Theor Appl Genet (1984) 68:531–539

Chromosomal assignment of genes coding for the wheat gliadin protein components of the cultivars 'Cheyenne' and 'Chinese Spring' by two-dimensional (two-pH) electrophoresis

D. Lafiandra [1, *], D. D. Kasarda [1] and R. Morris [2]

[1] Food Proteins Research Unit, Western Regional Research Center, Agricultural Research Service, US Department of Agriculture, Berkeley, CA 94710, USA
[2] Department of Agronomy, University of Nebraska, Lincoln, NE 68583, USA

Received February 20, 1984; Accepted March 18, 1984
Communicated by J. MacKey

Summary. The gliadin proteins of the hexaploid bread wheat cultivar 'Cheyenne' (*Triticum aestivum* L. var. *aestivum*), which has good mixing and baking characteristics, were analyzed by 2-dimensional (2-pH) polyacrylamide gel electrophoresis (pH 3.2 in the 1st dimension, pH 9.2 in the 2nd). Genes for most of the 35 resolved components were assigned to the chromosomes of homoeologous groups 1 and 6 through the use of various aneuploids and substitution lines. A similar analysis was carried out for the cultivar 'Chinese Spring', which does not have good quality, and which had been analyzed by a different 2-dimensional procedure by Wrigley and Shepherd (Ann NY Acad Sci 209:154, 1973). A coordinate system was devised for description of the components in the 2-dimensional patterns.

Key words: Wheat proteins – Gliadins – Chromosomal location – Aneuploids – 2-dimensional electrophoresis

Introduction

The pioneering studies of Shepherd (1968) and Wrigley and Shepherd (1973) used the aneuploids of Sears (1954) and 1- and 2-dimensional methods of gel electrophoresis to demonstrate that most (probably all) gliadin protein components of hexaploid common wheat (*Triticum aestivum* L. var. *aestivum*) endosperm are coded by genes located on chromosomes of homoeologous groups 1 and 6.

This finding has been supported by subsequent work (Kasarda et al. 1976; Mitrofanova 1976; Sozinov et al. 1978; Sozinov and Poperelya 1980; Brown and Flavell 1981; Brown et al. 1979, 1981). Most studies have focused on the cultivar (cv.) 'Chinese Spring' because of the availability of a wide range of aneuploid variants for this cv. (Sears 1954), and virtually complete assignment has been achieved for all gliadin components resolvable by 2-dimensional methods (Wrigley and Shepherd 1973). Although some assignments have been made for gliadins of other cvs. (Kasarda et al. 1976; Brown et al. 1981), these analyses are much less complete.

Because 'Chinese Spring' does not have any commercial importance (it is used mainly in genetic studies), we considered it desirable to carry out a more extensive analysis of the chromosomal locations of genes coding for gliadin components in another cv. with good baking quality. This will be helpful in understanding gliadin gene structure and organization, the evolution of hexaploid bread wheats, and differences in quality among cultivars that might result from differences in storage protein components.

We chose the US hard red winter wheat cv. 'Cheyenne' for our studies because of its good quality and because of the availability of the 'Cheyenne' chromosome substitution lines (in 'Chinese Spring'), as well as the 21 monosomics, some nullisomics, and some ditelosomics of this cultivar. 'Cheyenne' is still of commercial importance in the USA more than 50 years after its introduction by the Nebraska Agricultural Experiment Station (Briggle and Reitz 1963; Briggle et al. 1982), and is an ancestor of many cvs. grown

* Permanent address: Istituto del Germoplasma, C.N.R., I-70126 Bari, Italy

Abbreviations: 1-dimensional = one-dimensional; 2-dimensional = two-dimensional; 2-pH = two-pH; 1st = first; 2nd = second

extensively today ('Scout 66', 'Eagle', 'Newton', and so forth).

We have used the 2-dimensional electrophoretic method of Mecham et al. (1978) for our analysis of gliadin patterns. This polyacrylamide gel electrophoresis (PAGE) method combines a 1st-dimension separation in aluminum lactate buffer (pH 3.2) with a 2nd-dimension separation in Tris-glycine buffer (pH 9.2). Both dimensions are carried out in a single gel slab, which is in contrast to other 2-dimensional separations that involve attaching the 1st-dimension gel to a separate 2nd-dimension gel. This 2-pH method gives excellent resolution of gliadin proteins and is virtually free of the troublesome streaking that is common in the 2-dimensional method that combines isoelectric focusing and sodium dodecyl-sulfate PAGE (SDS-PAGE).

Because the 2-pH method of Mecham et al. (1978) has not been used previously for analysis of aneuploid patterns, we have included analysis of 'Chinese Spring' in our studies so that the results can be compared with those of other methods, such as that of Wrigley and Shepherd (1973). The comparison also indicates the relative complexities of the gliadin patterns of two different cultivars.

Materials and methods

Plant materials

The following materials were analyzed: substitution lines of 'Cheyenne' chromosomes 1A, 1B, 1D, 6A, 6B, and 6D in 'Chinese Spring', nullisomics 6A and 6D of 'Cheyenne', ditelocentrics 1A and 1B (short arms) of 'Cheyenne', all developed at the University of Nebraska; nullisomic-tetrasomic lines of all groups 1 and 6 homoeologous chromosomes of 'Chinese Spring' developed by E. R. Sears (1954).

Extraction of proteins

Proteins were extracted from single seeds by grinding them in a mortar with 1.5 M dimethylformamide (DMF). The ratio of weight of solvent to weight of seed was 12 : 1. The solution (usually about 0.4 ml) was centrifuged briefly in a 2.5 ml tube with a Fisher model 235 table top centrifuge. The clear supernatant was used for analysis. By storing the supernatant in a freezer at about −10 °C, several analyses could usually be carried out on a single extract without serious deterioration of the patterns.

Electrophoretic method

The 2-pH 2-dimensional electrophoretic method of Mecham et al. (1978) was used to analyze the gliadin patterns of extracts from seeds of the various aneuploids and substitution lines. The gliadin patterns were distinctly separated from other proteins of the extracts by their low mobilities, which made fractionation of the extracts unnecessary. A horizontal apparatus manufactured by E-C Apparatus Co. (USA) was used. The 7% polyacrylamide gel was polymerized in water, and then equilibrated overnight in 0.0085 M aluminum lactate buffer (pH 3.2). For the 1st dimension, the sample (about 35 µl) was loaded into a slot situated near the midpoint at one end (positive pole) of the gel, and electrophoresis was carried out for about 7 h at 65 mA with all protein components migrating toward the negative pole because all the proteins are positively charged at pH 3.2. Under these conditions only gliadin proteins remained in the gel; all other components have greater mobility and ran off the long end of the gel, which had dimensions of 22 × 15 × 0.6 cm.

After completion of the 1st-dimension electrophoresis, the gel was removed from the apparatus and equilibrated for 4 h with the 2nd-dimension buffer, Tris-glycine (0.125 M Tris; 0.025 M glycine, pH 9.2) that was 1.5 M in dimethylformamide (DMF). (The DMF was included to maintain solubility of gliadins in the basic buffer and during the pH change from acidic to basic as the proteins moved through their isoelectric points.) The gel was suspended above the bottom of the container on a stainless steel screen during the equilibration and the buffer was stirred with a magnetic stirring bar. The gel was then returned to the electrophoresis apparatus, turned 90° from the first position, and electrophoresis was carried out for 16 h at 30 mA. After completion of the 2nd-dimension electrophoresis, the gel was placed in 0.02% Coomassie Brilliant Blue R250 in 12% trichloroacetic acid for 48 h to stain the proteins. The gel was usually destained for a few hrs in 12% trichloroacetic acid before being photographed (Polaroid MP-3 camera, Polaroid Type 52 film, Wratten G-15 filter).

Photographs of the 2-dimensional gliadin patterns derived from the various chromosome variants were compared to determine which components were missing, enhanced, or substituted relative to the patterns of the disomic parental cultivars, 'Cheyenne' and 'Chinese Spring'.

The photographs of the patterns were used to assign x, y coordinates to the components. Two apparently common components ('Cheyenne' No. 29 and 'Chinese Spring' No. 30; see 'Results' for nomenclature) were used as internal standards for comparison of the relative mobilities of the components in the two cultivars. We measured the coordinates in mm and transformed the x coordinates (1st electrophoresis dimension) to correspond to the Bushuk and Zillman (1978) system (see Wrigley et al. 1982) by assigning our standard components a mobility (x′) of 73 Bushuk-Zillman units. This assignment was made by actual comparison of 1-dimensional analyses of the cv. 'Marquis', the standard cv. used by Bushuk and Zillman, with the equivalent patterns of 'Cheyenne' (CNN) and 'Chinese Spring' (CS) (see Fig. 1 of Kasarda 1980). The y coordinate system was calibrated by assigning to the common components (CNN No. 29 and CS No. 30) an arbitrary mobility (y′) in the 2nd electrophoresis dimension of 50 (measured perpendicularly to a line bisecting the streak that usually defines the track of the 1st dimension after completion of the 2-dimensional electrophoresis. We were able to reproduce our x′, y′ coordinates to ± 1 unit when we measured patterns of the same cv. obtained by independent analyses.

Results

The 2-dimensional patterns of gliadins from 'Cheyenne' and 'Chinese Spring' are shown in Figs. 1 and 2, respectively. All the components visible in the original gel may not be visible in these figures. Below the photograph of each pattern, is a diagram indicating the assignment of proteins in the patterns to genes of

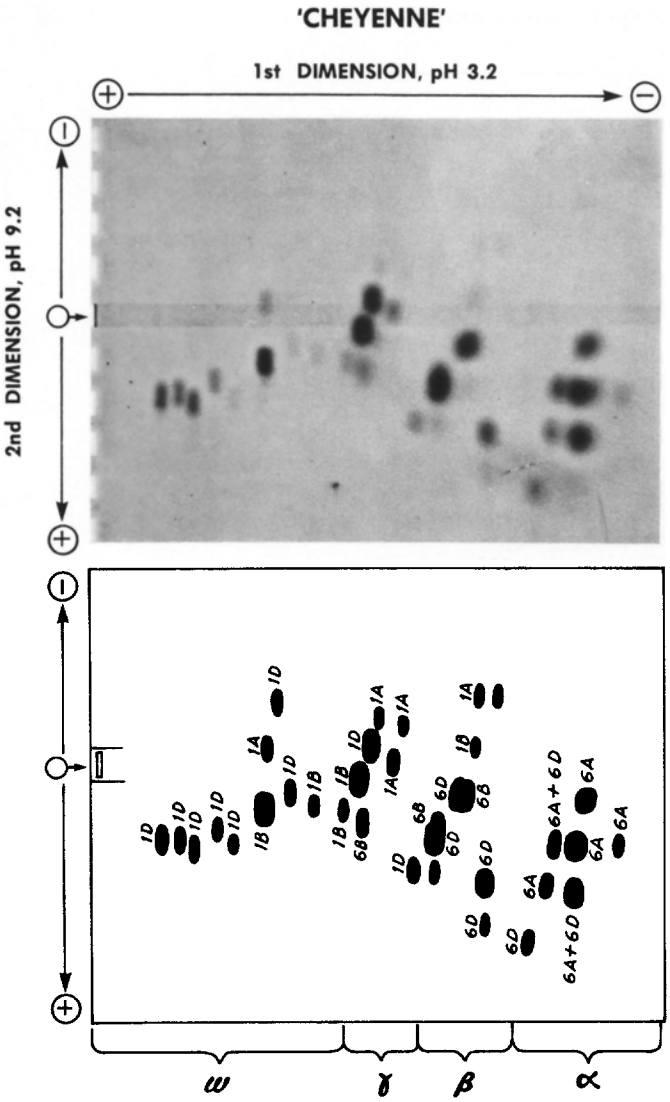

Fig. 1. Two-dimensional (2-pH) electrophoretic pattern of 'Cheyenne' gliadins. The *diagram below* the photograph indicates the genome and chromosome assignments of genes coding for most of the resolved components of the pattern. Assignments were made by comparisons of the electrophoretic patterns of groups 1 and 6 substitution lines, nullisomics, and ditelosomics. *Greek letters with brackets* indicate the ranges of mobilities in the 1st dimension (aluminum lactate, pH 3.2) corresponding to the usual electrophoretic mobility groupings of gliadins. The *small circle with short arrow* indicates the starting point of the 1st dimension

specific chromosomes. We assumed that the effects of aneuploidy and substitution on the gliadin patterns indicated the locations of the structural genes for the gliadin proteins.

We could readily distinguish 35 components in the pattern of 'Cheyenne'. There was an indication in some patterns of additional components, but these were only faintly evident. It seemed likely that additional components could be observed if the concentration of proteins in the extract were increased, but we did not pursue this aspect. We were able to assign control of 33 of the 35 'Cheyenne' components to chromosomes of homoeologous groups 1 and 6 by tests of the various substitution, nullisomic, or ditelosomic lines. We also showed (see below) that two of the spots contained two overlapping components coded by 6A and 6D so that the actual number of components identified for 'Cheyenne' was 37. In those instances when ditelosomics were used (1A and 1B), the genes coding for gliadin components were found to be located on the short arms of the chromosomes as is generally accepted for gliadin genes (for review, see Payne et al. 1982).

We were unable to assign a β-gliadin component of 'Cheyenne' that moved toward the anode in the 2nd

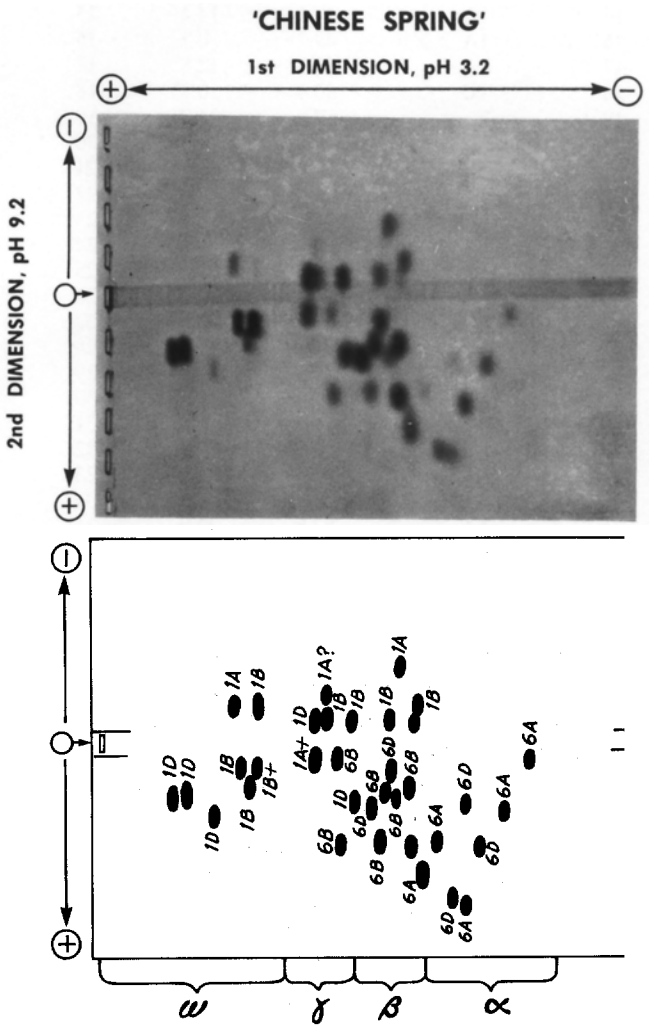

Fig. 2. Two-dimensional (2-pH) electrophoretic pattern of 'Chinese Spring' gliadins; other information is the same as in Fig. 1 except that assignments were made by comparisons of the electrophoretic patterns of nullisomic-tetrasomic lines

Table 1. Standardized coordinates (x',y') of 'Cheyenne' and 'Chinese' Spring' protein components in their 2-dimensional patterns

'Cheyenne'				'Chinese Spring'			
Component no.	Chromosome assignment	x'	y'	Component no.	Chromosome assignment	x'	y'
1	1D	10.8	24.2	1	1D	14.3	17.6
2	1D	13.7	22.6	2	1D	17.0	17.0
3	1D	16.3	25.8	3	1D	23.4	22.8
4	1D	20.9	19.6	4	1A	27.8	−9.4
5	1D	23.8	25.1	5	1B	28.8	8.7
6	1B	28.9	13.0	6	1B	30.9	14.0
7	1A	29.0	−3.7	7	1B+1D	32.6	9.1
8	1D	30.6	−17.1	8	1B	33.1	−8.7
9	1D	32.6	8.2	9	1A+	44.2	7.1
10	1B	37.6	11.2	10	1D	44.6	−5.0
11	1B	42.4	13.2	11	1A?	46.7	−13.8
12	6B	45.1	16.1	12	1B	47.2	−5.4
13	1B	45.1	4.3	13	6B	49.4	6.9
14	1D	46.5	−4.9	14	6B	49.8	32.6
15	1A	47.8	−14.4	15	1B	52.1	−4.9
16	1A	49.7	−0.7	16	1D+	52.3	20.1
17	1A	51.8	−12.8	17	6D	56.6	20.1
18	1D	53.4	30.7	18	6B	58.1	32.0
19	?	57.3	31.3	19	6B	59.4	16.2
20	6D	57.3	20.5	20	1B	60.4	9.5
21	6B	57.6	16.8	21	1B	60.6	−6.2
22	6D	61.4	9.2	22	6B	62.3	19.7
23	6B	62.6	8.8	23	1A	62.7	−20.3
24	1B	63.4	−4.7	24	6B	64.8	16.2
25	1A	64.5	−20.0	25	?	65.0	33.1
26	6D	65.6	45.0	26	?	66.2	−5.8
27	6D	65.6	33.6	27	?	66.4	−9.5
28	?	67.9	−19.9	28	6A	66.8	44.0
29	6D	73.0	50.0	29	6A	70.7	31.7
30	6A	75.5	33.3	30	6D	73.0	50.0
31	6A+6D	76.5	21.6	31	6A	75.6	52.4
32	6A+6D	80.4	35.4	32	6D	77.2	22.9
33	6A	80.6	22.2	33	6D	79.4	36.4
34	6A	81.3	9.4	34	6A	83.8	24.5
35	6A	87.8	22.0	35	6A	89.6	8.4

dimension (component No. 19, Table 1 and Fig. 3; from here on, component numbers refer to Table 1 and Fig. 3), and in addition, there was a component (No. 28) that moved toward the cathode in the 2nd dimension for which we were unable to make an assignment. Both were faint spots in the patterns, which made the analysis more difficult. Assignments for components 24 and 25, which were also faint, must be considered somewhat tentative.

We were readily able to distinguish 35 components in the pattern of 'Chinese Spring' by testing the nullisomic-tetrasomic lines. All but three were assigned to genes of particular chromosomes as indicated in Fig. 2, and we considered one of the three for which we were unable to make positive identification likely to be coded by chromosome 1A (No. 11). Also, component No. 25, which was a major component, must have represented at least two fairly major overlapping components; the corresponding spot in the pattern showed no obvious change for any of the chromosomal variants we examined. For three components (No's. 7, 9, and 16), a faint spot remained in the patterns of nullisomics 6A and 6D although the original intensity was greatly diminished. We assumed that the corresponding spots in the patterns represented overlapping protein components – with the major components coded by genes located on the designated chromosomes. We have added a plus sign to the chromosome designations for these three components to indicate this. The minor component of No. 7 is probably coded by a gene on chromosome 1D, as it appears to have the same mobility as component 9 of 'Cheyenne'. Thus, our 35

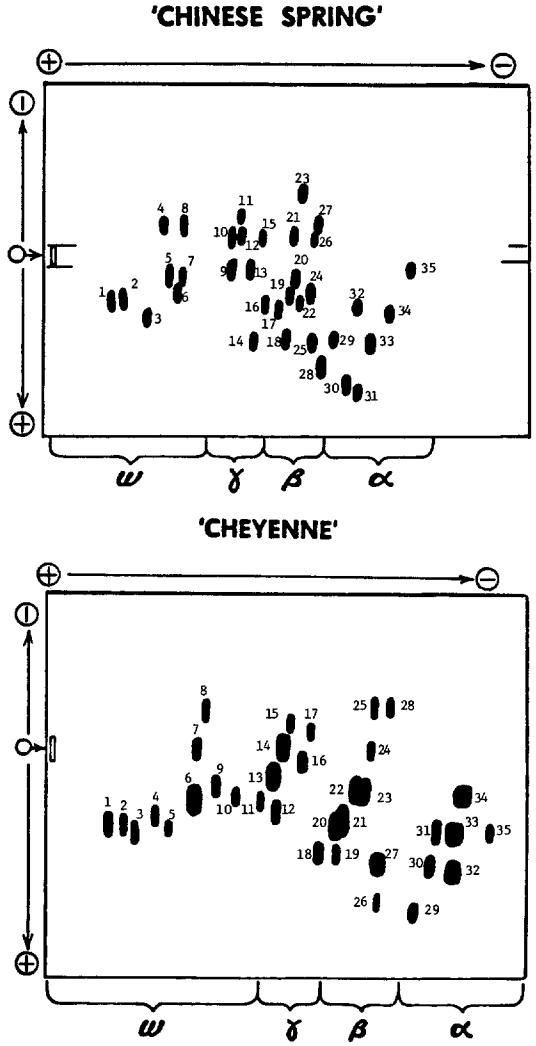

Fig. 3. Diagrams as in Figs. 1 and 2 of the 2-dimensional patterns of 'Cheyenne' and 'Chinese Spring' showing numbers assigned to the components in Table 1

spots in the 'Chinese Spring' pattern actually correspond to at least 38 components.

Our fortunate discovery of a nullisomic 6A seed amongst seeds produced by a monosomic 6A 'Cheyenne' plant while screening these seeds by 1-dimensional lactate-PAGE for those that were monosomic 6A (Lafiandra et al. unpublished results) permitted us to examine the overlaps in the region of α-gliadins more closely. The pattern of the nullisomic-6A seed is shown in Fig. 4 (the gel was unfortunately broken and the parts had to be fitted together for the photograph because no other nullisomic-6A seed was available for a duplication of the result). The nullisomic state suggested by the pattern was verified by germinating the embryo end of the seed and checking the root-tip chromosome count. It can be seen from Figs. 1, 4, and 5 that the 6A-coded α-gliadins of 'Cheyenne' (A-gliadin, Kasarda 1980) obscured 6D-coded α-gliadins in the euploid 'Cheyenne' pattern that appeared to be identical to those of 'Chinese Spring'. The pattern of A-gliadin from 'Scout 66', a cv. with an α-gliadin pattern similar to that of 'Cheyenne' is shown in Fig. 5 for comparison.

The pattern of 'Cheyenne' was substantially different from that of 'Chinese Spring', so we carried out electrophoresis on a mixture of equal volumes of extracts from each of these cvs. to determine which components of each cv. could be distinguished from those of the other. The result is shown in Fig. 6. Our analysis of the patterns indicated that there were only nine components in each variety that were clearly overlapping. (This may be difficult to determine from the reproduced photograph because some of the components appeared quite faintly in the original pattern.) Thus, it appears that the gliadins of our two cvs. comprise a mixture of about 66 distinguishable components (the overlapping components may be identical to one another), and this is likely to be an underestimate because some faint components have undoubtedly been overlooked.

Coordinates for the components of the 2-dimensional patterns of 'Cheyenne' and 'Chinese Spring' are given in Table 1 (see 'Materials and methods' for details).

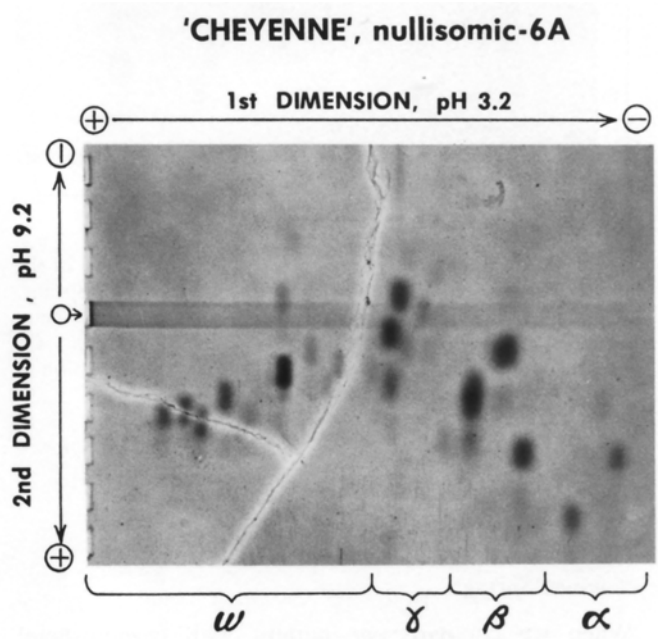

Fig. 4. Two-dimensional (2-pH) electrophoretic pattern of the gliadins extracted from a 'Cheyenne' seed that was nullisomic for chromosome 6A. Greek letters and origin of 1st-dimension pattern as in Fig. 1

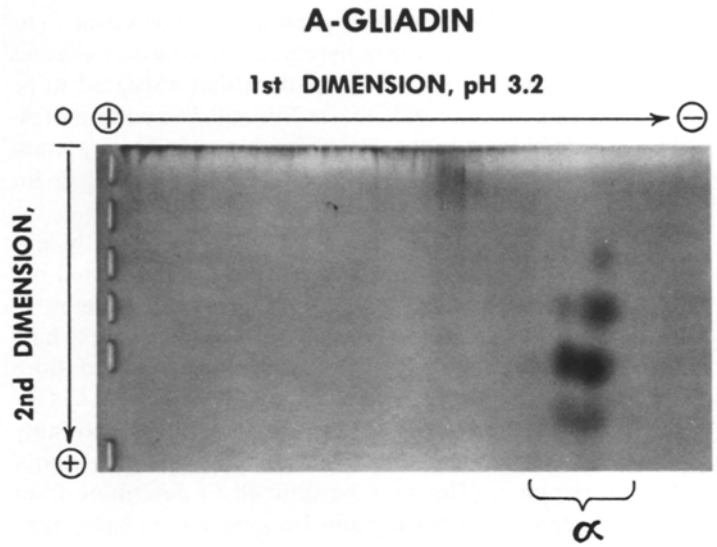

Fig. 5. Two-dimensional (2-pH) electrophoretic pattern of A-gliadin prepared from the cv. 'Scout 66'. The components correspond to genes of chromosome 6A (see text)

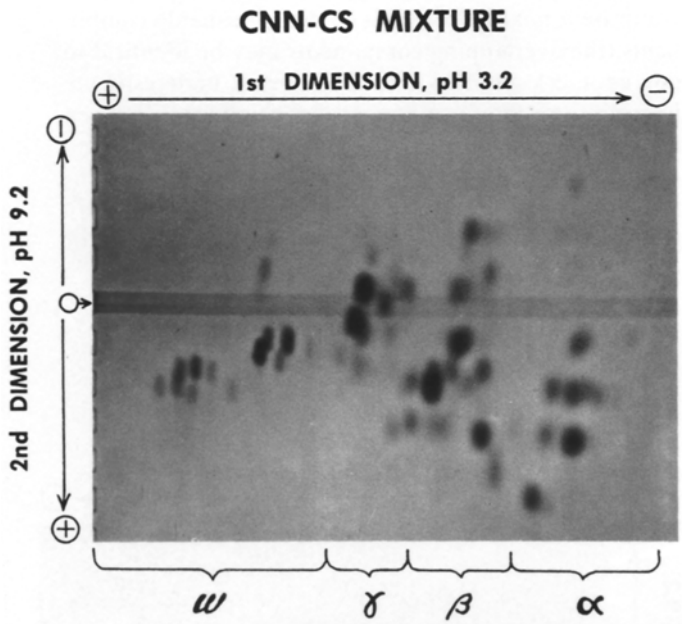

Fig. 6. Two-dimensional (2-pH) electrophoretic pattern of the mixture of gliadins from 'Cheyenne' and 'Chinese Spring'. Although intended to be a 1 : 1 mixture, the 'Cheyenne' components predominate slightly. *CNN* ('Cheyenne'); *CS* ('Chinese Spring'). *Greek letters* and origin of 1st dimension as in Fig. 1

Discussion

Comparison with the Wrigley-Shepherd results for 'Chinese Spring'

When we compare our pattern with chromosomal assignments for 'Chinese Spring' (Fig. 2) with that of Wrigley and Shepherd (1973; their Fig. 6), we find considerable similarity, particularly in general grouping of components according to their coding on particular chromosomes. We both used aluminum lactate electrophoresis for one of our dimensions (we for the 1st and they for the 2nd), and these dimensions should be comparable even though we used polyacrylamide gels and they used starch gels. Wrigley and Shepherd used iso-electric focusing for their other dimension (1st) whereas we used electrophoresis at pH 9.2 for ours (2nd).

Evidently, the migration of proteins at pH 9.2 under these conditions gives separations that are similar to the separations that result from isoelectric focusing. Electrophoresis in aluminum lactate at pH 3.2 under non-denaturing conditions separates proteins according to positive charge. Because the carboxyl groups are largely uncharged at this pH, the net charge on the molecule is positive, and approximately equivalent to the number of basic groups (histidine, lysine, and arginine) in the protein. At pH 9.2, most proteins will have all carboxyl side chains (and the C-terminal carboxyl group) in the ionized form; the resulting negative charge will be balanced to various degrees by the amount of arginine and lysine in the protein. At pH 9.2, all the histidine side chains (pK about 6.5) will have been titrated to neutrality; lysine and arginine with pK's of about 10 and greater than 12, respectively, will remain positively charged (Tanford 1961). Our observation that most gliadins separate into largely equivalent groupings by isoelectric focusing and by electrophoresis at pH 9.2 indicates that histidine must be present in the gliadins in approximate proportion to lysine and arginine, or that histidine must not vary much among the gliadins, or a combination of these possibilities.

Because of the high cost of ampholytes for isoelectric focusing, electrophoresis at pH 9.2 may be a

less costly alternative – at least for wheat storage proteins. The electrophoresis in the 2nd dimension must be carried out with a horizontal apparatus, however, in which proteins may migrate towards either pole without loss. It appears not to have been recognized by some workers that use of a vertical apparatus for electrophoresis with basic pH buffers will cause a fraction of the gliadins to be lost to the upper chamber electrolyte – which fraction depending upon the polarity defined by the experimenter. This can lead to misinterpretations of experimental data.

The main disadvantage of the procedure we used is the long time required for the analysis (24 h), but this time has been diminished to about 8 h by modifications of the procedure (Lafiandra and Kasarda, unpublished results). These modifications also adapt commercially available apparatus for use in the analysis.

Genetic relationships of protein components

The gliadin proteins are coded by groups of related genes that appear to have resulted from processes of DNA duplication followed by divergence of the duplicated DNA, which at first may have involved only short lengths of DNA that included mainly the codons for glutamine and proline, but eventually, whole gliadin genes (Kasarda 1980). Ultimately, similar groupings of gliadin genes, such as those coding for the α-type, the γ-type gliadins (Autran et al. 1979), or the ω-type gliadins (Kasarda et al. 1983) came to reside at complex loci located on the short arms of chromosomes of homoeologous groups 1 and 6 (for review, see Payne et al. 1982). The major gliadins are coded at these loci; however, there may be other loci on chromosomes of group 2 that control expression of a few gliadin components (Brown and Flavell 1981). The apparent involvement of group 2 chromosomes may be an artifact produced during production and maintenance of the critical stocks (Brown and Flavell 1981; Garcia-Olmedo et al. 1982). In addition, there may be loci coding for minor gliadins on chromosomes of groups 7 and 4 (Salcedo et al. 1979); these will be discussed later.

The clusters of closely-linked gliadin structural genes that make up the complex loci on chromosomes of groups 1 and 6 have been termed blocks by Sozinov and Poperelya (1979), isoloci by Mecham et al. (1978), and sections by Doekes (1973). These allelic blocks or polygenic loci (Sozinov and Poperelya 1980) act like single genes. Even though γ-gliadins and ω-gliadins are quite different in composition, the genes coding for these proteins apparently are clustered sufficiently closely on the short arms of group-1 chromosomes so that recombination of these genes on any one chromosome does not readily occur in crosses (Payne et al. 1982). The elucidation of the fine structure of this complex locus has not yet been achieved.

Our resolution in two dimensions provides additional information about the nature of allelic blocks, whereas our coordinate system provides a relatively simple way of describing both individual components and the allelic blocks, while enabling comparison with blocks of other cultivars. For example, component 29 of 'Cheyenne', which we chose as a standardizing protein, may be designated: CNN Gli 6D (73.0, 50.0), or CNN α-Gli 6D (73.0, 50.0), whereas the 6D block may written as Gld 6D1 (80.4, 35.4), (76.5, 21.6), (73.0, 50.0), (65.6, 33.6), (65.6, 45.0), (61.4, 9.2), (57.3, 20.5). It seems likely that the 6D-coded gliadins of 'Cheyenne' constitute the same block that Sozinov and Poperelya (1979) described for 'Bezostaya 1', although they reported only three components for their block Gld 6D1, whereas our 2-dimensional method resulted in definition of seven components. We have used the block notation of Sozinov and Poperelya (1979), but we suggest using the designation Gli for components to diminish confusion between components and blocks. Our coordinate system could be adapted equally well to the recently proposed gliadin nomenclature of Payne and Lawrence (1983) where complex gliadin loci on the short arms of group 1 chromosomes are designated as Gli-A1, Gli-B1, and Gli-D1, whereas those on group 6 chromosomes are designated Gli-A2, Gli-B2, and Gli-D2.

D-genome proteins in relation to formation of bread wheats

Our results obtained with nullisomic 6A 'Cheyenne' indicate that 'Chinese Spring' and 'Cheyenne' appear to have virtually the same block for 6D, although there is one additional component in 'Cheyenne' (No. 26) that does not appear to be present in 'Chinese Spring'. We have considered the possibility that component 26 is a deamidation product of component 27, which is also coded by 6D, but its absence in 'Chinese Spring' would then be puzzling because 'Chinese Spring' has a component (No. 25) that is likely to be equivalent (although consisting of at least two overlapping components). It seems most likely that the 6D blocks of 'Cheyenne' and 'Chinese Spring' represent minor variants of the same block. In view of the likely genetic separation of several thousand years for the two cultivars (Zurabishvili et al. 1978), this provides support for a monophyletic origin of the D genome of *T. aestivum*. Sozinov and Poperelya (1980) describe two different blocks for 6D, however, and we think that the block in 'Justin' (Kasarda 1980) constitutes a third. These, along with six different blocks for 1D (Sozinov and Poperelya 1980), could have resulted from a polyphyletic origin of the D genome of bread wheats, or from introgressive interactions after formation of the hexaploid.

Cathodic components in the 2nd dimension

There are a number of components, some of which are faint in our patterns, that migrate toward the cathode in the 2nd dimension at pH 9.2 (Figs. 1 and 2). These proteins must have more arginine (plus lysine) residues than glutamic plus aspartic acid residues. It is notable

that they seem to be coded by genes located on group 1 chromosomes even though some have the mobilities of β-gliadins, most of which are coded by genes of group-6 chromosomes. In general, the lactate patterns of gliadins can be divided into components coded on group-1 chromosomes (mainly γ- and ω-gliadins) and components coded on group-6 chromosomes (mainly α- and β-gliadins). It seems likely that the basic (cationic) gliadins that we observe at pH 9.2 have not been characterized. Although we have not been able to assign chromosomal locations for genes of all the basic components, they seem generally to be coded on chromosomes of group 1; this location would be typical of gliadins. They probably do not correspond to the low-molecular-weight gliadins of Salcedo et al. (1979) insofar as these proteins seem to be coded on chromosomes of groups 4 and 7 (Salcedo et al. 1980; Prada et al. 1982). It is possible, however, that some of our unassigned components do correspond to the low-molecular-weight gliadins.

Applications of chromosomal assignments

Our assignment of genome and chromosome locations for the genes coding for the major gliadins of 'Cheyenne', a cv. with excellent mixing strength and baking quality, provides a valuable addition to the previous assignments for 'Chinese Spring' gliadins by Wrigley and Shepherd (1973). This latter cv. has relatively poor flour and bread quality (Morris et al. 1966) and is usually grown only for scientific purposes. Accordingly, our assignments provide the possibility of a more sophisticated genetic analysis of the evolution of the genes coding for the protein components of the diploid species that were the progenitors of the A, B, and D genomes of hexaploid bread wheats, the mechanism by which they became combined in formation of the polyploid (Cole et al. 1981), and the potential relationship between gliadins and quality (Damidaux et al. 1978; Wrigley 1980; Sozinov and Poperelya 1980). Our assignments should be especially helpful in unraveling the complexities of amino acid and DNA sequencing information in relation to the families and subfamilies of gliadin genes that are clustered at six or more complex loci in the A, B, and D genomes of hexaploid wheat.

Acknowledgements. We thank E. R. Sears for providing the aneuploids of 'Chinese Spring' and for helpful comments on the manuscript. D. L. was a visiting scientist at WRRC in receipt of a C.N.R. fellowship during the course of this work. Reference to a company or product is for information purposes only and does not imply recommendation of the company or product to the exclusion of others that may be suitable.

References

Autran J-C, Lew EJ-L, Nimmo CC, Kasarda DD (1979) N-Terminal amino acid sequencing of prolamins from wheat and related species. Nature 282:527–529

Briggle LW, Reitz LP (1963) Classification of *Triticum* species and of wheat varieties grown in the United States. Tech Bull No 1278, US Department of Agriculture, Washington DC

Briggle LW, Strauss SL, Hamilton DE, House GH (1982) Distribution of the varieties and classes of wheat in the United States in 1979. Stat Bull No 676, Agricultural Research Service, US Department of Agriculture, Washington DC

Brown JWS, Flavell RB (1981) Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. Theor Appl Genet 59:349–359

Brown JWS, Kemble RJ, Law CN, Flavell RB (1979) Control of endosperm proteins in *Triticum aestivum* (var. 'Chinese Spring') and *Aegilops umbellulata* by homoeologous group 1 chromosomes. Genetics 93:189–200

Brown JWS, Law CN, Worland AJ, Flavell RB (1981) Genetic variation in wheat endosperm proteins: an analysis by two-dimensional electrophoresis using intervarietal chromosomal substitution lines. Theor Appl Genet 59:361–371

Bushuk W, Zillman RR (1978) Wheat cultivar identification by gliadin electropherograms. 1. Apparatus, method, and nomenclature. Can J Plant Sci 58:505–515

Cole EW, Fullington JG, Kasarda DD (1981) Grain protein variability among species of *Triticum* and *Aegilops:* quantitative SDS-PAGE studies. Theor Appl Genet 60:17–30

Damidaux R, Autran JC, Grignac P, Feillet P (1978) Mise en evidence de relations applicables en selection entre l'electrophoregramme des gliadins et les proprietes viscoelastiques du gluten de *Triticum durum* Desf. CR Acad Sci Paris, Serie D 287:701–704

Doekes GJ (1973) Inheritance of gliadin composition in bread wheat, *T. aestivum* L. Euphytica 22:28–33

Garcia-Olmedo F, Carbonero P, Jones BL (1982) Chromosomal location of genes that control wheat endosperm proteins. In: Pomeranz Y (ed) Advances in cereal science and technology, vol 5. Am Assn Cereal Chem, St Paul Minn, pp 1–48

Kasarda DD (1980) Structure and properties of α-gliadins. Ann Technol Agric 29:151–173

Kasarda DD, Bernardin JE, Qualset CO (1976) Relationship of wheat gliadin components to chromosomes in hexaploid wheats (*Triticum aestivum* L.). Proc Natl Acad Sci USA 73: 3646–3650

Kasarda DD, Autran J-C, Lew EJ-L, Nimmo CC, Shewry PR (1983) N-Terminal amino acid sequences of ω-gliadins and ω-secalins. Implications for the evolution or prolamin genes. Biochim Biophys Acta 747:138–150

Mecham DK, Kasarda DD, Qualset CO (1978) Genetic aspects of wheat gliadin proteins. Biochem Genet 16: 831–853

Mitrofanova OP (1976) Genetic control of the gliadin of soft wheat *T. aestivum* of the 'Chinese Spring' variety (in Russian). Tsitol Genet 10:244–247

Morris R, Schmidt JW, Mattern PJ, Johnson VA (1966) Chromosomal location of genes for flour quality in the wheat variety 'Cheyenne' using substitution lines. Crop Sci 6:119–122

Payne PI, Holt LM, Lawrence GJ, Law CN (1982) The genetics of gliadin and glutenin, the major storage proteins

of the wheat endosperm. Qual Plant – Plant Foods Hum Nutr 31:229–241

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1, Glu-B1,* and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Prada J, Sanchez-Monge R, Salcedo G, Aragoncillo C (1982) Isolation of the major low molecular weight gliadins from wheat. Plant Sci Lett 25:281–289

Salcedo G, Prada J, Aragoncillo, C (1982) Low MW gliadin-like proteins from wheat endosperm. Phytochemistry 18: 725–727

Salcedo G, Prada J, Sanchez-Monge R, Aragoncillo C (1980) Aneuploid analysis of low-molecular-weight gliadins from wheat. Theor Appl Genet 56:65–69

Sears ER (1954) The aneuploids of common wheat. Res Bull No 572, Mo Agric Exp Stn, Bull, Columbia Mo

Shepherd KW (1968) Chromosomal control of endosperm proteins in wheat and rye. In: Finley KW, Shepherd KW (eds) Proc 3rd Int Wheat Genet Symp. Aust Acad Sci, Canberra, pp 86–96

Sozinov AA, Poperelya FA (1979) Polymorphism of prolamins in breeding (in Russian). Vestn Skh Nauki (Moscow) 10: 21–34

Sozinov AA, Poperelya FA (1980) Genetic classification of prolamins and its use for plant breeding. Ann Technol Agric 29:229–245

Sozinov AA, Stel'makh AF, Rybalka AI (1978) Hybridological and monosomic analyses of gliadins in common wheat varieties (in Russian). Genetika 14:1955–1967

Tanford C (1961) Physical chemistry of macromolecules. Wiley and Sons, New York

Wrigley CW (1980) The genetic and chemical significance of varietal differences in gluten composition. Ann Technol Agric 29:213–227

Wrigley CW, Shepherd KW (1973) Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209: 154–162

Zurabishvili TG, Iordansky AB, Badaev NS (1978) Linear differentiation of cereal chromosomes. 2. Polyploid wheats. Theor Appl Genet 51:201–210