

Studies of Glutenin. VII. Inheritance of Its Physicochemical Factors in Triticale¹

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ABSTRACT

Glutenins of one variety of Triticale (6A190) and its rye and durum parents were studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, amino acid analysis, and scanning electron microscopy. Results of all techniques showed that glutenin of Triticale is simply inherited from its parents. All glutenin subunits of the Triticale could be accounted for by corresponding subunits in one, or both, of its parents. The amino acid composition of the Triticale glutenin was essentially intermediate between that of its rye and durum parents. Distinct differences in the ultrastructure of glutenin of spring rye (cv. Prolific) and amber durum (cv. Stewart) were noted. Both types of ultrastructure were evident in the micrographs of Triticale glutenin.

Hexaploid Triticale (AABBRR) is a synthetic amphiploid produced by crossing a durum wheat (AABB) and rye (RR). The resulting Triticale has 42 chromosomes, 28 from the durum wheat and 14 from the rye. Because this hybrid has a higher lysine content than wheat (1-3), its development into a commercial crop for human consumption and animal feeding is an attractive prospect. However,

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initial studies have indicated that Triticale does not possess good breadmaking quality (4, 5). Recently, Lorenz et al. (6) and Tsen et al. (7) have demonstrated that Triticale can be used to produce acceptable bread.

Chen and Bushuk (8-10) fractionated the flour proteins of a Triticale and its parents using a modified Osborne procedure. Since electrophoresis of the soluble proteins showed that all proteins of the Triticale are present in the parents, Chen and Bushuk (10) concluded that the soluble proteins of Triticale are simply inherited from its parents. Electrophoresis of intact glutenins was not satisfactory, making interpretation of their inheritance impossible.

Recent advances in electrophoresis of reduced glutenins (11) allows determination of the subunit composition of glutenin of cereal grains. Wall et al. (12) reviewed much of the biochemistry of Triticale and reported on the glutenin subunits of rye, durum, and Triticale flours. They found the Triticale glutenin was composed of subunits typical of the rye and durum glutenins and also contained a subunit of molecular weight (MW) corresponding to the largest glutenin subunit in bread wheat (11,13). Wall et al. (12) concluded that this subunit may have been introduced when the Triticale was crossed with a bread wheat.

Scanning electron microscopy showed characteristic structures (14) of glutenins of different cereals. Bread wheat glutenin was composed of parallel fibrils grouped in

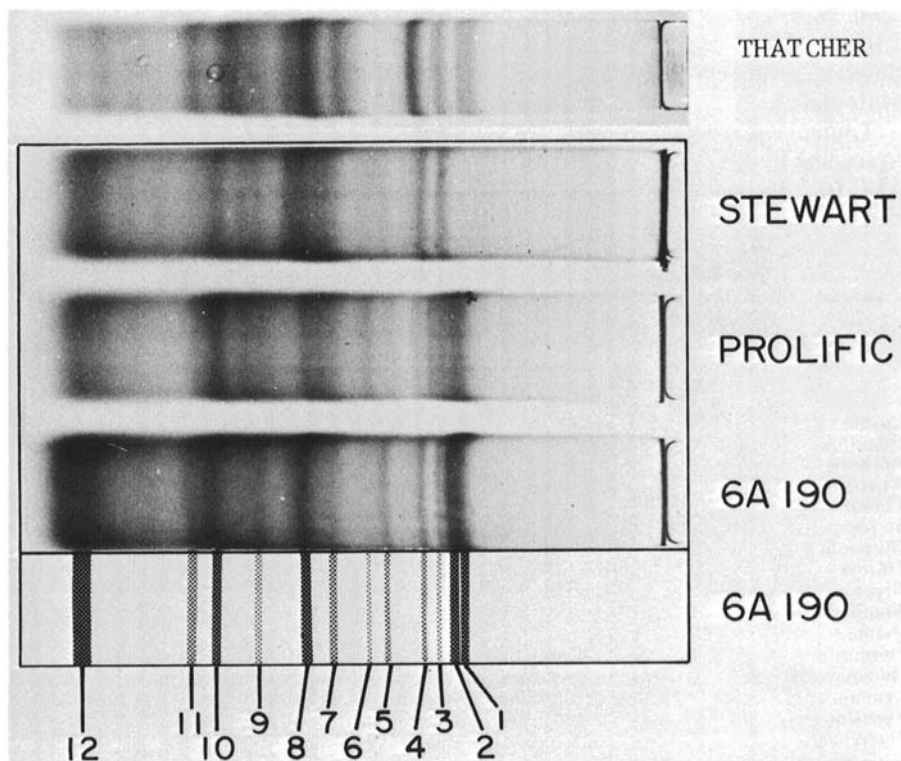


Fig. 1. SDS-PAGE patterns of reduced glutenins of Triticale (6A190), its parents Prolific (rye), Stewart (durum), and Thatcher (a bread wheat).

aggregates. Durum wheat glutenin was composed of ribbons in random orientation, and rye glutenin was in the form of short rods. These ultrastructures could be correlated with the rheological properties of the flours of these cereals.

This paper reports on electrophoretic studies of reduced glutenins, and amino acid composition and scanning electron microscopy of intact glutenins of one variety of Triticale, its durum wheat and rye parents, and a variety of hexaploid wheat.

MATERIALS AND METHODS

Flour Samples

Grain samples of one line of Triticale (6A190), its spring rye (cv. Prolific) and durum wheat (cv. Stewart) parents, and a bread wheat (cv. Thatcher) were obtained from plants grown together on University of Manitoba experimental plots. The grain was milled into flour on a Buhler experimental mill after overnight tempering to 15.5% moisture.

Preparation of Glutenins

Ten grams of flour was mixed into a dough with distilled water, and the gluten separated by hand-washing in running distilled water. The gluten was kneaded by hand until a ball was formed. The separation of the gluten from the rye and Triticale flour was considerably more difficult than from the durum wheat. However, with care the yellow glutenous material was separated from most of the white starch.

Glutenin was isolated from the gluten by the pH-precipitation method (15). According to this procedure the gluten from 10 g. of flour is dispersed in 200 ml. of AUC (0.1 acetic acid, 3M urea, 0.01M hexadecyltrimethylammonium bromide) by

TABLE I. AMINO ACID COMPOSITION OF GLUTENINS^a

	Mole Percent on an Ammonia-Free Basis		
	Durum wheat (Stewart)	Spring rye (Prolific)	Triticale (6A190)
Lysine	2.90	3.36	2.97
Histidine	1.97	1.94	1.94
Arginine	4.12	3.60	3.61
Aspartic acid	5.63	5.86	5.62
Threonine	3.49	3.71	3.58
Serine	6.44	6.16	6.25
Glutamic acid ^b	26.27	24.54	26.04
Proline	11.37	13.13	12.70
Glycine	8.12	8.21	8.35
Alanine	5.77	5.93	5.69
Valine	5.34	5.47	5.32
Methionine	1.39	1.38	1.28
Isoleucine	3.66	3.45	3.50
Leucine	7.18	6.92	6.88
Tyrosine	2.78	2.64	2.82
Phenylalanine	3.57	3.71	3.48

^aTryptophan, cysteine (and cystine) were not determined.

^bGlutamic acid and glutamine.



Fig. 2. Scanning electron micrograph of rye (cv. Prolific) glutenin.

overnight stirring and centrifuged ($5,000 \times g$, 1 hr.) to remove insoluble material. The glutenin in the clarified supernatant is precipitated by adjusting to 70% (v./v.) in ethanol, and to pH 6.4 by dropwise addition of 1M NaOH.

Reduction and Electrophoresis of Glutenins

Reduction of glutenin with β -mercaptoethanol, complexing of the subunits with sodium dodecyl sulfate, and electrophoresis were performed by the methods described previously (13). MWs were determined from standards run on the same gel.

Amino Acid Analysis

Glutenin samples were weighed into hydrolysis tubes and hydrolyzed at 110°C . in 4 ml. of 6N hydrochloric acid for 24 hr. The hydrolysates were dried over sodium hydroxide pellets and the residue dissolved in 8 ml. of citrate buffer, pH 2.2, and centrifuged. A Beckman 121 analyzer with automatic integration of peak areas was used to determine the amino acid composition. Since these glutenins were prepared from solutions containing urea, results are reported as mole percent on a zero-ammonia basis.

Scanning Electron Microscopy

Freeze-dried glutenin material was attached to circular stubs with double-sided tape and coated with gold to a thickness of 20 to 25 nm. The mounted specimens



Fig. 3. Scanning electron micrograph of durum wheat (cv. Stewart) glutenin.

were examined in a Cambridge Stereoscan Mk IIa scanning electron microscope at an accelerating potential of 10 kv. and photographed on Panatomic X film. Micron markers on all micrographs illustrate the degree of magnification.

RESULTS AND DISCUSSION

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Reduced Glutenins

Figure 1 shows the SDS-PAGE patterns of the reduced glutenin of one line of Triticale (6A190), the durum wheat parent (cv. Stewart), the rye parent (cv. Prolific), and a hexaploid bread wheat (cv. Thatcher). The bands of the Triticale pattern are schematically shown and numbered for identification during discussion.

Comparison of the SDS-PAGE patterns for the glutenins of Stewart and Prolific (Fig. 1) showed distinctly different subunit compositions, especially in the high-MW region, as well as some similarities between a number of bands in the two electrophoretic patterns. Accordingly, it may be possible to determine if any of these subunits are simply inherited in Triticale, and if the Triticale contains any subunits not in either of the parents.

Band 1 in the 6A190 pattern corresponds to a MW of 140,000. The largest bread wheat glutenin subunit has a MW of 152,000 (16); this was readily distinguishable from the largest rye and Triticale subunit by SDS-PAGE as shown in Fig. 1. This first band in Triticale has the same mobility as the slowest-moving band in the Prolific pattern, so we can conclude that this glutenin subunit is



Fig. 4. Scanning electron micrograph of Triticale (6A190) glutenin.

inherited directly from the rye parent. Likewise, band 2 is present in the 6A190 and Prolific patterns but not in that of the Stewart.

The subunits comprising band 3, 4, and 5 appear to be inherited mainly from the durum parent, although in each case there are faint bands of corresponding mobility in the rye pattern. Band 6 is inherited from the rye and durum parents, whereas band 7 could be from either or both parents.

Band 8 was present only in the durum wheat parent. Finally, the protein bands 9, 10, 11, and 12 can be attributed to both parental species.

It should be noted that equal mobility in SDS-PAGE does not necessarily mean complete identity of the polypeptides, since the basis of this separation is primarily size. Every subunit of glutenin of 6A190 that can be resolved by SDS-PAGE can then be attributed to subunits in one or both parents. There are no "new" protein bands in the Triticale and all prominent bands in the parents are present in the progeny. This observation supports the conclusion of Chen and Bushuk (8-10) that in hexaploid Triticale, the rye and durum genomes function independently and its proteins are simply inherited from the parents.

The largest glutenin subunit of Prolific and 6A190 observed could be mistaken for the largest subunit of bread wheat glutenin (11,13). It is possible that the conclusion of Wall et al. (12) that the Triticale they studied contained a glutenin subunit inherited from the bread wheat used in producing the hybrid, rather than from the rye glutenin is based on this erroneous identity.

Amino Acid Composition of Glutenins

Table I compares the amino acid compositions of glutenins from Stewart, Prolific, and 6A190. In general, the three glutenins are similar in amino acid composition, but there are a number of significant differences. The proportions of five amino acids—lysine, threonine, glutamic acid, proline, and arginine—are significantly different for rye and durum wheat glutenins. The first four of these are present in 6A190 at an intermediate level, whereas the proportion of arginine is approximately the same as that in the glutenins of Prolific. The proportions of the remaining 11 amino acids in the 6A190 are not significantly different from the proportions in the glutenins of the other three grains. These results are in general agreement with those of Chen and Bushuk (8) obtained for total flour proteins.

Scanning Electron Microscope Studies

Glutenins of a number of hexaploid and tetraploid wheats, diploid rye, and *Aegilops squarrosa* (the diploid progenitor of the D-genome of bread wheat) exhibited characteristic ultrastructures when examined by scanning electron microscopy (14). Their ultrastructures appear to be related to the rheological properties of the doughs prepared from the same flours.

Figures 2 to 4 show micrographs of glutenins of 6A190 and its durum wheat and rye parents. The characteristic rodlike ultrastructure of rye glutenin and the ribbonlike strands of durum wheat glutenin are distinctly different. The glutenin of 6A190 appears to be a mixture of glutenins of its parents; both rods and ribbons are evident. This observation is consistent with the mutual influence of both parents on the subunit and amino acid composition of Triticale glutenin discussed in this paper.

CONCLUSIONS

The glutenins of one variety of Triticale and its durum wheat and rye parents were studied by SDS-PAGE, amino acid composition, and scanning electron microscopy. All of the subunits of Triticale glutenin are present in the glutenins of either, or both, of the parents. In general, the proportions of amino acids in Triticale glutenin are intermediate between the proportions of the same acids in the glutenin of the parents. Scanning electron micrographs showed that Triticale glutenin ultrastructure was characteristic of that of the parents. It is concluded that Triticale glutenin is simply inherited from the durum wheat and rye parents.

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