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Restriction fragment analysis of 'null' forms at the *Gli-1* loci of bread and durum wheats

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Summary. Wheat accessions lacking some of the ω- and γ-gliadin components encoded by the *Gli-1* loci on the short arm of chromosome 1D in bread wheat and chromosome 1A in durum wheat were studied by two-dimensional polyacrylamide gel electrophoresis and restriction fragment analysis. Digested genomic DNAs of 'normal' and 'null' forms were probed with a cDNA clone related to ω-/γ-gliadins and with a genomic clone encoding an LMW subunit of glutenin. The hybridisation patterns with the ω-/γ-gliadin probe were similar to those of cvs 'Chinese Spring' and 'Langdon' used as standards for bread and durum wheats, respectively, but several restriction fragments located on the 1D chromosome of bread wheat and the 1A chromosome of durum wheat were absent in the 'null' forms. In addition, specific LMW glutenin fragments encoded by the same chromosomes were also absent in the 'null' forms, suggesting that simultaneous deletions of blocks of genes for both ω-/γ-gliadins and LMW glutenins had occurred. Comparisons of the protein and RFLP patterns enabled some proteins to be mapped to specific restriction fragments.

Key words: 'Null' forms – RFLP – Gliadins – LMW glutenins – Deletion

Introduction

The *Gli-1* loci on the group 1 chromosomes of wheat are complex families of genes encoding three groups of proteins: the γ-type gliadins, the low molecular weight (LMW) subunits of glutenin (both sulphur-rich prolamins) and the ω-type gliadins (S-poor prolamins) (Payne 1987). We have reported detailed studies of these loci in bread and durum wheats in which restriction fragment analysis of euploid and aneuploid lines was carried out using DNA probes (Sabelli and Shewry 1991). The presence of homologous sequences in γ- and ω-gliadins required the use of specific subclones and hybridisation conditions in order to discriminate between the two classes of genes.

In the present paper we use the same approach to analyse a series of 'null' forms at the *Gli-1* loci in bread and durum wheats, which were identified by screening germ plasm collections for the absence of specific gliadin proteins (Lafiandra et al. 1987). The results provide new information on the structures of the *Gli-1* loci as well as on the nature of the deficiencies themselves.

Materials and methods

Plant material was from the wheat collection of the Germ plasm Institute, National Research Council of Italy, Bari. Bread wheat accessions MG27079, MG27094, MG27081 and MG27104 were collected in Nepal in 1979 (Erskine et al. 1979), and durum wheat accession MG41078 in Algeria (Lafiandra et al. 1987). For convenience, the 'normal' and 'null' forms of the above accessions will be referred to as (+) and (−) respectively. *Triticum aestivum* cv 'Chinese Spring', its aneuploid nullisomic-tetrasomic lines and *Triticum durum* cv 'Langdon' were used as standards.

Two-dimensional (2-pH) polyacrylamide gel electrophoresis of gliadins extracted from single seeds was performed according to the method of Lafiandra and Kasarda (1985). Methods for DNA isolation and Southern blot analysis and the probes for the γ-type gliadins (pKAP1a) (Bartels et al. 1986) and LMW glutenin subunits (pLMWTG2) (Colot et al. 1989) are as described in Sabelli and Shewry (1991). All hybridisation experiments were carried out at medium stringency.

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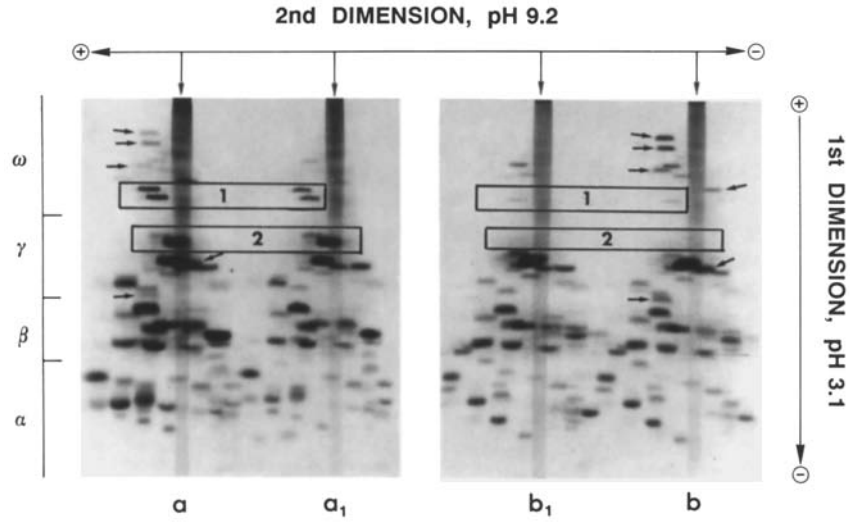

Fig. 1. Two-D (2-pH) gel electrophoresis analyses of gliadins of bread wheat accessions MG27079 and MG27094. 'Normal' and 'null' form of the same accession are analysed on the same gel. *a* and a_1 represent MG27079 (+) and (−), respectively; *b* and b_1 MG27094 (+) and (−), respectively. *Arrows* indicate the $\omega-/\gamma-$ components that are absent in the 'null forms'. *Boxes 1* and *2* are discussed in the text

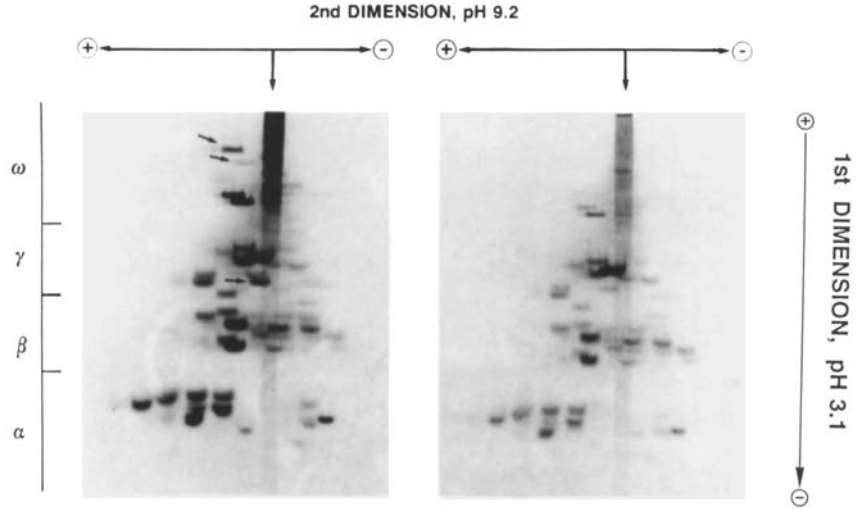

Fig. 2. Two-D (2-pH) gel electrophoresis analyses of gliadins of durum wheat accession MG41078. *Arrows* indicate the $\omega-/\gamma-$ components of the 'normal' form (*left*) that are missing in the corresponding 'null' form (*right*)

Results

Absence of gliadin components in the 'null' lines

The patterns of gliadins from 'normal' and 'null' forms of the bread wheat accessions MG27079 and MG27094 separated by two-dimensional (2-pH) polyacrylamide gel electrophoresis are shown in Fig. 1. The patterns of MG27081 and MG27104 were identical to those of MG27079 and MG27094, respectively, and are not shown. The 'normal' and 'null' forms of the durum wheat accession MG41078 are shown in Fig. 2. In all cases the 'null' forms lack specific ω- and γ-gliadin components, which are indicated by arrows. In the bread wheat lines these components had the same electrophoretic properties as certain chromosome 1D-encoded ω- and γ-gliadins of cv 'Chinese Spring' (Fig. 1), while the 'null' line of durum wheat lacks components with similar migration to certain chromosome 1A-encoded ω- and γ-gliadins of 'Langdon' (Fig. 2) (Lafiandra et al. 1987).

Restriction fragment analysis of MG27079 and MG41078

ω-/γ-Type sequences. Although pKAP1a encodes a γ-type gliadin (Bartels et al. 1986), previous analysis showed that it hybridised to γ-gliadin and ω-gliadin genes under conditions of medium stringency (Sabelli and Shewry 1991). The hybridisation patterns of pKAP1a to bread wheat (left and centre) and durum wheat (right) DNAs digested with *Hin*dIII, *Eco*RI and *Bam*HI are shown in Fig. 3. The central parts of this figure show the hybridisation patterns on gels which have been run for a long time in order to improve the resolution of large frag-

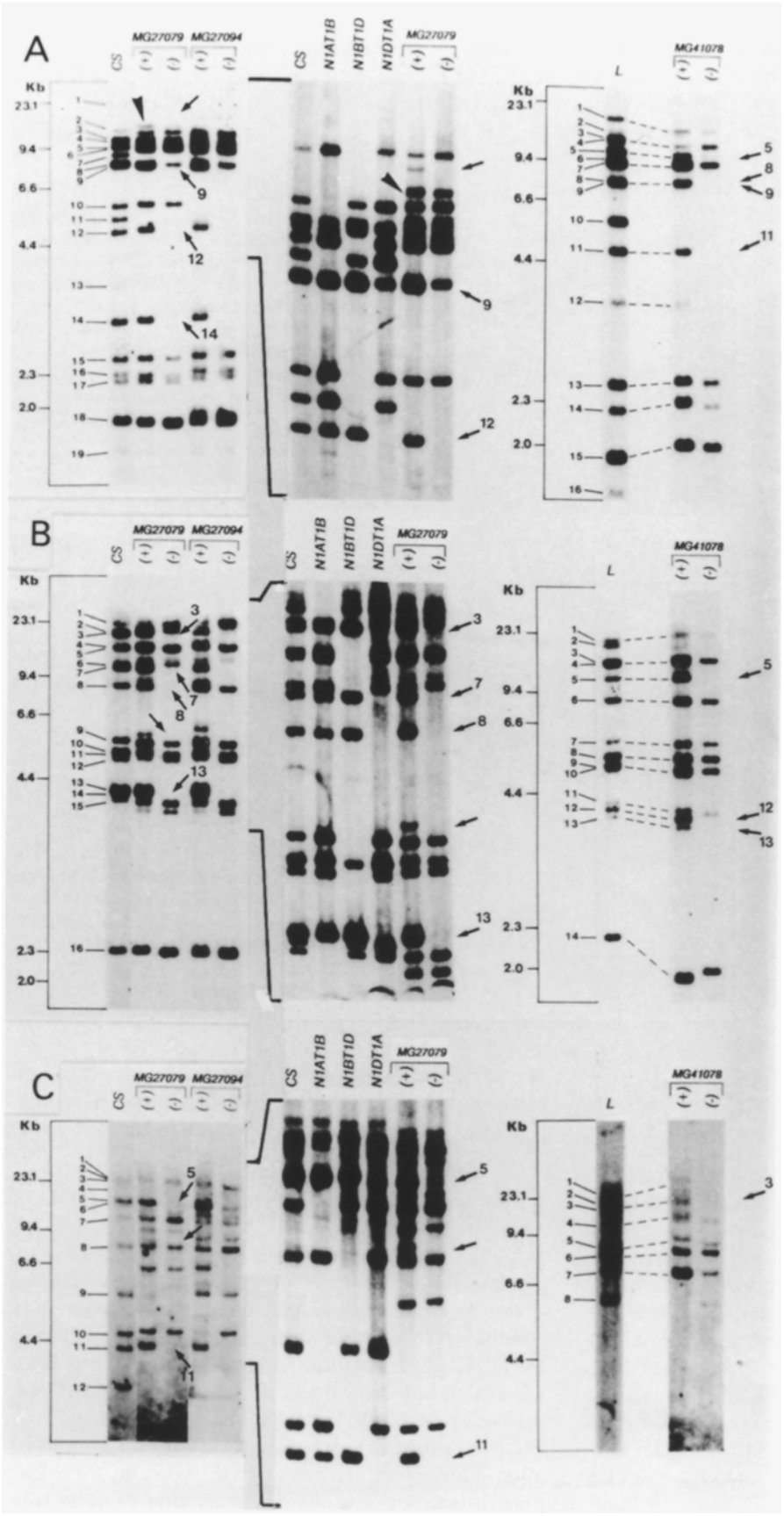

Fig. 3A–C. Restriction analysis of 'normal' and 'null' forms of bread and durum wheat DNAs digested with *Hin*dIII (**A**), *Eco*RI (**B**) and *Bam*HI (**C**) and probed with the $\omega-/\gamma-$ clone pKAP1a. Bread wheat cv 'Chinese Spring' (*CS*), the group 1 nulli-tetra aneuploids and cv 'Langdon' (*L*) are used as standards. The central parts of the figure show long gel analyses. *Arrows* indicate the restriction fragments that are absent in the 'null' forms (−) of the bread wheat accession MG27079 and the durum wheat accession MG41078. They are numbered according to corresponding fragments of 'Chinese Spring' and 'Langdon', respectively. *Unnumbered arrows* indicate fragments of unknown chromosomal locations. The *arrowheads* in **A** indicate the fragment which may contain genes encoding the γ-gliadins in box 2 (Fig. 1)

ments. 'Chinese Spring' and its group 1 aneuploid lines are used as standards for bread wheat, and 'Langdon' for durum wheat. Broken lines between 'Langdon' and accession MG41078 indicate fragments with identical relative mobilities. The restriction fragments are numbered according to Sabelli and Shewry (1991). The hybridisation patterns of pKAP1a to the normal forms of MG27079 and MG41078 are broadly similar to those of 'Chinese Spring' and 'Langdon', respectively, although some polymorphism is present. For example, *Hin*dIII fragments corresponding to 7 and 11 in 'Chinese Spring' are absent from MG27079, while bands corresponding to 10 and 16 of 'Langdon' are absent from MG41078 and the band corresponding to 3+4 is less intense. MG27079 also shows several fragments that are not apparently present in 'Chinese Spring'. Comparisons of the 'normal' and 'null' forms of MG27079 and MG41078 demonstrate that some hybridising fragments are absent from the latter. These are summarised in Table 1 and are indicated by numbered arrows in Fig. 3. Comparisons with "standard" DNAs indicate that these sequences are located on chromosome 1D in MG27079 and on chromosome 1A in MG41078 (Table 1). In addition, the 'null' form of MG27079 lacks single fragments in all three digests that do not correspond to fragments present in 'Chinese Spring'. These could not be mapped to a chromosome and are indicate by unnumbered arrows in Fig. 3. The 'normal' and 'null' lines of these accessions also differ in the intensities or relative mobilities of some restriction fragments; for example the relative mobilities of fragments 11 and 14 in MG41078 DNAs digested with *Eco*RI.

LMW glutenin sequences.

The same membranes were also hybridised with the LMW glutenin probe pLMWTG2 as shown in Fig. 4 (A, B and C). Sabelli and Shewry (1991) showed that this probe probably hybridises only to LMW glutenin genes. The patterns of hybridisation to MG27079 and MG41078 were again similar to those of 'Chinese Spring' and 'Langdon', respectively, with the 'normal' and 'null' forms showing minor differences in intensity. The 'null' lines also differed in the absence of certain restriction fragments, indicated by numbered arrows in Fig. 4. Comparisons with 'Chinese Spring' and 'Langdon' indicate that most of these sequences are located on chromosome 1D of MG27079 and chromosome 1A of MG41078 (Table 1). In addition, two fragments in MG27079 (no. 8 with *Hin*dIII and no. 3 with *Eco*RI) correspond to fragments located on chromosome 1B in 'Chinese Spring', while the 'null' form of MG41078 also contained some fragments not apparently present in the 'normal' form (indicated by * in Fig. 4).

Table 1. Restriction fragments absent from the 'null' forms, relative to the patterns of 'Chinese Spring' and 'Langdon'

pKAP1a								
*Hin*dIII			*Eco*RI			*Bam*HI		
MG27079	MG27094	MG41078	MG27079	MG27094	MG41078	MG27079	MG27094	MG41078
9(D)	9(D)	5(A)	3(D)	3(D)	5(A)	5(D)	5(D)	3(A)
12(D)	12(D)	8(A)	7(D)	7(D)	12(A)	11(D)	11(D)	
14(D)	14(D)	9(A)	8(D)		13(A)			
		11(A)	13(D)	13(D)				
pLMWTG2								
*Hin*dIII			*Eco*RI			*Bam*HI		
MG27079	MG27094	MG41078	MG27079	MG27094	MG41078	MG27079	MG27094	MG41078
2(D)		6(A)	3(B)?	3(B)?	2(A)	4(D)	4(D)	5(A)
8(B)?			7(D)	7(D)		6(D)?	6(D)?	
9(D)	9(D)?		9(D)	9(D)		10(D)	10(D)	
13(D)	13(D)							
15(D)	15(D)							

Chromosomal locations of the numbered restriction fragments are indicated by the letters in parentheses

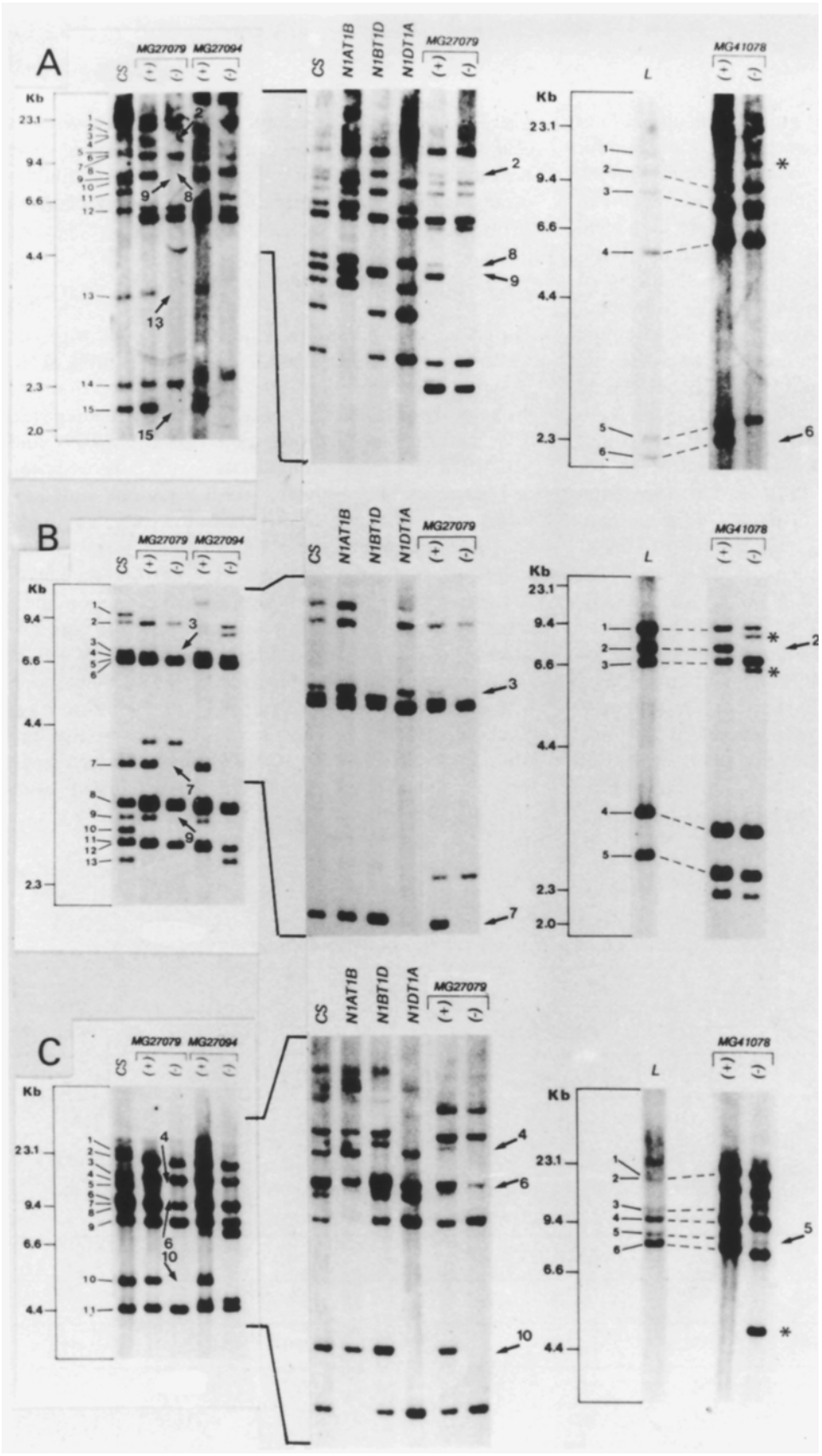

Fig. 4A–C. Restriction fragment analysis of 'normal' and 'null' forms of bread and durum wheat DNAs probed with the LMW glutenin clone pLMWTG2. Samples and restriction enzymes are as in Fig. 3. *Arrows* indicate the restriction fragments absent in the 'null' forms of MG27079 and MG41078 numbered according to corresponding fragments in 'Chinese Spring' and 'Langdon', respectively. Some fragments present in the 'null' form of the durum wheat accession MG41078 are absent from the 'normal' form and are indicated by *

Restriction fragment analysis of other bread wheats

The hybridisation patterns of MG27094 with pKAP1a and pLMWTG2 are compared with those of 'Chinese Spring' and MG27079 in Figs. 3 and 4, respectively. The overall patterns were similar to those of MG27079, although some polymorphism was observed. Some fragments were absent from the 'null' form of only one of the accessions, while others were present in the 'null' and 'normal' forms of one accession but in neither form of the other. In addition, some fragments were present only in the 'null' form of one accession but absent from both forms of the other.

Further variation occurred in the intensity of hybridisation, both between the two accessions and between 'normal' and 'null' lines within one accession. However, for both probes the overall sequence copy number was consistent with that reported for 'Chinese Spring' by Sabelli and Shewry (1991). The hybridisation patterns of MG27081 and MG27104 were similar to those of MG27079 and MG27094, respectively, which is consistent with their protein phenotypes.

Discussion

Several authors have reported electrophoretic analyses of 'null' lines lacking specific gliadin components (Wrigley and Shepherd 1974; Autran 1975; Damania et al. 1983; Payne et al. 1984a, b; Pogna et al. 1985; Lafiandra et al. 1989) including Lafiandra et al. (1987) who observed the absence of ω- and γ-gliadins in lines of bread and durum wheat. They assigned the missing proteins to genes on chromosome 1D of bread wheat and 1A of durum wheat and proposed that the deficiencies might be due to the deletion of genes as well as the stable repression of gene expression. The analyses reported here demonstrate that genomic fragments hybridising to pKAP1a (a γ-gliadin clone) are absent from the 'null' lines and that most of these indeed appear to be located on chromosomes 1A and 1D in the durum and bread wheats, respectively (Table 1). Previous studies have shown that pKAP1a cross-hybridises to ω-gliadin genes under conditions of medium stringency, and some of the hybridising fragments which were absent from the 'null' lines could therefore encode ω-type gliadins. In fact, several of the restriction fragments which are absent from the 'null' lines do correspond in mobility to putative ω-gliadin genes in the standard lines 'Chinese Spring' and 'Langdon' (Sabelli and Shewry 1991), and these are underlined in Table 1. There is, however, disagreement between the proportions of γ-type and ω-type fragments identified with the three enzymes (there are, for example, no ω-type fragments in the *Hin*dIII digests of bread wheat), which indicates that the situation is more complex. *Hin*dIII fragment 9 is present in high copy number (about eight per haploid genome) in 'Chinese Spring' and shows a dramatically decreased intensity of hybridisation with the γ-gliadin specific probe pKAP1a-3′ (Sabelli and Shewry 1991). It is possible, therefore, that this band contains several fragments encoding γ- and ω-type prolamins in 'Chinese Spring' and in MG27079 and MG27094 ('normal' forms). Because the fragment size (7.4 kb) is sufficient to contain several genes we cannot conclude whether the γ- and ω-type sequences are present on the same or different fragments.

The 'null' forms of bread and durum wheat also lacked fragments hybridising to pLMWTG2, a probe that appears to hybridise only to LMW subunit genes (Sabelli and Shewry 1991). Comparisons with 'Chinese Spring' and 'Langdon' indicate that most of these fragments are located on chromosome 1A in the durum wheat and 1D in the bread wheats, but several *Hin*dIII and *Eco*RI fragments in the bread wheats appear to be located on 1B. None of the *Bam*HI fragments from the same lines corresponded to 1B-derived fragments of 'Chinese Spring', so they may be polymorphic variants of 1D fragments that correspond in mobility to 1B fragments of 'Chinese Spring'. The LMW subunits of glutenin are poorly resolved by SDS-PAGE of total prolamin preparations, but such analyses showed simpler patterns in the 'null' forms (results not shown), which are consistent with the absence of some proteins.

The absence of fragments encoding ω-/γ-gliadins *and* LMW glutenin subunits suggests that considerable amounts of DNA have been deleted from the 'null' lines. We do not know whether this has occurred by single or multiple deletion events, but the former seems more likely as multiple events within a single locus must occur very rarely. The lines did not exhibit any differences in chromosome number (Lafiandra et al. 1987), but detailed karyotype analyses have not been carried out.

Comparison of the hybridisation patterns of MG27079 and MG27094 (Figs. 3 and 4) showed restriction fragment polymorphism consistent with that observed in the protein patterns (Fig. 1). By combined evaluation of the DNA and protein polymorphisms it is possible to tentatively assign some proteins to specific DNA fragments. In particular the two major chromosome 1B-encoded ω-gliadins present in MG27079 (indicated by box 1 in Fig. 1) are absent from MG27094 and are probably encoded by genes on *Hin*dIII fragment 10, *Eco*RI fragment 6 (the absence of this fragment is clearly seen on the autoradiograph, but is not clear in the reduced photograph in Fig. 3) and the additional *Bam*HI fragment between 7 and 8. Similarly, the slowest γ-gliadins of MG27079 (box 2 in Fig. 1) are absent from MG27094 and 'Chinese Spring' (Lafiandra et al. 1987) and are possibly encoded by genes on the fragment indicated by arrowheads in Fig. 3A.

In conclusion, comparisons of the 'null' and 'normal' forms of bread and durum wheats by combined protein and DNA analyses have demonstrated that all the 'null' forms appear to derive from deletions of structural genes on chromosomes 1A (durum wheat) and 1D (bread wheat). Comparisons of the bread wheat accessions also allow us to allocate individual gliadin proteins to DNA restriction fragments, which has not been possible previously due to the high degree of polymorphism and structural homology of the gliadin gene families. The 'null' forms identified in this and previous studies (Lafiandra et al. 1987, 1989; Payne et al. 1984a) will also facilitate the analysis of wheat gluten structure and functionality by allowing the construction of isogenic lines to correlate the presence or absence of specific proteins with various characteristics.

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