aestivum* or in its AA progenitor subsequent to the initial production of hexaploid wheat, or that the three genomes may have contributed somewhat unequally to hexaploid wheat's glutenin composition. Examination of additional genetic stock by the techniques we have described may shed further light on generic, specific, and varietal relationships among cereals.

The results obtained using SDS-PAGE at pH 8.9 differ from those we obtained at pH 7.3, and from those of Orth and Bushuk (18,26). This difference may be partially due to the methods of extraction used: SDS plus

mercaptoethanol quantitatively extracts proteins, whereas AUC extracts only about 93% (29). Since glutenin is heterogeneous, incomplete extraction, such as with dilute acetic acid, can yield preparations with subunit compositions atypical of whole glutenin (24). The AUC-extracted glutenin, however, was similar to total glutenin in subunit composition in the varieties we examined, so the method of extraction does not seem to be a major difference between the studies.

Rather, the differences in results seem to be primarily in the analytical conditions themselves. Resolution, such as of glutenin subunits 3 and 4, seems slightly better at pH 8.9. A major difference, however, is in the staining; since high mol wt bands stain much less intensely at pH 7.3 than at pH 8.9, pH 7.3 gels are much more difficult to interpret. Conceivably, this is a deleterious effect of phosphate buffer as compared to Tris-borate. For these reasons, but primarily because we have less difficulty obtaining good, sharp, reproducible results, we prefer SDS-PAGE at pH 8.9 rather than at pH 7.3.

In conclusion, the purification and analysis of glutenin from single kernels of hexaploid and tetraploid wheats with normal and modified chromosome complements have permitted the assignment of several high mol wt glutenin subunits to specific chromosome arms, and have led to observations on variability and quality-controlling factors in wheat. These observations and the described methods should prove valuable to the wheat geneticist and breeder.

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