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Genetics

Relationship of gliadin protein components to chromosomes in hexaploid wheats (*Triticum aestivum* L.)

(celiac disease/wheat grain/gel electrophoresis/substitution lines)

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ABSTRACT **The synthesis of the A-gliadin protein fraction derived from the endosperm of the grain of hexaploid bread wheats (*Triticum aestivum* L.), which is toxic in celiac disease, was associated with the α arm of the 6A chromosome through use of the substitution lines of 'Cheyenne' chromosomes in 'Chinese Spring'. The association was made through the use of ditelocentric stocks of Chinese Spring. The synthesis of many other gliadin components in the gel electrophoretic patterns of these two varieties could be associated with particular chromosomes as well. All genes detected were located in the chromosomes of homoeologous groups 1 and 6. It is possible to remove some of the proteins toxic to people with celiac disease from wheat (flour) by chromosome manipulation. If the toxic factor is not widely distributed among the storage protein components, it may be possible to produce a wheat that would be safe for celiac patients to eat.**

A-gliadin is an α-gliadin protein fraction derived from the endosperm of the grain of bread wheat (*Triticum aestivum* L.); it has unusual aggregation properties and a distinctive electrophoretic pattern (1–3). It is present in some of the most important United States varieties of hard red winter wheat and in 'Cheyenne', a progenitor of some of them, but absent in many other wheat varieties (such as 'Chinese Spring') (3). We have carried out an extensive characterization of this wheat storage protein fraction in our laboratory (see review of ref. 4). The toxicity of A-gliadin in celiac disease has recently been demonstrated (5, 6).

In this paper we report the association of A-gliadin synthesis with genes on chromosome 6A of hexaploid bread wheats through the use of genetic stocks in which chromosomes of the variety Cheyenne were individually substituted in Chinese Spring (7). By means of these chromosome substitution lines and various aneuploid stocks of Chinese Spring, we associated many other gliadins of the parent varieties with particular chromosomes.

MATERIALS AND METHODS

Seeds of 20 of the 21 substitution lines of Cheyenne chromosomes in Chinese Spring were obtained from Rosalind Morris, University of Nebraska, Lincoln. The 2B substitution line was unavailable. These lines were described by Morris *et al.* (7), but the material supplied to us had been backcrossed two additional times, making six in all. Seeds were increased at Davis, Calif. Nullisomic-tetrasomic variants of Chinese Spring in which the 6A chromosome was absent and compensated for by two extra homoeologous chromosomes (6B or 6D) and the nullisomic-6D-tetrasomic-6A variant were obtained from E. R. Sears, United States Department of Agriculture, Columbia, Mo. E. R. Sears also supplied the 6Aα ditelocentric variant of Chinese Spring in which the β arm of the 6A chromosome was missing.

Proteins were extracted from single seeds (after the germ end had been cut off) by grinding the dry seed in a small mortar and pestle, adding the extracting solvent, then grinding the mixture for a few minutes. Ordinarily, about 20 mg of seed were extracted with 0.40 ml of 8.5 mM aluminum lactate (obtained from ICN Pharmaceuticals, Inc.) that contained enough lactic acid to bring the pH to 3.2. The extract was spun in a 1-ml centrifuge tube at 25,000 × *g* for 15 min to remove starch and other insoluble material. About 35 μl of the clear supernatant was placed in one of the sample slots of a 7% polyacrylamide gel‡ slab (27 × 12 × 0.6 cm) that had been polymerized in water, soaked overnight in 4 liters of water, and then equilibrated with 4 liters of the aluminum lactate buffer by an overnight soaking. This aluminum lactate buffer was then used for the electrophoresis, which we carried out for 5–8 hr at a voltage drop of about 12.5 V/cm using a horizontal gel electrophoresis apparatus (EC Apparatus Co.). These conditions provided good resolution of gliadins, while the albumins and globulins, which have greater mobilities than gliadins, were run off the end of the gel.

Gels were stained with Coomassie brilliant blue (1% Coomassie brilliant blue in absolute ethanol, diluted 20-fold with 12% trichloroacetic acid, ref. 8) for 48 hr and then destained for a few hours in 12% trichloroacetic acid before being photographed. Extensive destaining to remove all background was not necessary for a good photographic record; it caused the disappearance of faint bands.

At least two seeds, each from a different plant, were examined for each of the substitution lines, but usually a greater number of seeds were examined—about 10 seeds for each of the groups 1 and 6 substitution lines. Comparisons of the electrophoretic patterns of the substitution lines were made with patterns of the parent varieties that were included on the same gel slab.

RESULTS

The approach is illustrated in Fig. 1 where the electrophoretic patterns of the seven substitution lines of the A-genome chromosomes are compared with the pattern of Chinese Spring on the same gel slab. The most notable difference from the patten of Chinese Spring was found for the 6A substitution line where the A-gliadin pattern of Cheyenne (see Fig. 2) substituted for the α-gliadin pattern of Chinese Spring. The only other difference was a minor one in the pattern of the 1A substitution line.

By this approach, we found differences from the gliadin pattern of Chinese Spring only in the patterns of substitution

‡ Contains 16.6 g of acrylamide, 0.88 g of bis-acrylamide, 0.10 ml of *N,N,N′,N′*-tetramethylethylenediamine, and 0.10 g of ammonium persulfate in 250 ml of H_2O.

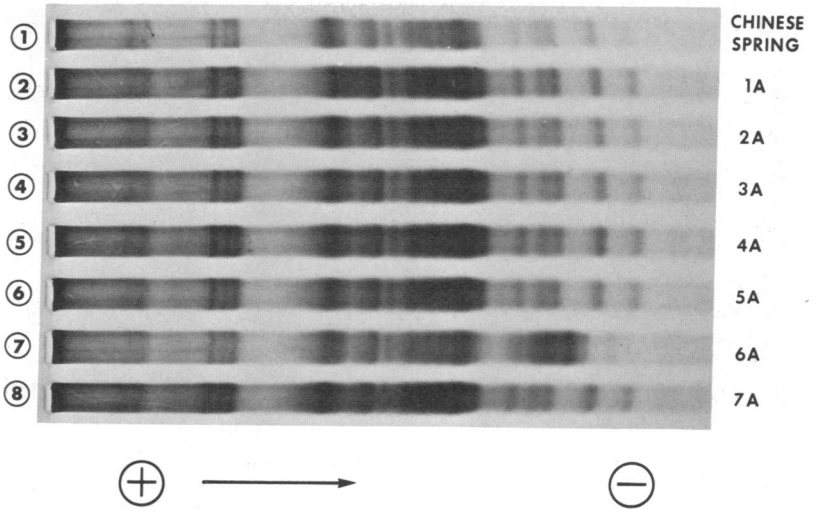

FIG. 1. Gel electrophoretic patterns of gliadin proteins extracted from the A genome substitution lines of 'Cheyenne' in 'Chinese Spring' (designated 1A through 7A) compared with the gliadin pattern of 'Chinese Spring'.

lines 1A, 1B, 1D, 6A, and 6B. The patterns of substitution lines for homoeologous groups 1 and 6 are shown in Figs. 2 and 3, where they are compared with the patterns of the parent varieties. The results are summarized in the diagram of Fig. 4, where the 25 bands of Cheyenne that were distinguishable in our electrophoretic patterns are compared with the 22 distinguishable bands of Chinese Spring according to their mobilities relative to the most intense A-gliadin band, which was assigned a mobility of 1.00. The bands in the two patterns were given a rating of 1 to 5 in order of increasing intensity by a visual inspection of photographs of the patterns. From here on we shall refer to the bands of the electrophoretic pattern as gliadin components even though we recognize that gel electrophoresis in one dimension is not capable of resolving all the many components of the gliadin mixture (9) and that any one band may include more than one protein component. We found excellent agreement when we compared the ratings for one of the patterns with intensities obtained by densitometry of the negative. The chromosome associations of gliadin components unique to one or the other parent variety are given in Fig. 4. We were able to associate 13 of the 25 components of Cheyenne and 11 of the 22 components of Chinese Spring with particular chromosomes.

Although we did not find a significant difference between the patterns of the 6D substitution line and Chinese Spring, we found that protein components 18 and 20 of Chinese Spring (Fig. 4) were missing from the nullisomic-6D-tetrasomic-6A line and enhanced in intensity in the nullisomic-6A-tetrasomic-6D; these protein components were evidently controlled by the 6D chromosome. Protein components 17 and 19 (Fig. 4) were missing from nullisomic-6A-tetrasomic-6B and nullisomic-6A-tetrasomic-6D, but were enhanced in the patterns of nullisomic-6D-tetrasomic-6A; these components are evidently controlled by the 6A chromosome. The intensity of band 16 (Fig. 4) was affected by changes in the dosage of the 6A and 6D chromosomes in such a way as to suggest to us that band 16 may represent two unresolved components—one controlled by the 6A chromosome and the other by the 6D chromosome.

The pattern of the 6Aα ditelocentric variant of Chinese Spring, which lacked the β arm of the 6A chromosome, did not

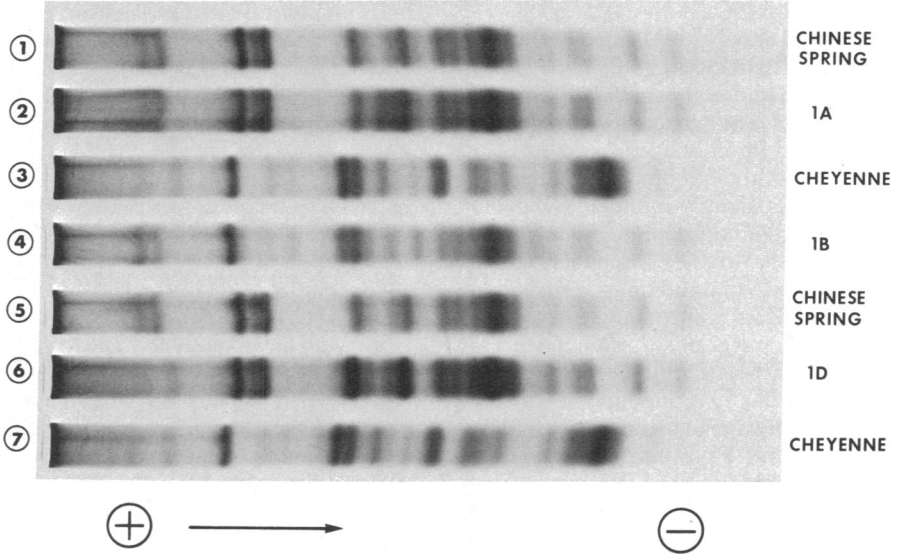

FIG. 2. Gel electrophoretic patterns of gliadins extracted from the 1A, 1B, and 1D substitution lines compared with the gliadin patterns of 'Cheyenne' and 'Chinese Spring'.

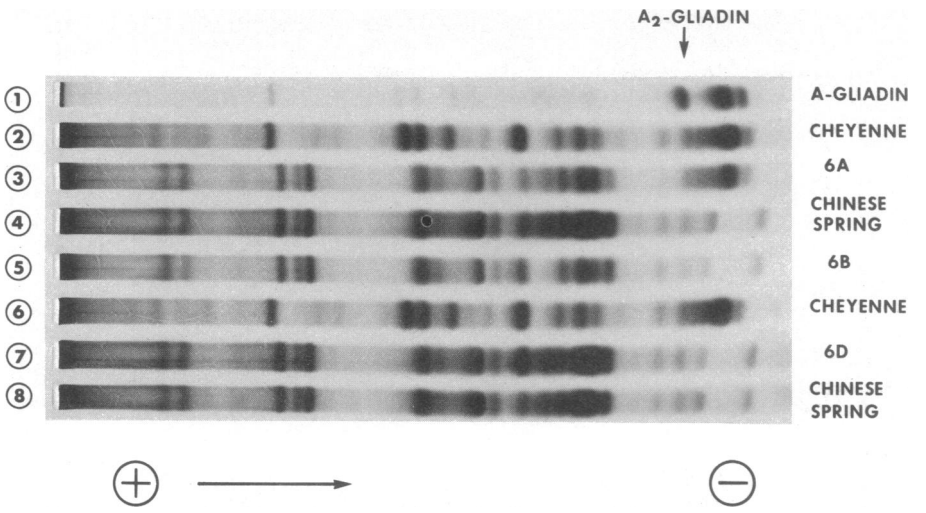

FIG. 3. Gel electrophoretic patterns of gliadins extracted from the 6A, 6B, and 6D substitution lines compared with gliadin patterns of 'Cheyenne' and 'Chinese Spring' and with the pattern of the A-gliadin fraction.

differ qualitatively from the electrophoretic pattern of Chinese Spring. We conclude that the genes which encode the A-gliadin are located on the α arm of the 6A chromosome.

DISCUSSION

The substitution lines of Cheyenne chromosomes in Chinese Spring that had been prepared by Morris *et al.* (7) provided us with a highly suitable means to associate synthesis of A-gliadin with a particular chromosome. Although Eastin *et al.* (10) have published electrophoretic patterns for gliadin fractions that had been prepared from these substitution lines, their patterns were of relatively low resolution; we found them unsuitable for a detailed analysis of the relationship between the protein bands of the gel electrophoretic patterns and chromosomes. Our patterns of much higher resolution have enabled us to associate A-gliadin synthesis with the 6A chromosome of Cheyenne and to associate about one-half of the resolved gliadin components of each of the parent varieties with particular chromosomes as well. Because the remaining components of the parent varieties did not differ in electrophoretic mobility when the two varieties were compared, we were unable to associate these components with chromosomes.

Wrigley and Shepherd (9) and Shepherd (11) used aneuploid derivatives of Chinese Spring (12) to assign genes involved in the synthesis of most of the gliadin components of Chinese Spring to particular chromosomes. They concluded that gliadin protein components were coded for only by chromosomes of homoeologous groups 1 and 6. Although our electrophoretic pattern for Chinese Spring differs slightly from theirs (possibly because they included urea in their aluminum lactate buffer whereas we did not), we found that all the components whose synthesis we were able to assign to chromosomes were coded for by chromosomes of homoeologous groups 1 and 6 in support of their conclusions. The assignment of gliadin components to chromosomes of other groups by Solari and Favret (13), who used substitution lines of 'Thatcher' chromosomes in Chinese Spring in their study, was probably due to inadequacies in their genetic material, as was considered a possibility by them.

Our results provide assignments for many protein components in the electrophoretic pattern of Cheyenne, which is close in pattern to some of our most important commercial wheats, such as the various selections of 'Scout', as well as many components of Chinese Spring, which is not grown commercially. This should be of help in choosing protein components for amino acid sequencing studies (14) where knowledge of genome assignments will be needed to explore evolutionary relationships of the amino acid sequences of the storage protein components of hexaploid wheats.

The toxicity of A-gliadin in celiac disease has been demonstrated by the work of Hekkens *et al.* (5, 15) and Falchuk *et al.* (6). Celiac disease is a condition wherein susceptible individuals suffer adverse changes in the epithelial tissue of the small intestine upon eating wheat and some closely related cereals such as rye and barley (see review of ref. 16). These changes interfere with the absorption of nutrients, and this malabsorption is usually accompanied by intestinal distress. The only satisfactory long-term treatment of celiac disease is to remove wheat completely from the diet.

The toxic factor responsible for the production of symptoms in celiac disease has for some time been known to be associated with the gliadin proteins or with peptides derived from gliadins in the digestive process. The toxic factor is probably a particular sequence of amino acids in the polypeptide chain of a gliadin protein that is capable of producing a specific immune response localized in the small intestine; this immune response results in tissue destruction and the changes of the intestinal mucosa characteristic of celiac disease.

Hekkens *et al.* (5, 15) instilled an α-gliadin preparation (5) that was similar to our A-gliadin or a fraction derived from the preparation by tryptic digestion (15) directly into the small intestine of celiac patients. Samples of epithelial tissue from the portion of the intestine exposed to the gliadin fractions were obtained by biopsy at regular intervals following the installation. Changes in the tissue characteristic of celiac disease were noted within hours of the beginning of the instillation. Falchuk *et al.* (6) developed a test for toxicity of gliadin proteins or peptides based on changes in cultured epithelial tissues obtained by biopsy from celiac patients. They found that levels of alkaline phosphatase and other enzymes in the tissues increased over a 48-hr period of culture in the absence of gliadin proteins or peptides; the increase was significantly less in the presence of gliadin proteins or an enzymatic digest of gliadins and this was taken as indicating toxicity for such preparations. A-gliadin prepared by the method of Bernardin *et al.* (1) was toxic on the basis of this organ culture test (6). The results of Hekkens *et al.* (5, 15) and of Falchuk *et al.* (6) carried out with intact proteins suggest to us that both the intact A-gliadin molecule and a

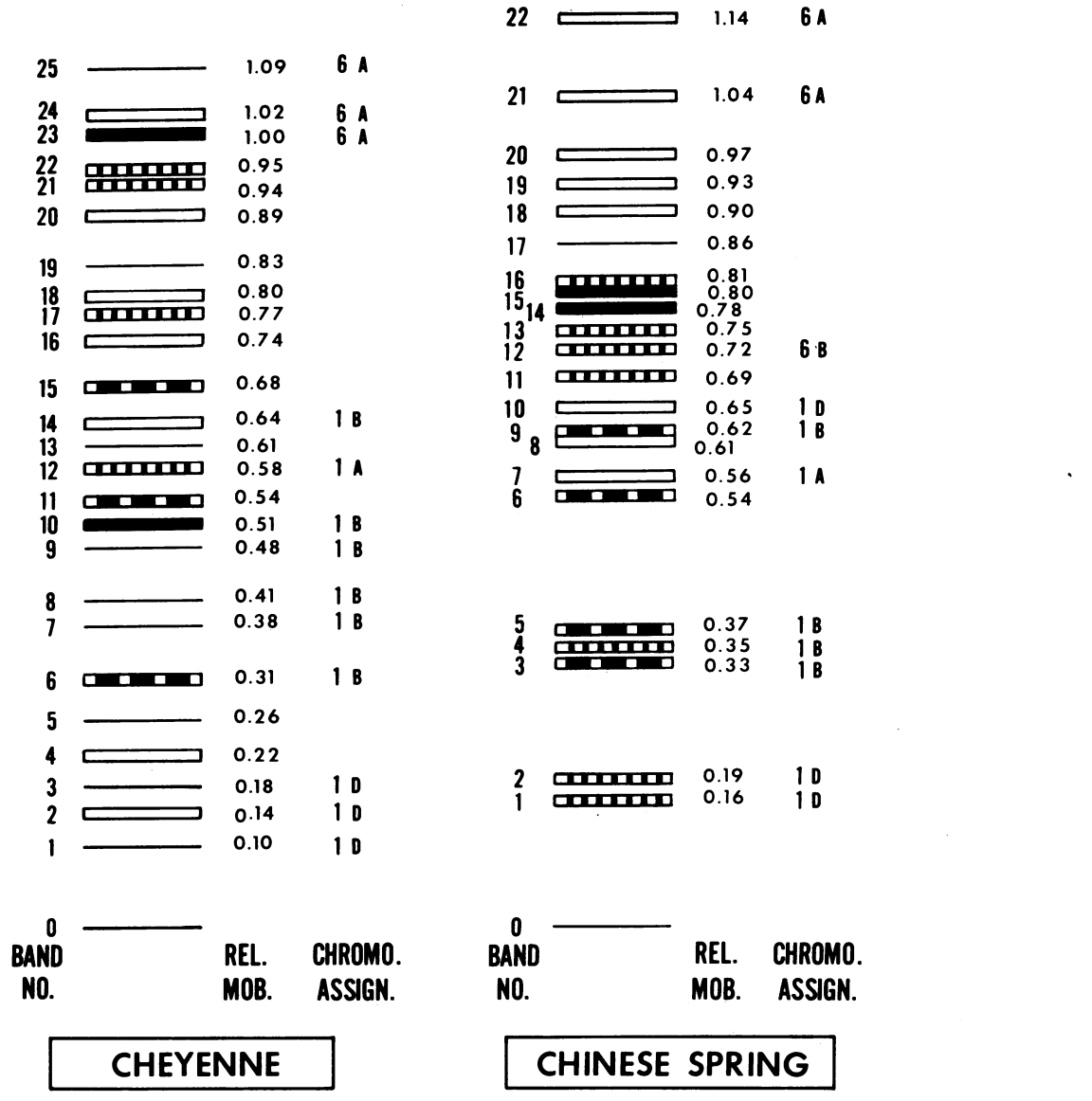

FIG. 4. Diagram of gliadin patterns from 'Cheyenne' and 'Chinese Spring'. Protein components are numbered sequentially according to increasing mobility (left side of patterns) and assigned a relative mobility (right side of patterns) by comparison with the most intensely stained component of A_1-gliadin. The chromosome assignment based on comparison of substitution line patterns is given on the right. Intensities (on a visual scale of 1 to 5) of the bands in the patterns are indicated in order of increasing intensity as follows: —— 1; ▭ 2;, ▭ 3; ▭ 4; ▭ 5.

peptide (or peptides) derived from it are capable of triggering the immune response. Strober *et al.* (17) have discussed the possible nature of this immune response, which might involve binding of the toxic factor to cell surface antigens along the lines suggested by Schrader *et al.* (18) for interactions of viral antigens with tumor cell surfaces.

In Cheyenne, the A-gliadin makes up most of the α-gliadin protein and a substantial part (perhaps as much as 30%) of all the gliadin protein, whereas in Chinese Spring, the amount of protein represented by α-gliadins (components 17 to 22 in Fig. 4) is small. We think it likely, however, that the α-gliadins of Chinese Spring that are controlled by chromosome 6A (components 17, 19, 21, and 22 in Fig. 4) are close in primary structure to the A-gliadins; they probably also contain the toxic sequence of amino acids. We found that some of the α-gliadins of Chinese Spring (components 18 and 20 in Fig. 4) were contributed by the 6D chromosome, but none were contributed by the 6B chromosome. Wrigley and Shepherd (9) found one component in the region of α-gliadins that had been contributed by 6B.

It is not known whether all, or only some, of the many protein components in the gliadin mixture contribute the toxic factor. There is evidence from peptide mapping that many gliadin components have partial sequences in common (19, 20). If we assume that the toxic factor is a particular sequence of amino acids, then it is possible that many gliadin protein components contain this sequence. On the other hand, there are clearly differences in sequence among the components, as evidenced by their separation upon electrophoresis; it is not necessary that the toxic sequence be common to many, or all, components. Kendall *et al.* (21) reported that only α-gliadins may be toxic. They separated gliadin proteins on a carboxymethylcellulose column and found that only a small fraction of the total mixture was toxic when fed to celiac patients (estimates of toxicity were based on xylose excretion tests). They noted that proteins of the toxic fraction had mobilities equivalent to those of α-gliadins.

If only a small fraction of the gliadins is toxic, it may be possible to remove these proteins by chromosome manipulations of the sort used to prepare nullisomic-tetrasomic variants of hexaploid wheat (12).

It is clear from our results that the donor of the A genome contributed toxic proteins to hexaploid wheats. Since the other diploid species that contributed the B and D genomes to hexaploid wheats may have been as closely related to the donor of the A genome as are rye and barley, species that are known to be toxic in celiac disease, it seems possible that these other diploid species would also have contributed toxic fractions to bread wheats. There is evidence, however, that synthesis of some storage protein components may have been suppressed in polyploid formation (4, 22). It is conceivable that the only toxic proteins expressed in the hexaploid are those derived from the A genome. If this were so and if toxic proteins were limited to those controlled by the 6A chromosome, then the nullisomic-tetrasomic variants of Chinese Spring prepared by Sears (12) in which the 6A chromosome is absent and compensated for by two extra doses of the 6B or 6D chromosome, would not be toxic to celiac patients.

Further work is needed to test our speculations. Exact definition of the toxic peptide and determination of its distribution in gliadin proteins would be of great value both in leading to a more detailed understanding of the immunological reactions that produce the symptoms of celiac disease and in guiding development of a nontoxic wheat.

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