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

1. One-dimensional electrophoresis of glutenins and gliadins

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Summary. A new methodology to determine the chromosomal location and allelic differences of endosperm proteins in common wheat cultivars by analysis of monosomic intervarietal hybrids is reported. Endosperm proteins from the common wheats Chinese Spring, Capelle Desprez, Holdfast and Pane 247 are studied using monosomic F1 for the chromosomes of homoeologous groups 1 and 6. The proteins were fractionated by two electrophoretical techniques, SDS- and A-PAGE. The use of monosomic offers a remarkable advantage over the utilization of intervarietal substitution lines, because it permits a quicker characterization and earlier evaluation of basic material to be used in breeding programmes.

Key words: Glutenin – Gliadin – F1 monosomic analysis – Chromosomal assignment – Electrophoresis

Introduction

Triticum aestivum L., common or bread wheat is an allohexaploid species containing three sets (seven pairs each) of chromosomes (genome AABBDD; $2n=6x=42$ chromosomes).

Homeology between genomes and chromosomes permits the existence of aneuploid forms that have lost part of their genetic information. Sears (1954, 1965) obtained several types of aneuploids (nullisomics, nullitetrasomics, monosomics, etc.) in the cv Chinese Spring, Monosomes are viable aneuploids in common wheats that lack 1 chromosome out of 21 pairs. Frequencies of monosomic offspring from monosomic plants range from 71,5%–74,5%. The frequency depends about 75% on female transmission of $n-1$ eggs, while male transmission is limited to between 1% and 7%.

Unrau (1950) described a method to produce a monosomic series of a new variety using the monosomic lines of Chinese Spring. The latter is crossed with the selected cultivar functioning as male parent. The F1 monosomic plants are backcrossed again with the male parent, a procedure continued until exchange of the genetic background is reached. By this method, production of monosomic series of different local varieties can be obtained. Material thus produced offers opportunities for genetic analyses and might function as basic material in wheat breeding programmes.

Monosomic analysis has been employed to study quantitative characters (Snape et al. 1983) and to analyse the genetics behind heading date and spikelet number in common wheat (Millet 1987). Law et al. (1981) used the method of reciprocal monosomic analysis to study chromosomal locations of some genes governing agronomic traits in the varieties Capelle Desprez and Norin 10.

Endosperm is an important wheat grain tissue. It has a triploid chromosome constitution, two doses contributed by the female and one by the male gamete. Glutenins and gliadins are the main fractions among the endosperm storage proteins (Wall 1979). Glutenins, probably the main fraction of rheological importance, are molecules held together by disulphide bonds. They are conveniently separated into two groups: low-molecular-weight (LMW) and high-molecular-weight (HMW). Gliadins have been more extensively investigated since they are the main constituents of kernel proteins and have some rheological but predominantly nutritional significance. They are smaller than glutenins, have no disulfide bonds and are divided into four groups (ω, γ, β and α) when fractionated in gel electrophoresis at acid pH (Payne 1987).

Payne et al. (1981) and Branlard and Dardevet (1985a, b) have found correlations between allelic consti-

tution as to glutenins and gliadins and several bread-making qualities in tests such as SDS-sedimentation and Chopin alveograph. Payne (1987) reviewed the studies on the genetic control of endosperm proteins that were carried out, using the nullitetrasomic and ditelosomic sets of Chinese Spring. Lafiandra et al. (1984), Galili and Feldman (1985) and Brown et al. (1981) studied the genetic control by means of different electrophoretical techniques and used intervarietal substitution lines as experimental material. The involvement of four loci was shown and located to chromosomes of the homoeologous groups 1 and 6.

We report here an F1 monosomic analysis of glutenins and gliadins in four varities of bread wheat. This method has, at least, two advantages over the traditional use of intervarietal substitution lines: (1) it saves the time necessary to obtain the plant material; and (2) it avoids the requirement of prior knowledge of the genetic control of the character under study in the recipient variety.

Materials and methods

Plant material

The monosomic series of four cultivars of *Triticum aestivum* L., namely chinese Spring (CS), Capelle Desprez (CD), Holdfast (H) and Pane-247 (P), were used to obtain the monosomic F1s. The CS series was developed by Sears (1954). CD and H monosomic series were produced by and have been maintained at the Plant Breeding Institute (Cambridge, UK) and the monosomics of P were produced in our department. The monosomic of CS, H and CD were kindly supplied by T. E. Miller (P. B. I., Cambridge, UK).

We produced F1 hybrids between homoeologous group 1 and 6 monosomic (m1A to m6D) female plants of each variety and disomic plants from the other 3 cultivars as males. The list of parents and intervarietal hybrids used in the study is listed below:

Chinese Spring	Holdfast	Capelle Desprez	Pané-247
CS ($2n=42$)	H ($2n=42$)	CD ($2n=42$)	P ($2n=42$)
CSm1A × P	Hm1A × CS	CDm1A × P	Pm1A × H
CSm1A × H	Hm1A × P	CDm1A × H	Pm1A × CD
CSm1B × P	Hm1B × P	CDm1B × P	Pm1B × H
CSm1D × P	Hm1B × CS	CDm1B × H	Pm1B × CD
CSm1D × H	Hm1D × CS	CDm1D × P	Pm1B × CS
CSm6A × P	Hm1D × P	CDm1D × CS	Pm1D × CS
CSm6A × H	Hm6A × CS	CDm1D × H	Pm1D × H
CSm6B × P	Hm6A × P	CDm6A × H	Pm6A × H
CSm6D × P	Hm6B × P	CDm6A × CS	Pm6A × CS
CSm6D × H	Hm6B × CS	CDm6B × H	Pm6B × CD
CSm6D × CD	Hm6D × CS	CDm6B × P	Pm6D × H
	Hm6D × P	CDm6D × H	
		CDm6D × P	

Methods

Chromosomal control. Half kernels containing embryo were germinated, and the chromosome number was determined on root somatic metaphases after conventional Feulgen staining.

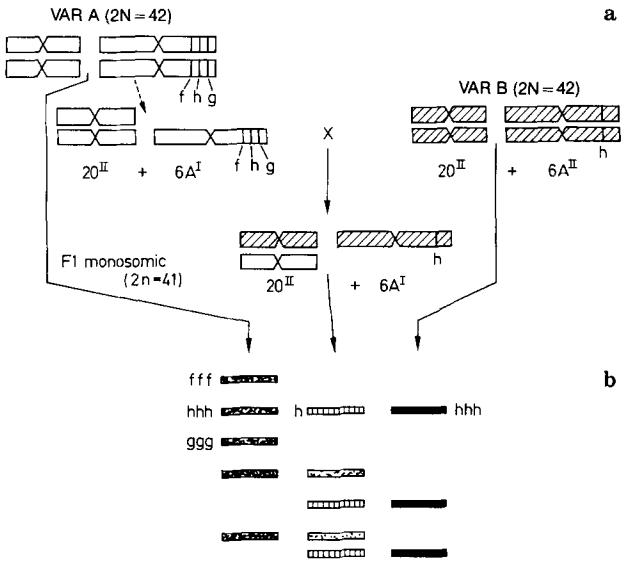

Fig. 1 a and b. Scheme of procedure used in the present work to determine the chromosomal location of endosperm protein genes (see text)

Polyacrylamide gel (10%) electrophoresis in presence of Sodium Dodecyl Sulphate (SDS-PAGE). Extraction of protein and the electrophoretic method of Payne et al. (1981) were used to analyze the glutenin and gliadin patterns of seeds of the various F1 monosomics. This method separates the protein subunits by molecular size. The molecular weight markers (Sigma) employed were: Phosphorilase b (94 Kilodalton (Kd)), Bovine Serum Albumin (67 Kd), Ovalbumin (43 Kd) and Carbonic Anhydrase (29 Kd).

Polyacrylamide gel (7%) electrophoresis with pH=3.1 aluminium lactate buffer (A-PAGE). The extraction conditions and electrophoretic method described by Lafiandra and Kasarda (1985) was followed with minor modifications. In these conditions gliadins were separated by the charge/mass ratio.

Results

Localization of protein genes

The method employed to determine the chromosomal location of genes controlling endosperm proteins is shown in Fig. 1. As an example, the female parent A is monosomic for chromosome 6A that codes for the f, h and g genes. Euploid plants from variety B are used as male parents and carry the gene for h, also present in variety A. Of the F1 offspring, 75% are expected to be monosomics with the single chromosome 6A coming from the variety B (Fig. 1 a).

The hypothetic electrophoretic patterns of varieties A, B and F1 monosomics are shown in Fig. 1 b. The f and g components, which characterize variety A, are not present in the pattern of the F1 monosomic. By comparing the electrophoretic pattern of the parental varieties and F1, information is provided as to the location of

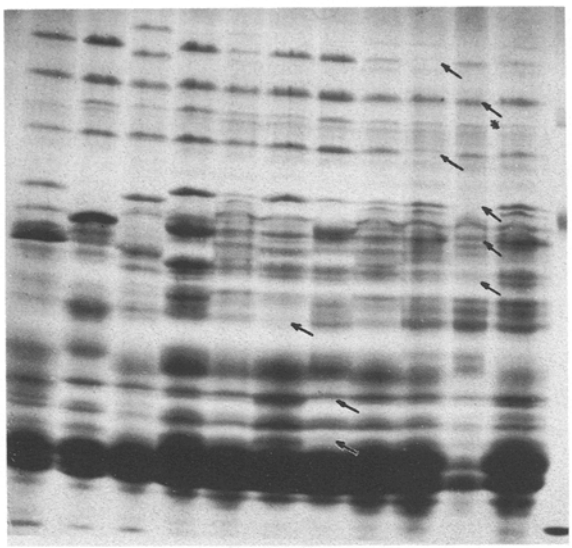

Fig. 2. SDS-PAGE migration pattern of total endosperm protein subunits extracted from (*a*) CD, (*b*) P, (*c*) H, (*d*) CS, (*e*) CSm1A × H, (*f*) CSm6D × CD, (*g*) CSm6B × P, (*h*) CSm6A × H, (*i*) CSm1D × H, (*j*) CSm1B × P, (*k*) CSm1A × H, (*MW*) molecular weight markers. *Arrows* point out CS bands that disappear in different F1 monosomics or decrease in their relative staining intensity (signed with *)

protein genes and eventual allelic differences between cultivars.

Additional information is offered by the relative reduction of staining intensity in the proteins coded by the aneuploid chromosome in the F1 monosomics depending on the source of gene-dosage (⅔ via female parent and ⅓ via male parent).

SDS-PAGE

Figure 2 shows the electrophoretic patterns of the four varieties and the different F1 monosomics derived from the monosomic plants of Chinese Spring. The group of bands having the lowest mobility are HMW-glutenins. Those with the highest mobility are LMW-glutenins and different types of gliadins. *Arrows* point out bands that disappear or decrease in their relative staining intensities (marked by *) in monosomic F1s for different chromosomes.

Figures 3–5 are SDS-PAGE photographs with monosomic F1s derived from varieties CD, H and P. A schematic representation of the electrophoretic pattern of each variety is given in Fig. 6. The chromosomal location is pointed out to the right of each band. The pattern of HMW-glutenins from Chinese Spring permits us to assign bands characteristic of the other three cultivars to those described by Payne and Lawrence (1983). CS and P have the same HMW-glutein alleles while CD lacks the subunit type y from *Glu-B1* (each *Glu-1* locus codifies for

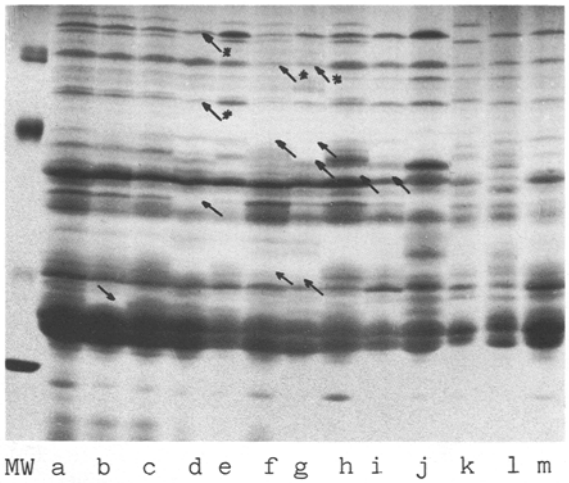

Fig. 3. SDS-PAGE migration pattern of total endosperm protein subunits extracted from: *MW*, (*a*) CDm6D × H, (*b*) CDm6B × H, (*c*) CDm6A × H, (*d*) CDm1D × P, (*e*) CD × H, (*f*) CDm1B × H, (*g*) CDm1B × P, (*h*) CDm1A × H, (*i*) CDm1A × P, (*j*) P, (*k*) H, (*l*) CS, (*m*) CD

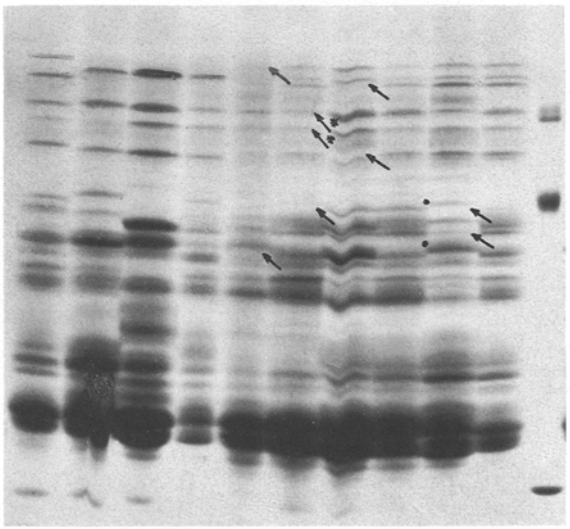

Fig. 4. SDS-PAGE migration pattern of total endosperm protein subunits extracted from: (*a*) H, (*b*) CD, (*c*) P, (*d*) CS, (*e*) Hm1A × P, (*f*) Hm1B × CS, (*g*) Hm1D × CS, (*h*) Hm6A × CS, (*i*) Hm6B × CS, (*j*) Hm6D × CS, *MW*

two types of HMW-glutenin subunits x and y with x characterized by a lower mobility than y). H is the only variety that does not carry the null allele at the *Glu-A1* locus. Its *Glu-B1* allele is probably the same as that of CS and P, but its subunits at chromosome 1D are different from those of the other varieties. Only band 7 is common to all varieties. Accordingly, in order to locate it, it is necessary to look for its reduction in relative staining intensity.

In F1 monosomics derived from the Holdfast mono-6B, some bands previously located on 1A or 1B chromo-

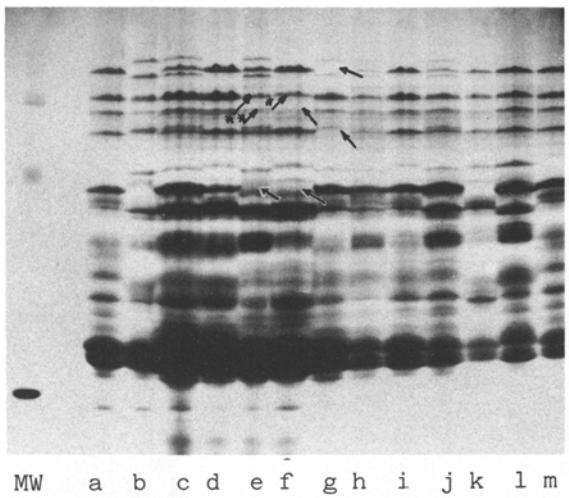

Fig. 5. SDS-PAGE migration pattern of total endosperm protein subunits extracted from: *MW*, *a*) P, (*b*) H, (*c*) Pm1A × H, (*d*) Pm1A × CD, (*e*) Pm1B × H, (*f*) Pm1B × CD, (*g*) Pm1D × H, (*h*) Pm6A × H, (*i*)Pm6D × CD, (*j*) Pm6D × H, (*k*) CD, (*l*) CS, (*m*) P

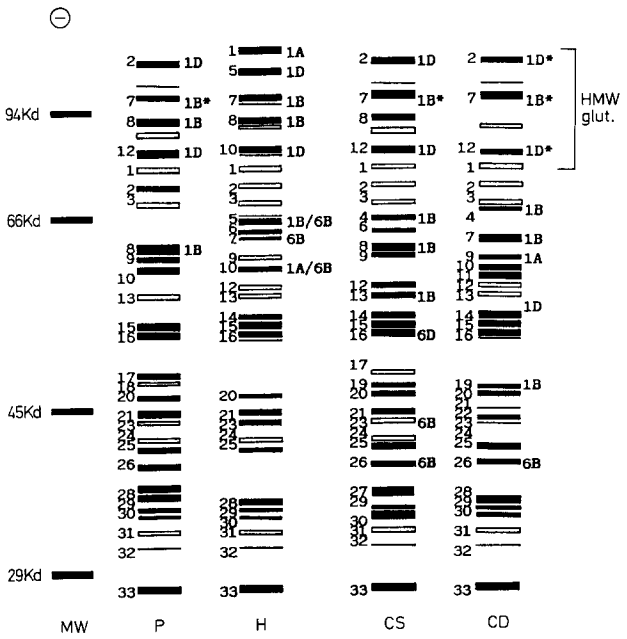

Fig. 6. Diagram of the SDS-PAGE fractionation pattern of total endosperm proteins extracted from the four cultivars examined. Molecular weight markers are signalized at the left. HMW-glutenins are named according to Payne and Lawrence (1983). Chromosomal assignment ist specified at the right of each band

somes did not show up; instead new bands having a higher molecular weight were observed. Bands that display such a behaviour are denoted as 1A/6B or 1B/6B in Table 1 where the results are summarized.

A-PAGE

Figure 7 illustrates an A-PAGE separation of gliadins for the varieties CS, P and the CS × P F1 monosomic. Similar results are shown for varieties CD, H and P in Figs. 8–10. *Arrows* mark positions of both the absence of CS bands and CS and P common bands that show a decrease in relative staining intensity when the corresponding CS chromosome is absent. Two cases can be observed for a block of proteins in Pane-247 mono 1B × Holdfast (Fig. 10). In this cross some P ω-bands disappear, while the same bands that are common to CD decrease in their relative staining intensity in Pane-247 mono 1B × Capelle Desprez. The first γ-zone band, exclusive of P, disappears in both cases.

Figure 11 summarizes the results obtained with the present method. The bands are ordered and numbered

Table 1. Presence (+)/absence (−) and chromosomal location of protein subunits separated in the SDS-PAGE. The HMW-glutenin subunits are named according to the Payne and Lawrence catalogue (1983). The remaining bands are numbered by increased mobility order

Allele	Band	CS	CD	H	P	Present in no. of varieties
H. M. W. glutenins						
GluA1a	1	−	−	1A	−	1
GluA1c	null	+	+	−	+	3
GluB1b	7, 8	1B/+	−	1B?	1B	3
GluB1a	7	−	1B	−	−	1
GluD1a	2, 12	1D	1D	−	1D	3
GluD1d	5, 10	−	−	1D	−	1
	1	+	+	+	+	4
	2	+	+	+	+	4
	3	+	+	+	+	4
	4	1B	1B	−	−	2
	5	−	−	1B/6B	−	1
	6	+	−	+	−	2
	7	+	1B	6B	−	3
	8	1B	−	−	1B	2
	9	+	1A	+	+	4
	10	−	+	1A/6B	+	3
	11	−	+	−	−	1
	12	+	+	+	−	3
	13	1B	+	+	+	4
	14	+	1D	+	−	3
	15	+	+	+	+	4
	16	6D	+	+	+	4
	17	+	−	−	+	2
	18	−	−	−	+	1
	19	+	1B	−	−	2
	20	+	+	+	+	4
	21	+	+	+	+	4
	22	−	+	−	−	1
	23	6B	+	+	+	4
	24	+	+	+	+	4
	25	+	+	+	+	4
	26	6B	6B	−	+	3
	27	+	−	−	−	1
	28	−	+	+	+	3
	29–33	+	+	+	+	4
	Total	9/27	9/26	8/21	5/23	31/97

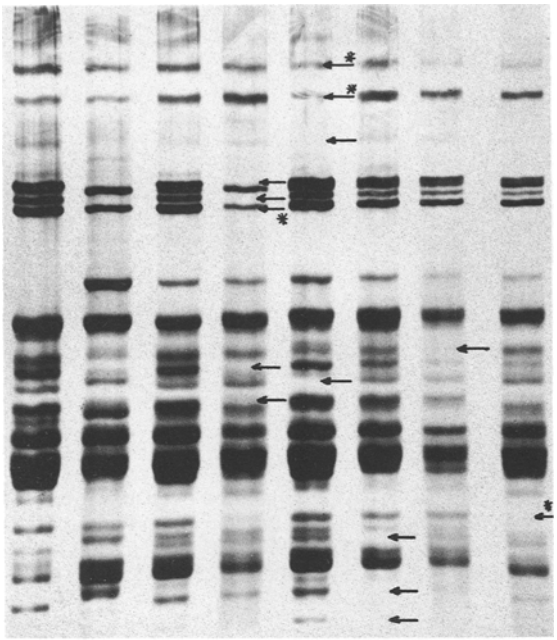

Fig. 7. A-PAGE separation of gliadins from: (*a*) CS, (*b*) P, (*c*) CSm1A × P, (*d*) CSm1B × P, (*e*) CSm1D × P, (*f*) CSm6A × P, (*g*) CSm6B × P, (*h*) CSm6D × P, *Arrows* point out CS bands that disappear in different F1 monosomics or decrease in their relative staining intensity (signed with *)

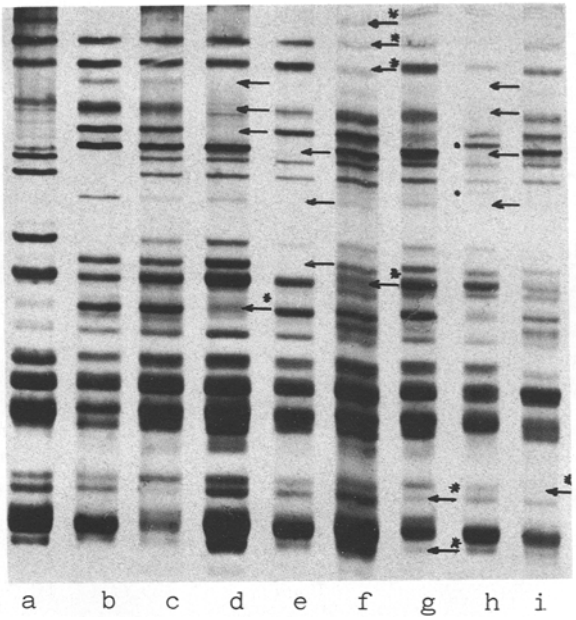

Fig. 9. A-PAGE separation of gliadins from: (*a*) P, (*b*) H, (*c*) H × P, (*d*) Hm1A × P, (*e*) Hm1B × P, (*f*) Hm1D × P, (*g*) Hm6A × P, (*h*) Hm6B × P, (*i*) Hm6D × P

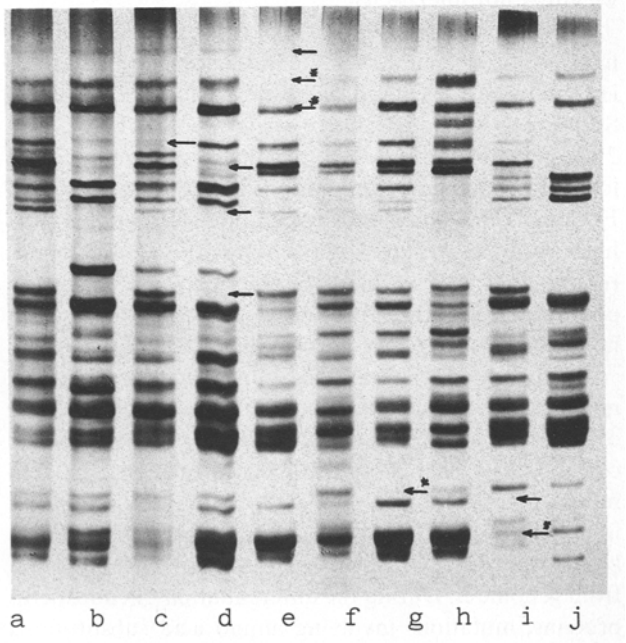

Fig. 8. A-PAGE separation of gliadins from: (*a*) CD, (*b*) P, (*c*) CDm1A × P, (*d*) CDm1B × P, (*e*) CDm1D × H, (*f*) CDm6B × H, (*g*) CDm6D × H, (*h*) H, (*i*) CDm6A × CS, (*j*) CS

according to their mobility. Like in SDS-PAGE, F1s derived from Holdfast mono-6B lack bands previously located to chromosomes 1A or 1B (Fig. 9). The same result appeared when the male parental was CS instead of P. New bands, absent in both parents, appeared in F1 and were named ω-7′ and ω-15′.

Discussion

Chromosome location studies of endosperm protein genes have been performed mainly on aneuploids of the variety Chinese Spring (nullitetrasomics and ditelosomics) by correlating the absence of a specific band in the electrophoreitc pattern with the deficiency of either the whole chromosome or one of its arms. Further genetic studies have been made by using intervarietal substitution lines with CS as recipient variety and cultivars with good quality as chromosome donors. Such lines cannot be used directly in breeding programmes.

The method described here will allow genetic mapping whenever differences are observed between any available monosomic series. Moreover, an analysis of reciprocal F1 monosomics (Mackewan and Kaltsikes 1970) permits an early evaluation of the effects of a series of possible intervarietal chromosomal substitutions (genomic interferences, increased quality, etc.). The use of substitution lines is a technically more cumbersome method.

The genes controlling the most stained HMW-glutenins were located in homoeologous group 1 in ac-

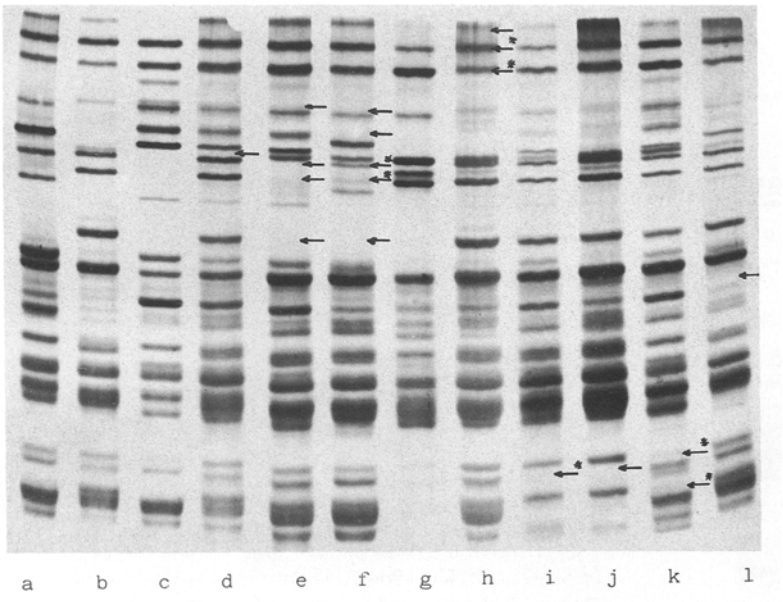

Fig. 10. A-PAGE separation of gliadins from: (*a*) CD, (*b*) P, (*c*) H, (*d*) Pm1A × H, (*e*) Pm1B × H, (*f*) Pm1B × C, (*g*) CS, (*h*) Pm1D × CS, (*i*) Pm6A × H, (*j*) Pm6A × CS, (*k*) Pm6D × H, (*l*) Pm6B × CD

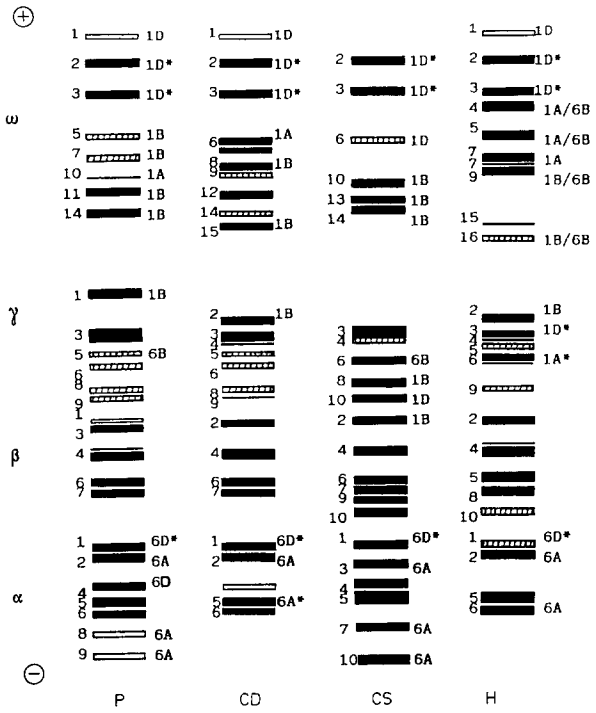

Fig. 11. Diagram of A-PAGE separation of gliadins from the four varieties. Chromosomal assignment of each band is specified on the right

cordance with previous reports (Lawrence and Sheperd 1980; Payne et al. 1980; Brown et al. 1981). The less stained HMW-subunits have not been analysed because of their faint staining intensity. The concerned bands could be post-translationally modified forms coming from major subunits (Payne 1987).

HMW-glutenins play a fundamental role in wheat flour processing. The alleles *Glu-A1a* (band 1) and *Glu-D1d* (bands 5 and 10) in cv H could be correlated with high breadmaking quality by Lorenzo et al. (1987). There are few differences between the electrophoretic patterns of the four varieties for the remaining protein fractions separated by SDS-PAGE. If endosperm proteins originate from duplication followed by diversification, such a finding of few intervarietal divergences in molecular weight is not unexpected.

Similar analyses using other types of wheat material have not been superior. Galili and Feldman (1983) studied nullitetrasomics and ditelosomics forms of Chinese Spring, but were only able to assign 1 component out of 21 to chromosome 6B. The remaining subunits were all located to homoeologous group 1. In addition, Galili and Feldman (1985) used substitution lines for studying the homoeologous group 1 by one-dimensional electrophoresis in the presence of SDS, a technique offering too poor of a resolution for γ- and β-gliadins located on homoeology group 6 of chromosomes.

Variation in relative staining intensity does not permit location of bands below 75 Kd, probably because overlapping subunits are controlled by more than one chromosome. The disappearance of one protein in a monosomic F1 is thus hidden by others. The greatest intervarietal variability was found in the 65 Kd zone: bands were predominantly controlled by chromosomes from genome B. During the differentiation of endosperm proteins, mutations involving amino acid substitutions were more frequent than changes in size. A technique, like A-PAGE, that separates gliadins by its charge/mass ratio, is able to demonstrate such intervarietal differ-

ences. For each variety, between 10 and 14 bands could be assigned to chromosomes. The more easily handled zones are ω and α. In contrast, the γ and especially the β zone do not allow for identification of bands in different monosomic F1s by a decrease in relative staining intensity. The results can be explained, if such bands represent several proteins with their genetic control located on different chromosomes. In such a case the disappearance of $^2/_3$ of the relative activity of one of them cannot be observed and the use of two-dimensional (two-pH) electrophoresis has to be applied (Sanz et al. 1988).

We want to emphasize that some bands (like ω-6, ω-7 and γ-6) are related to different chromosomes in different varieties. In this case, the use of substitution lines do not permit the unequivocal location of genes arising from donor varieties.

Two possible hypothesis could explain the abnormalities found in Holdfast mono-6B:

(1) Gliadins of cv 'Holdfast' could be controlled by more than one chromosome, and proteins codified by structural genes in chromosome 6B could be post-transcriptionally modified. This could explain the appearance of new bands with greater molecular weights substituting the normal gliadins.

(2) The mono-6B strain lost some structural genes of the ω-gliadins and would thus not turn up in the electrophoretical pattern. The strain seems, however, to have some unchanged gliadin genes located on chromosomes 1A and 1B.

The present results do not provide enough evidence in favour of one or another hypothesis. More information to solve this problem will come from analysis of endosperm proteins by two-dimensional electrophoresis (Sanz et al. 1988).

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