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Chromosomal location by F_1 monosomic analysis of endosperm proteins in bread wheat

2. Two-dimensional fractionation of gliadins

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Summary. The gliadin components from four bread wheat cultivars: Chinese Spring, Capelle Desprez, Holdfast and Pane-247 and their monosomic F1s for the chromosomes of homoeologous groups 1 and 6 have been analyzed by two-dimensional (2-pH) polyacrylamide gel electrophoresis. Chromosomal location of gliadin genes and the allelic differences were well established by analyzing the different F1 monosomic hybrids, electrophoretical patterns and differences in relative staining intensity. A new gliadin encoded by a gene located on chromosome 6B in Chinese Spring is described. The two-dimensional patterns of gliadin in the other three varieties and the chromosomal location of their genes are reported for the first time. Relationships between gliadins in the two-dimensional patterns and the traditional system for their nomenclature are discussed.

Key words: Gliadin – Two-dimensional electrophoresis – F1 monosomic analysis – chromosomal assignment – wheat

Introduction

Gliadins are the major storage protein fraction of wheat endosperm. They are small and do not have disulfide-bonds in their subunit structure (Wall 1979). Classical gliadins have been classified into ω, γ, β and α components based on fractionation by gel electrophoresis at low pH.

There are two complex loci involved in the genetic control of these proteins. *Gli-1* codes for ω- and γ-gliadins and is located on the short arms of group 1 chromosomes. β- and α-gliadins are coded by locus *Gli-2* located on the short arms of group 6 chromosomes (for a review see Payne 1987).

Each gliadin-coding unit is assumed to consist of several tightly linked genes that arose through duplication and subsequent divergence (Kasarda 1980). There is no intra-locus recombination that implies the gliadin components are inherited as units (Metakovsky et al. 1984).

Branlard and Dardevet (1985) demonstrated a positive correlation between gliadin composition and some bread-making quality tests. Their implication in determining dough charachteristics call for further studies of their allelic variation. Thus, Lafiandra et al. (1984) have reported on genes coding for wheat gliadins in the bread cultivars Chinese Spring and Cheyenne after using two-dimensional (2-pH) electrophoresis. Moreover, Lafiandra et al. (1987), by analysis of intervarietal substitution lines, have located chromosomal control of gliadins in the durum wheat variety Langdon, Cheyenne is the only bread wheat variety distinctive to Chinese Spring in which the genetic control for the proteins has been established.

An F1 monosomic analysis to determine chromosomal location of endosperm protein genes in four varieties of bread wheat is described in a previous paper (Hueros et al. 1988). In the present work, two-dimensional (2-pH) electrophoresis is used to compare the Chinese Spring gliadin components with those found in three other wheat cultivars.

Materials and methods

Plant materials

The plant material used in the present study consisted of the following: (1) seeds from the bread wheat varieties Chinese

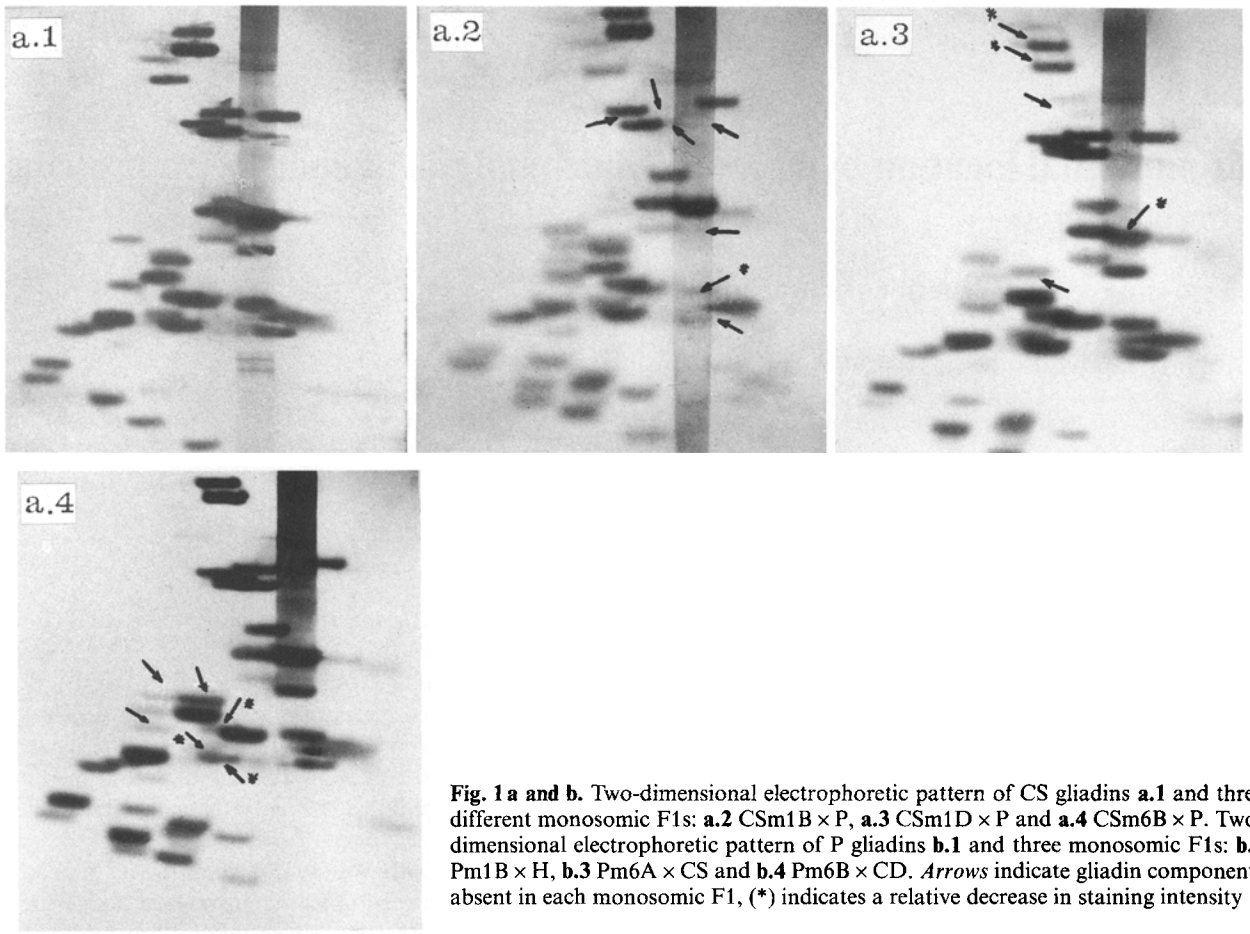


Fig. 1 a and b. Two-dimensional electrophoretic pattern of CS gliadins **a.1** and three different monosomic F1s: **a.2** CSm1B × P, **a.3** CSm1D × P and **a.4** CSm6B × P. Two-dimensional electrophoretic pattern of P gliadins **b.1** and three monosomic F1s: **b.2** Pm1B × H, **b.3** Pm6A × CS and **b.4** Pm6B × CD. *Arrows* indicate gliadin components absent in each monosomic F1, (*) indicates a relative decrease in staining intensity

Spring (CS), Capelle Desprez (CD), Holdfast, (H) and Pane-247 (P). Due to the great number of studies on genetic control of endosperm proteins in CS, this variety was used as control; and (2) monosomic (m) progenies from crosses between monosomic plants (female) for chromosomes of homoeologous groups 1 or 6 and euploids (male), as follows:

CSm1A × P	Hm1A × CS	CDm1A × P	Pm1A × H
CSm1A × H	Hm1A × P	CDm1B × P	Pm1B × H
CSm1B × P	Hm1B × P	CDm1D × CS	Pm1B × CS
CSm1D × P	Hm1D × P	CDm6A × H	Pm1D × CS
CSm6A × P	Hm6A × CS	CDm6A × CS	Pm6A × H
CSm6B × P	Hm6B × P	CDm6B × H	Pm6A × CS
CSm6D × P	Hm6D × CS	CDm6B × P	Pm6B × CD
CSm6D × H		CDm6D × H	Pm6D × H
		CDm6D × P	

The CS monosomic series was developed by E. R. Sears, the CD and H series by C. N. Law and the P series by one of the present authors (N. Jouve). The monosomic set of CS, CD and H were kindly supplied by T. E. Miller (PBI, Cambridge, UK).

Chromosomal control

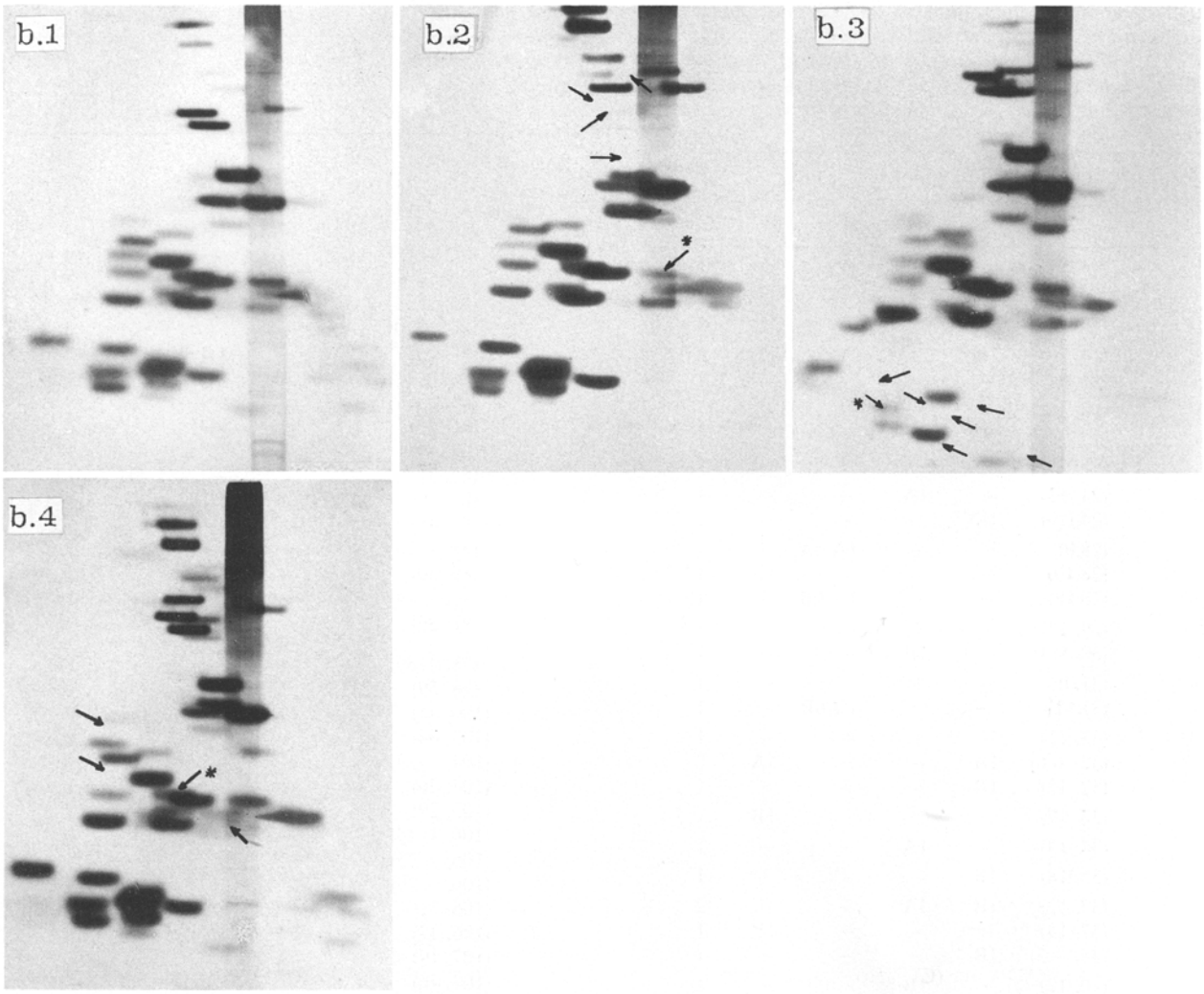
The chromosome number was determined by conventional Feulgen staining of root tips at metaphase, SDS-PAGE or A-PAGE could also be used for control of a monosomic condition through endosperm protein markers. The C-banding technique was applied for identification and chracterization of somatic chromosomes in Holdfast and Hm6B, according to a procedure previously reported (Jouve et al. 1980).

Extraction of proteins and electrophoresis method

The two-dimensional (2-pH) electrophoresis technique developed by Lafiandra and Kasarda (1985) was used. Their mode of extraction was also largely followed. Minor modifications were made in gels that were 0.9 mm instead of 1.5 mm thick. A reduction in time for the buffer equilibration in the second dimension (10 min) was also applied. Gels were prerun for 1.5 h at constant voltage (400 V). In the first dimension (aluminium lactate buffer) voltage was 500 V for 3 h at 4 °C. In the second phase (tris-glycine buffer) voltage was 300 V during 16 h (overnight) at 6 °C.

Results

Figures 1 a and b show two examples of how monosomic F1 hybrids are used to assign gliadin bands to chromosomes. When one band disappears or decreases in its relative staining intensity in a monosomic F1, it can be



assumed that its locus is located on a chromosome with a monosomic female parent. Thus, the absence of the critical chromosome from the female parent in the monosomic F1s permits the assignments.

The two-dimensional pattern of gliadins from CS is shown in Fig. 2. The schema includes the assignment of protein coding genes to chromosomes. It also shows the coordinates (x, y) for bands that were located through data of their relative mobility to gliadin α-1 (common to the four varieties). The relative positions of bands that were very close were determined by studying the two-dimensional patterns of the reciprocal hybrids between any two varieties. Nearly 40 components in CS could be identified within the 4 groups ω, γ, β and α. Symbol (*) indicates that chromosomal location was determined by a relative decrease in staining intensity. Symbol (+) was used to design bands absent in the male parent but with low activity in monosomic F1; such bands are probably made up of overlapping proteins related to different chromosomes.

Figures 3 to 5 show photographs and diagrams of two-dimensional separations of gliadins from CS, H and P, respectively. Chromosomal locations are also indicated.

The line Hm6B × P did not show some ω and γ gliadins codified by chromosomes 1A and 1B. There was also one ω gliadin (28,0) controlled by the 1A chromosome that was absent in Hm6A × CS.

Table 1 summarizes the results obtained. Sixty-five out of 100 distinct components revealed from the 4 varieties were assigned to chromosomes. Between 29 and 33 bands were located in each variety. Single bands resolved into components in the second dimension from one-dimensional electrophoresis are specified in the left column. The set of alleles present in each variety and their chromosomal location is also shown. The right column

Table 1. Chromosomal location of single bands resolved by two-dimensional electrophoresis. For each band, coordinates (see text) and number of varieties in which it is present are indicated. Symbol – indicates that the band is not present in that variety, + indicates that a band is present but has not been located

A-PAGE band	Coordinates	Varieties				Present in varieties
		CS	CD	H	P	
ω-1	(2, 20)	–	1D	1D	1D	3
ω-2	(8, 19)	1D*	1D*	1D*	1D	4
	(8, 24)	–	1D	–	1D	2
ω-3	(14, 19)	1D*	1D*	1D*	1D*	4
	(14, 22)	+	–	–	–	1
ω-4	(18, 28)	–	–	1A/6B	–	1
ω-5	(22, 27)	–	–	1A/6B	–	1
	(23, 13)	–	–	1A/6B	1B	2
ω-6	(24, 13)	–	1A	–	–	1
	(24, 19)	1D	–	–	–	1
ω-7	(28,0)	–	–	1A/6A	–	1
	(28,10)	–	–	–	1B	1
	(28,18)	–	–	1A/6B	–	1
ω-8	(29, 14)	+	+	–	–	1
	(30, 19)	–	1B	–	–	1
ω-9	(31,0)	–	+	–	–	1
	(31,11)	–	–	1B/6B	–	1
ω-10	(32, 11)	–	+	–	–	1
	(32, −6)	1A	–	–	1A	2
	(32, 12)	1B	–	–	–	1
ω-11	(33, 17)	–	–	–	1B	1
ω-12	(34, 13)	–	1A	–	–	1
ω-13	(35, 18)	1B	–	–	–	1
ω-14	(37, 12)	1B	1A	–	–	2
	(37, 14)	–	–	–	1B	1
	(38, −3)	1B	–	–	–	1
ω-15	(40, 12)	–	1B	–	–	1
ω-16	(42, 13)	–	–	1B/6B	–	1
γ-1	(51, 6)	–	–	–	1B	1
γ-2	(52, 12)	–	–	+	–	1
	(54, 6)	–	1B	1B	–	2
γ	(58, 12)	1A*	–	–	1A	1
	(59, −11)	1A	–	–	1A	2
	(59, 0)	1D*	1D*	1D*	1D*	4
	(59, 35)	–	6B	+	–	2
γ-4	(60, 0)	+	–	–	–	1
	(60, 24)	–	–	–	1A	1
	(61, 12)	–	+	+	–	2
γ 5–9	(62, −21)	–	–	–	+	1
	(62, 36)	–	–	–	6B	1
	(64, 11)	6B	–	1A+6B	6B?	3
	(64, 36)	6B	6B	6B	–	3
	(66, 24)	–	6B	6B	+	4
	(67, −2)	–	1A	–	–	1
	(67, 0)	1B	–	1A/6B	–	2
	(67, 34)	–	1D	+	–	2
	(67, 24)	6B	–	–	–	1
	(68, 35)	–	–	–	1D	1
γ-10	(68, 23)	1D	–	–	–	1
β-1	(72, 38)	–	–	–	6B	1
β 2–3	(75, 26)	+	+	+	+	4
β	(77, 37)	6B	–		–	1
	(78, −11)	–	–	1A	–	1
	(78, 38)	–	6B*	6B*	+	3
	(79, 20)	6B*	6B*	6B*	6B*	4
	(80, −1)	1B*	1B*	+	1B*	4
	(80, 14)	+	6D*	6D/6B	6D*	4
	(82, 8)	–	1B	–	–	1
	(84, −10)	1A	+	–	1A	3
	(84, 26)	6B	–	6B	–	2
	(86, 0)	–	6B	–	–	1
	(86, 25)	+	6B*	+	+	4
	(86, 40)	+	6D*	6D*	+	4
	(87, −5)	1B	–	–	–	1
	(87, 3)	–	–	–	6B	1
	(87, 20)	6B*	6B*	6B*	+	4
	(87, 45)	6A	–	–	–	1
	(88, −18)	–	+	+	–	2
	(89, 50)	6A	–	–	–	1
	(90, −14)	–	–	–	+	1
	(91, 36)	6A	–	–	–	
α=	(98, −30)	–	–	–	+	1
	(98, 59)	6D*	6D*	6D*	6D*	4
	(100, 41)	–	6A	6A	6A	3
	(103, 64)	6A	–	–	–	1
	(104, −29)	–	–	–	+	1
	(104, 26)	+	–	–	6D	2
	(105, 29)	–	–	–	6A	1
	(106, −35)	–	–	–	+	1
	(106, −25)	–	+	+	–	2
	(106, −20)	–	–	–	+	1
	(106, 31)	–	6A	6A	6A	3
	(106, 43)	–	6A	+	–	2
	(107, 19)	–	6A	6A	6A	3
	(107, 46)	+	–	6A	6D*	1
	(108, 30)	–	+	6A	6A	3
	(112, 43)	–	–	–	6D	1
	(114, 30)	6A	–	–	6A	2
	(116, −28)	–	–	–	+	1
	(117, 5)	–	–	–	6A	1
	(118, 11)	6A	–	–	–	1
Ratio between located and present band		30/37	30/39	29/39	34/47	122/162
ω-7′	(29,10)					
ω-15′	(38, 11)	Only present in Hm6B				
γ-	(64, 41)					

shows the number of varieties that hold the specific component.

Discussion

Lafiandra et al. (1984, 1987) reported chromosomal location of genes coding for wheat gliadins using nullitetrasomic lines of Chinese Spring. The monosomic F1

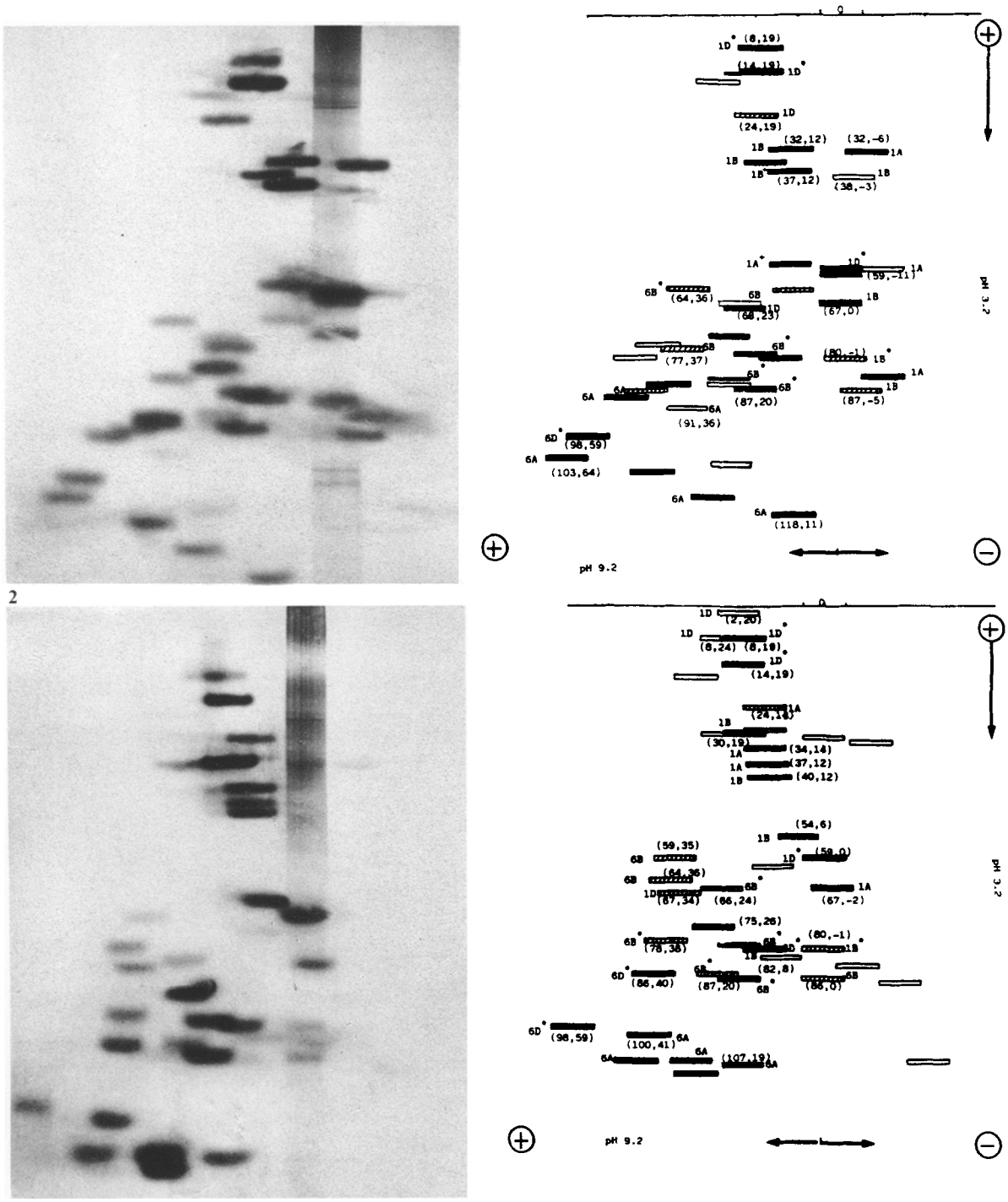
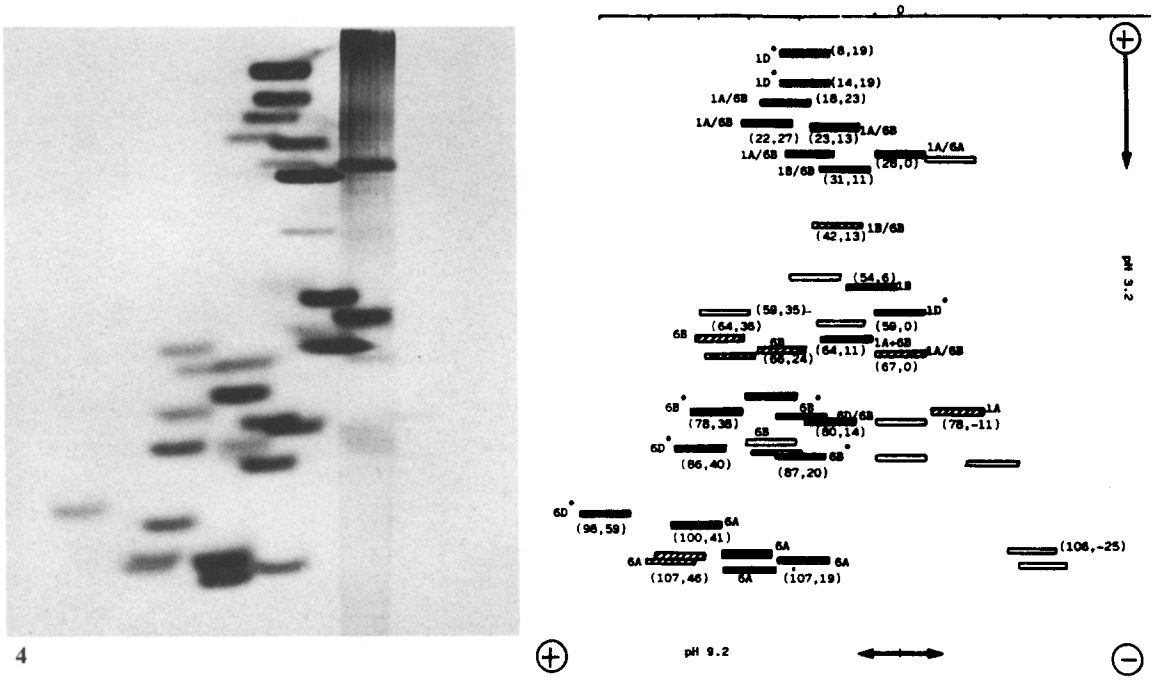


Fig. 2. Two-dimensional pattern of CS gliadins. The diagram shows chromosomal locations performed. The coordinates of some individual bands are also included

Fig. 3. Two-dimensional pattern of CD gliadins. The diagram shows chromosomal locations performed. The coordinates of some individual bands are also included



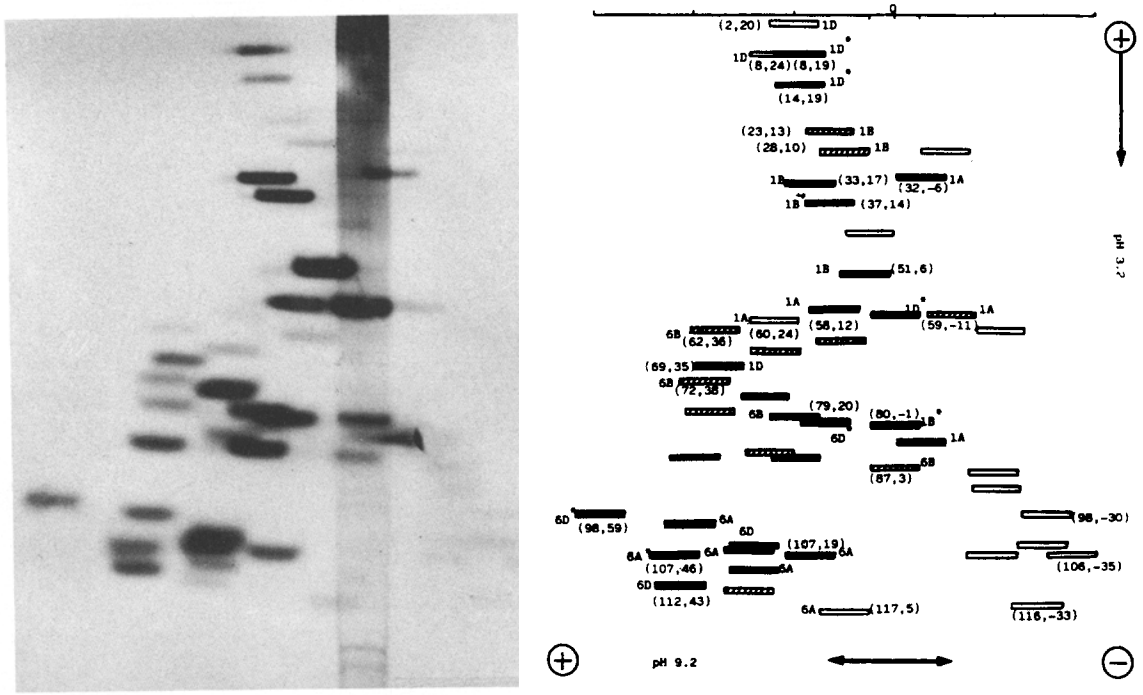


Fig. 4. Two-dimensional pattern of H gliadins. The diagram shows chromosomal locations performed. The coordinates of some individual bands are also included

Fig. 5. Two-dimensional pattern of P gliadins. The diagram shows chromosomal locations performed. The coordinates of some individual bands are also included

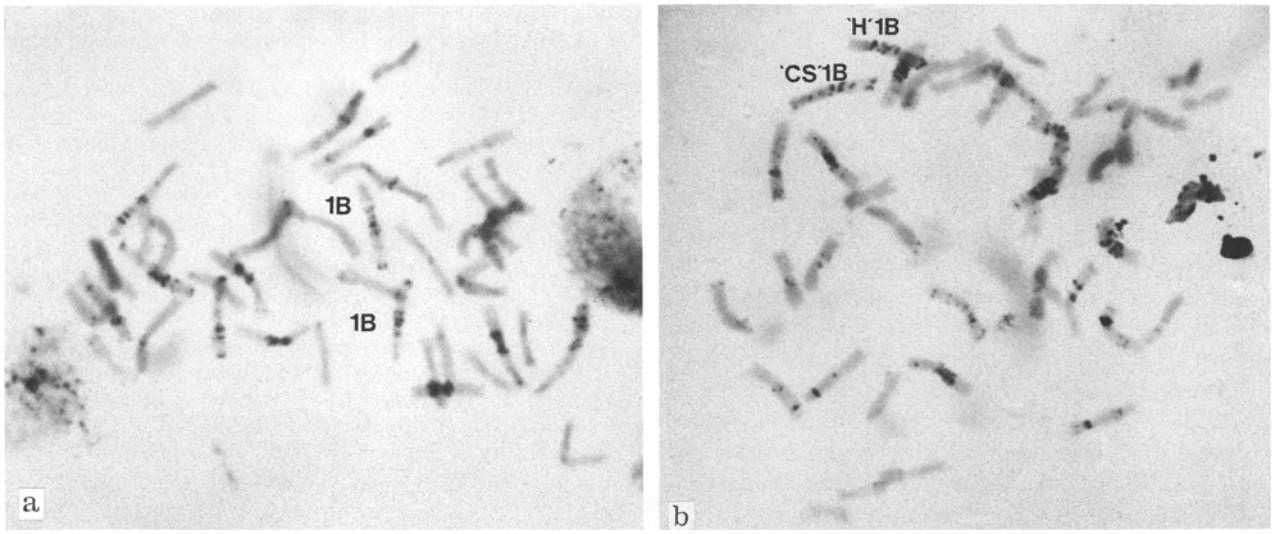


Fig. 6a and b. Microphotographs of somatic metaphase. C-banding of **a** Holdfast and **b** 'Hm6Bx' line

analysis used in the present work has confirmed those assignments. However, one difference is pointed out: the component named 1D+ by Lafiandra et al. (1987) (this band decreases in its relative staining intensity but does not disappear in nullisomic grains for the chromosome 1D) is revealed as a duplicate in our analysis. The most cathodic gliadin (68, 23) is controlled by the 1D chromosome with the other assigned to chromosome 6B (Figs. 1a.3, 1a.4 and 2).

The genetic control of Capelle Desprez proteins was studied by Brown et al. (1981) using two-dimensional electrophoresis (IEF × SDS) and substitution lines. However, this technique has a poor resolution for gliadins with a molecular weight that ranges from 30–40 Kd. Two-dimensional (2-pH) electrophoresis permits a better separation of this group of proteins.

The gliadin constitution of Holdfast and Pane-247 is examined here for the first time. H has good bread making quality, at yet only correlated with its HMW-glutenin subunits. P is a Spanish wheat cultivar well adapted to local conditions but with low quality. Bread-making quality tests of reciprocal F1 monosomics for the two varieties could be useful to correlate rheological characteristics to specific gliadin units.

In a previous paper (Hueros et al. 1988) we reported some anomalies in the Holdfast mono-6B strain. The two-dimensional analysis here helps to explain that situation. The band ω-7 controlled by chromosome 1A appeared to be single when studied by one-dimensional electrophoresis, but is now proved to include two components. The most anodic one could be related to chromosome 1A and 6B and the other one to 1A and 6A. Two alternative hypotheses could explain these observations:

(1) A complementary effect between genes located on chromosomes 1A or 1B and 6B could offer one explanation. The simultaneous activity of both genetic systems could be necessary for the expression of some gliadins. Nevertheless, seeds of plants with $2n=42$ and $2n=41$ from the Holdfast mono-6B line have the same pattern; it must be assumed that this strain has damaged its hypothetic regulatory gene(s). Similar interrelations have been reported by Gupta and Shepherd (1987). The group E glutenin subunits controlled by genes on the short arm of homoeologous group 1 are only expressed under the presence of the large arm of chromosome 1B.

(2) The loci *Gli-B1* and *Gli-A1* are totally or partially inactive in the Holdfast mono-6B strain. Some bands, e.g. (54,6), (28,0) and (78, −11), are controlled by chromosomes 1B and 1A do not disappear in this line. The results suggest that the multigene family *Gli-A1* and *Gli-B1* is not totally inactivated or that such bands are controlled by another genetic system, possibly *Glu-2*, which lies in the short arm of chromosomes 1A and 1B (Payne 1987). Galili and Feldman (1984) described proteins with ω-gliadin-like characteristics located in this locus. Similar deletions have been found in other varieties of common wheat by Wrigley and Shepherd (1974), Autran (1975), Payne et al. (1984) and Pogna et al. (1985). Recently Lafiandra et al. (1987) described a similar phenomenon in a variety of durum wheat. They found that ω-gliadins located in chromosomes 1A and 1B were missing without a change in γ, β and α components. However, the occurrence of a twofold deletion affecting the same region in two different chromosomes is very unlikely.

Moreover, the C-banding technique was applied to study the chromosome region where *Gli-1* is located. The chromosome 1B (1A remained unidentified by C-banding) showed the same pattern in Holdfast and Hm6B (Fig. 6). There was no cytogenetic evidence in

favour of a deletion in any region of that chromosome in Holdfast mono-6B.

Our results and those from Lafiandra et al. (1984, 1987) reveal that the classical separation of gliadins in four groups according to their mobility at low pH does not give sufficient information about their genetic control. The ω gliadins (S-poor prolamins classified by Shewry and Miflin 1985) are definitely located in chromosome group 1. The S-rich prolamins corresponded to the γ, β and α gliadins and their N-terminal amino acid sequences can be grouped into two nonhomoeologous groups: (1) the α-type occurs in α and in some β and γ gliadins; and (2) the γ-type is generally found only among γ-gliadins (Shewry and Miflin 1985). Our chromosomal assignments in different bread wheat varieties show that some classical γ gliadins (generally the most anodic ones in second dimension separation) have their related genes in chromosomes of homoeologous group 6. The most cathodic components of β gliadins are, however, related to chromosomes of homoeologous group 1. Finally, all α gliadins are controlled by the homoeologous group 6. It is surprising that never, in five varieties of hexaploid and one variety of tetraploid wheat, has the chromosome 6B been involved in the control of α gliadins.

The four varieties show minor modifications as to the *Gli-D1* and *Gli-D2* loci. Such observations provide support for a monophyletic origin of the D genome in hexaploid wheat. A polyphyletic origin of the B genome is also supported by the fact that the most important intervarietal variation is found in genes belonging to *Gli-B1* and *Gli-B2*.

The most cathodic proteins, which are also the least stainable, have not been located on any chromosome until now. Some of these components could correspond to low-molecular-weight gliadins described by Salcedo et al. (1980).

The methodology applied here has some advantages over the use of intervarietal substitution lines: (1) it permits expansion of the genetic analysis to all varieties where a monosomic series is available; (2) the analysis of a two-dimensional gliadin pattern in reciprocal monosomic crosses can help to establish relationships between endosperm protein blocks and bread-making quality; and (3) it has a better resolution capacity, allowing for an accumulation of more detailed information.

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