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RFLP patterns of gliadin alleles in *Triticum aestivum* L.: implications for analysis of the organization and evolution of complex loci

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Abstract A correspondence between RFLP patterns and gliadin alleles at the *Gli-1* and *Gli-2* loci was established in a set of 70 common wheat (*T.aestivum* L.) cultivars using γ-gliadin (K32) and α-gliadin (pTU1) specific probes. All *Gli-B1* and *Gli-D1* alleles which differed in encoded γ-gliadins showed definite RFLP patterns after hybridization with the K32 probe. Two groups of *Gli-B1* alleles, *Gli-B1b*-like and *Gli-B1e*-like, were identified, and these could originate from distinct genotypes of the presumptive donor of the B-genome. Intralocus recombination and/or gene conversion as well as small deletions, gene silencing and gene amplification were assumed to be responsible for the origin of new gliadin alleles. Silent γ-gliadin sequences were shown to exist in all of the genotypes studied. K32 also differentiated *Gli-A1a* from all other *Gli-A1* alleles as well as the *Gli-B1l* allele in cultivars carrying the 1B/1R (wheat/rye) translocation. pTU1 was shown to recognize several *Gli-A2* alleles, but not the *Gli-B2* or *Gli-D2* alleles. Moreover, this probe hybridized to chromosome 1R sequences suggesting the existence of rye gene(s), probably silent, for α-gliadin-like proteins on chromosome 1R.

Key words RFLP · Gliadin alleles · Organization of *Gli-1* loci · *Gli-2*-type sequences on chromosome 1R Common wheat

Introduction

Highly polymorphic, alcohol-soluble seed storage proteins, gliadins, from a common wheat genotype can be electrophoretically separated into more than 40 individual components (Wrigley and Shepherd 1973; Payne et al. 1982). Gliadin-encoding loci (*Gli*) are located on the short arm of the chromosomes of the first (*Gli-1*) and sixth (*Gli-2*) homoeologous groups (Payne et al. 1982). Each locus is complex and controls the synthesis of a group ("block") of jointly inherited gliadins (Sozinov and Poperelya 1980). Multiple alleles (up to 25 at each locus) result in gliadins being the most polymorphic genetic markers in wheat. Alleles differ in number, relative electrophoretic mobility and intensity of the encoded gliadin protein bands (Sozinov and Poperelya 1980; Metakovsky 1991). However, at the DNA level, the allelic differences at each *Gli* locus have not been described. Analysis of the organization of these loci is limited by the low frequency of intralocus recombination and by extensive sequence homology existing between some gliadin genes (Harberd et al. 1985; Okita et al. 1985; Sabelli and Shewry 1991).

There are 100 or more gliadin-encoding sequences belonging to at least three main families (α-, γ-, and ω-genes), which have been identified primarily on the basis of their N-terminal protein sequences (Kreis et al. 1985). Genes of each group have specific primary sequences and domain organization (see Kreis et al. 1985; Shewry and Tatham 1990, for reviews) and differ by single nucleotide substitutions and small deletions/insertions in their coding sequences (Kasarda et al. 1983; Okita et al. 1985; Sumner-Smith et al. 1985; Scheets and Hedgcoth 1988). Gliadin pseudogenes have also been described (Rafalski 1986; Anderson 1991).

The restriction fragment length polymorphism (RFLP) technique (Beckmann and Soller 1986; Sharp et al. 1989) may be useful for gliadin allele identification and might give new information on the organization of the *Gli* loci (Vaccino et al. 1993).

The aim of the work presented here was to reveal gliadin alleles in convenient RFLP patterns and to obtain new data about the organization and evolution of the *Gli* loci at the DNA level.

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Materials and methods

Four lines and 54 cultivars of common wheat (*Triticum aestivum* L.) grown in Italy were studied (Table 1). Cultivar 'Chinese Spring' and

Table 1 *T. aestivum* genotypes and, in brackets, their allelic composition at the *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* loci

Adria	(*a, g, k, g, o, j*)	Liocorno	(*m, b, k, g, o, m*)
Alpe	(*b, b, b, b, y, b*)	Loreto	(*a, c, a, c, c, u*)
Aquileja	(*a, k, d, g, o, r*)	Maestra	(*f, l, b, b, c, a*)
Ardito	(*a, f, f, g, ?, j*)	Manital	(*a, b, k, g, c, t*)
Arquà	(*a, b, k, g, o, r*)	Mara	(*a, g, k, g, o, r*)
Artù	(*a, g, k, g, p, a*)	Marzotto	(*a, g, k, g, o, r*)
Asiago	(*s, b, k, b, o, r*)	Mec	(*a, g, k, e, h, g*)
Aurelio	(*a, e, b, g, o, a*)	Mentana	(*a, k, a, e, h, j*)
Bolero	(*f, f, b, l, ?, h*)	Mirtos	(*a, b, k, b, b, b*)
Brasilia	(*a, e, a, e, v, r*)	N. Strampelli	(*f, k, b, h, y, j*)
Centauro	(*a, e, k, g, y, j*)	Nobel	(*a, g, k, e, h, g*)
Chiarano	(*a, f, d, o, ?, j*)	Oderzo	(*o, e, a, g, p, m*)
Costantino	(*a, m, k, g, o, r*)	Orso	(*a, e, f, g, j, j*)
Eridano	(*a, e, b, c, v, c*)	Pandas	(*a, m*, f, g, o, b*)
Etruria	(*o, g, k, b, b, b*)	Pegaso	(*a, k, f, b, o, r*)
Farnese	(*a, e, f, g, j, b*)	Produttore	(*a, f, h, e, e, j*)
Farneto	(*a*, b, a, f, c, m*)	Sal 6	(*a, c, b, p, ?, r*)
Fiocco	(*f, l, b, b, c, a*)	Sal 8[a]	(*a, r*, j+k, g, c, n*)
Fiorello	(*a, e, f, g, ?, u*)	Sal 57	(*a, k, a, g, o, a*)
Frassineto	(*f, g, k, o, o, n*)	Sal 180	(*b, b, b, p, ?, a*)
Gallo	(*a, q, b, z, j, j*)	Saliente	(*a, b, f, b, b, b*)
Gemini	(*a, e, d, g, o, j*)	Salmone	(*l, s, b, e, aa, o*)
Gladio	(*a, g, b, b, b, b*)	Salvia	(*a, g, k, g, o, b*)
Golia	(*a, e, k, g, c, t*)	Santerno	(*a, e, d, g, v, n*)
Granarolo	(*a, g, k, g, j, r*)	Saul	(*o, k, b, o, o, r*)
Impeto	(*a, g, k, g, o, a*)	Spada	(*?, n*, b, c, o, m*)
Inallettabile 96	(*o, f, b, p, c, g*)	S.Pastore	(*a, k, d, g, j, a*)
Irnerio	(*a, e, k, g, v, n*)	Tiberio	(*a, d, a, b, ?, a*)
Leopardo	(*a, k, k, g, v, a*)	Veronese	(*f, e, a, e, e, a*)
		Ch. Spring	(*a, a, a, a, a, a*)

[a] 'Sal 8' is a mixture of two biotypes differing at *Gli-D1*

its ditelosomic (DT1AS and DT1AL) and nullitetrasomic (N1BT1D, N1DT1B) lines were used to allocate DNA fragments to individual chromosomes. Both one-dimensional acid (pH 3.1) polyacrylamide gel electrophoresis of gliadin proteins (APAGE) and the RFLP technique (six probe/enzyme combinations) were applied to this set of genotypes. Twelve other Italian and international cultivars were analysed with different probe/enzyme combinations in order to confirm the RFLP compositions of several rare alleles.

The APAGE procedure and the gliadin allele identification were performed according to Metakovsky and Novoselskaya (1991) and Metakovsky (1991), respectively.

DNA was extracted from 7-day-old seedlings according to a modified CTAB procedure (Murray and Thompson 1980). DNA digestion, gel electrophoresis, blotting and hybridization were carried out as described by Gebhardt et al. (1989). After hybridization, membranes were washed three times in 1×SSC, 0.1% SDS (20 ml, 65°C, 15 min each time), two times in 5 m*M* sodium phosphate, 1 m*M* EDTA, 0,2% SDS, pH 7.0 (800 ml, room temp, 15 min) and then exposed (–70°C, 3–10 days) to Kodak X-OMAT AR films using an intensifying screen.

The K32 probe (pTag 1436), a cDNA clone recognizing γ-gliadin sequences (Bartels et al. 1986), was obtained from R. Thompson (Max Planck Institut, Köln). The pTU1 probe provided by O. Tanzarella (Università della Tuscia, Viterbo) was derived from a *T.urartu* genomic library and recognizes *Gli-2* DNA sequences (D'Ovidio et al. 1992). It was previously found that relatively high levels of polymorphism could be detected using the four-cutter enzymes *Hae*III, *Rsa*I and *Taq*I (Vaccino et al. 1993). Each membrane was independently hybridized with both probes.

The correlation between presence/absence of certain DNA fragments and particular gliadin alleles was analysed. Reproducible, well-identified RFLP bands were considered. All correlations found were doubtless, so that the presence of an allele is always accompanied by the presence of definite fragments. The only exception was cv 'Liocorno' at *Gli-B1* (see Results). The statistical analysis of data was performed as described by Castagna et al. (1994).

Results

APAGE analysis of gliadin alleles

Amongst the 58 Italian common wheat genotypes and cv 'Chinese Spring' analysed, 8, 15, 6, 11, 14, and 12 alleles were found at the *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* loci, respectively. Each allele encodes a different combination of gliadin polypeptides (Fig. 1). Some *Gli-1* alleles differed only for the presence/absence (for example, *Gli-D1b* and *Gli-D1f*) or relative mobility (*Gli-B1q* and *Gli-B1n*) of ω-gliadins. Other alleles differed, however, only in the presence/absence or mobility of γ-gliadins (*Gli-B1e* and *Gli-B1f*, or *Gli-D1a*, *Gli-D1f* and *Gli-D1k*) (Fig. 1).

Two main types of α-gliadins can be described in APAGE patterns (Kasarda 1980): the 'Cheyenne'-type (encoded by alleles *Gli-A2c*, *Gli-A2g*, and several others in the set of genotypes studied) and the 'Chinese Spring'-type (*Gli-A2a* and others, Fig. 1).

RFLP analysis using the K32 probe

Using the *Hae*III, *Taq*I, and *Rsa*I restriction enzymes, 31, 37, and 30 polymorphic bands 0.35–0.75 kb, 0.45–1.2 kb and 0.6–0.95 kb in size, respectively, were scored in the RFLP patterns (Fig. 2). The upper part of each membrane, which contained fragments larger than 1 kb, gave less reproducible patterns and had a more intense background; bands 3′, 19′, 21 in Fig. 2A and 1 and 2 in Fig. 2B were not scored, being difficult to follow.

Gli-A1 *locus*

Six bands in *Hae*III digests were assigned to chromosome arm 1AS in cv 'Chinese Spring' using its aneuploid lines; some minor differences between euploid and the ditelosomic 1AL line were also visible in the *Taq*I digest (Fig. 3). 1AS-derived *Hae*III bands 6 and 8 (Fig. 2A), absent in the DT1AL line of 'Chinese Spring', were polymorphic in the set of cultivars: the presence or absence of these bands separated all cultivars into two groups, those having alleles *Gli-A1a* or *Gli-A1l*, and all the others, respectively.

Gli-B1 *locus*

Analysis of the nullitetrasomic lines of 'Chinese Spring' in *Hae*III, *Taq*I and *Rsa*I digests allowed us to allocate at least 2, 5 and 5 strong bands to chromosome 1B, respectively (Fig. 3). Several other polymorphic fragments were

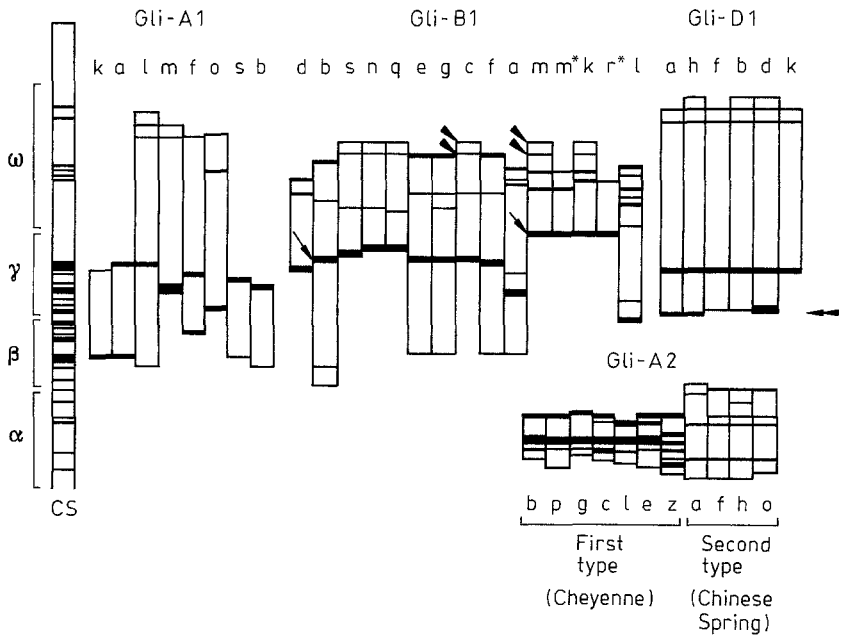


Fig. 1 Schemes of allelic blocks of gliadin bands encoded by the *Gli-A1*, *Gli-B1*, *Gli-D1*, and *Gli-A2* loci in the set of cultivars fractionated by APAGE. CS cv 'Chinese Spring', α-, β-, γ- and ω-gliadins are indicated in the patterns. Some particular bands are shown: γ40 (*short arrow*), γ43.5 (*long arrow*), *Gli-B5*-encoded ω-gliadins (*arrowheads*). Alleles at the *Gli-A2* locus are divided into two different types: 'Cheyenne' (first type) and 'Chinese Spring' (second type)

revealed in the set of cultivars studied (Fig. 2). A good correspondence between the presence of definite fragments and the *Gli-B1* alleles was found (Table 2). Alleles differing only in ω-gliadins, such as *Gli-B1e* and *Gli-B1g* (Fig. 1), had identical RFLP patterns of scored bands, indicating the specificity of the K32 probe to γ-gliadin genes.

For example, cultivars containing γ-gliadin 40 encoded by *Gli-B1k* and *Gli-B1m* (Fig. 1) had bands 17–20, 33 and 35 in their *Taq*I patterns (Table 2 and Fig. 2B, lanes 14 and 15). The same DNA fragments occurred in cultivars with *Gli-B1o* and *Gli-B1p* alleles, which also code for γ40 (data not shown). Therefore, this combination of bands is probably a characteristic of *Gli-B1* alleles encoding the γ40 polypeptide. In contrast, alleles which code for γ-gliadin 43.5 (Fig. 1) were divided into two distinct groups. One group is represented by *Gli-B1b* and some other alleles; the second includes *Gli-B1e*-like alleles (*Gli-B1e*, *Gli-B1g*, *Gli-B1c* and *Gli-B1f*) (Fig. 2B, lanes 4–7; see Fig. 1). Alleles *Gli-B1s* (Fig. 2B, lane 3), *Gli-B1n* (lane 18) and *Gli-B1q* shared some bands with *Gli-B1b*, while *Gli-B1a* had 2 bands specific for *Gli-B1e*-like alleles (Table 2). The presence of band 36 and the absence of band 33 differentiated *Gli-B1f* from other *Gli-B1e*-like alleles (Fig. 2B, lanes 8 and 9).

Similar results were obtained in *Rsa*I digests (Fig. 2C, Table 2). The unpredicted difference between *Gli-B1b* and *Gli-B1e*-like alleles (judging from APAGE where both alleles have apparently identical γ-gliadin) was confirmed. One exception from RFLP/gliadin allele congruity was found: bands 21 and 23 assigned to *Gli-B1b* had a slightly decreased mobility in cultivar 'Liocorno' when compared with 8 other *Gli-B1b* cultivars analysed (Fig. 2C, lanes 11 and 12). These fragments may derive from silent genes.

*Hae*III provided an additional remark for this locus. The occurrence of bands 4 and 5 in *Gli-B1c* (Fig. 2A, lane 13), but not in *Gli-B1g* and *Gli-B1e* (lanes 15 and 17) differentiated these 3 alleles into two groups (Table 2). As shown in Fig. 1, the *Gli-B1c* allele, in contrast to *Gli-B1e* and *Gli-B1g*, includes two ω-gliadins that have recently been found to be encoded by the *Gli-B5* locus (Pogna et al. 1993). This suggests that DNA fragments 4 and 5 contained sequences linked to, or derived from, the *Gli-B5* locus. The absence of these fragments in *Gli-B1m** supports this hypothesis (Figs. 1; 2A, lanes 10 and 16). Moreover, bands 4, 5 and *Gli-B5*-encoded ω-gliadins were present in *Gli-B1q* and *Gli-B1n* altogether (Table 2).

The peculiarity, at the protein level, of allele *Gli-B1l* coding for secalins (Metakovsky 1991) was confirmed at the DNA level for each of the three enzymes used (Fig. 2A, lane 2; Fig. 2B, lane 10; Fig. 2C, lane 9; Table 2).

In conclusion, there are two main groups of alleles (Fig. 4). The first group consists of *Gli-B1b*, *Gli-B1n*, *Gli-B1q* and *Gli-B1s*. The core of the second group includes four *Gli-B1e*-like alleles. *Gli-B1a* is an obvious derivative of the second group whereas *Gli-B1k* and *Gli-B1m* are distantly related to the first group.

Gli-D1 *locus*

In the *Rsa*I and *Taq*I patterns, the fragments assigned to *Gli-D1* were located in "zone A" (Fig. 2B, C). In this zone, we found 4 RFLP patterns, which corresponded to the four variants of the γ-gliadin marked with a double arrowhead in Fig. 1.

Cultivars with *Gli-D1a* and *Gli-D1h* had specific groups of bands in "zone A" (Figs. 5; 2B, lanes 6, 9 and 19; 2C, lanes 5, 8, 18 and 19). These 2 alleles can be distinguished in *Hae*III digests by the absence or presence, respectively, of bands 12 and 14 (data not shown).

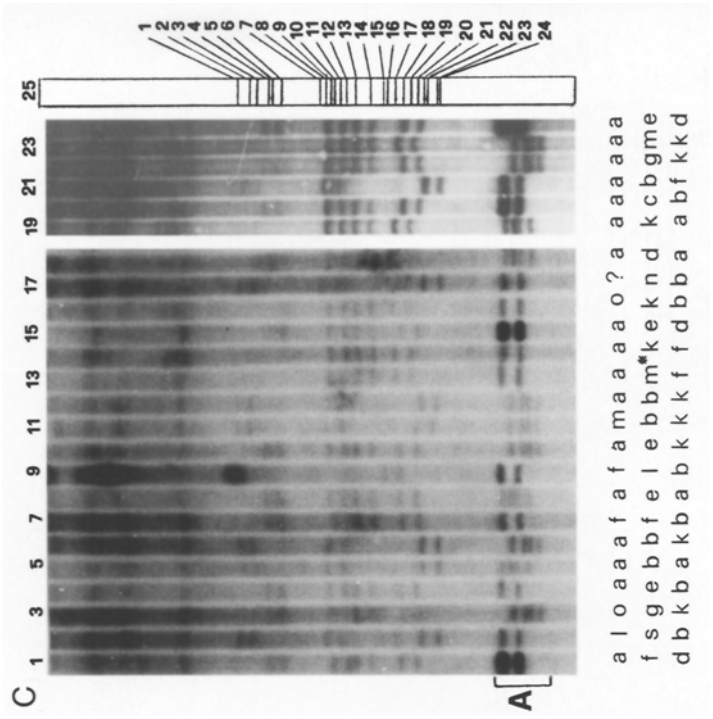


Fig. 2 Southern blot of common wheat DNAs restricted with *Hae*III (**A**), *Taq*I (**B**), *Rsa*I (**C**) and hybridized to probe K32. **A** *lanes 2–19* 'Maestra', 'Pegaso', 'Etruria', 'Gallo', 'Granarolo', 'Irnerio', 'Oderzo', 'Orso', 'Pandas', 'Sal 8', 'Sal 57', 'Sal 6', 'Saliente', 'Salvia', 'Costantino', 'Gemini', 'Leopardo', 'Salmone', **B** *lanes 2–19* 'Chiarano', 'Salmone', 'Etruria', 'Eridano', 'Farneto', 'Arquà', 'Bolero', 'Brasilia', 'Fiocco', 'Golia', 'Liocorno', 'Mirtos', 'Pandas', 'Pegaso', 'Santerno', 'Saul', 'Spada', 'Tiberio', **C** *lanes 1–24* 'Chiarano', 'Salmone', 'Etruria', 'Eridano', 'Farneto', 'Arquà', 'Bolero', 'Brasilia', 'Fiocco', 'Golia', 'Liocorno', 'Mirtos', 'Pandas', 'Pegaso', 'Santerno', 'Saul', 'Spada', 'Tiberio', 'Sal 57', 'Sal 6', 'Saliente', 'Salvia', 'Costantino', 'Gemini'. **A,B** *lane 1* scheme of bands assigned to chromosome 1R in cultivars with 1B/1R translocation, **A–C** *lanes 20, 20* and *25*, respectively: scheme of all polymorphic bands. *Bands 21, 23, 28* and *29* in **B** are specific for cv 'Chinese Spring' (not shown)

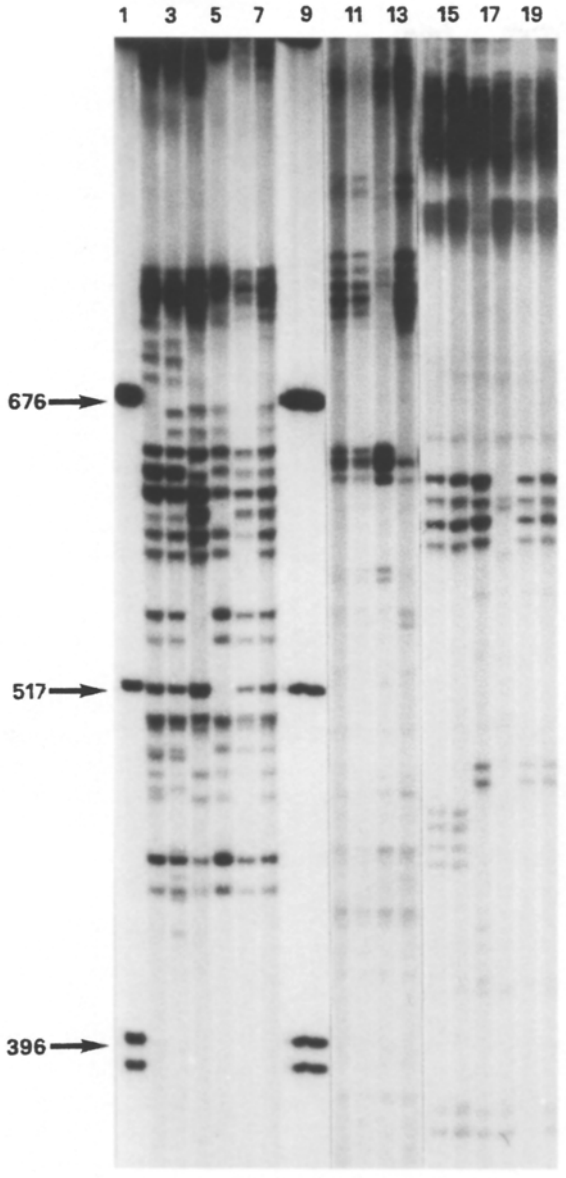


Fig. 3 Southern blot of common wheat DNAs digested with *Hae*III (*lanes 2–7*), *Rsa*I (*10–13*), *Taq*I (*14–19*) and hybridized to probe K32. *Lanes 7, 10* and *19* 'Chinese Spring', *6, 11* and *18* DT1AL, *5, 12* and *17* N1BT1D, *4, 13* and *16* N1DT1B, *2, 3, 14* and *15* cvs 'Tiberio', 'Jecora', 'Golia', 'Brasilia', respectively. *Lanes 1, 8* and *9* molecular markers

As expected, *Gli-D1b* and *Gli-D1f*, differing in ω-gliadins, gave identical K32 patterns of *Gli-D1*-derived bands (for example, Fig. 2A, lanes 13 and 14; 2B, lanes 15 and 17; Fig. 2C, lanes 14 and 16). All 6 cultivars with allele *Gli-D1d* had a remarkable increase in intensity of the "zone A"-fragments specific for *Gli-D1b* and *Gli-D1f* (Fig. 2B, lanes 2 and 3; 2C, lanes 1 and 2; see also Fig. 5). Furthermore, *Hae*III fragments 1, 2, 25 and 26 were stronger in *Gli-D1d* (Fig. 2A, compare lines 17 and 19). Only an increase in intensity of one γ-gliadin distinguished *Gli-D1d* from *Gli-D1b* at the protein level (Fig. 1). These results

Table 2 Presence (+) or absence (–) of K32 RFLP fragments in different *Gli-B1* alleles

DNA fragment		Alleles at the Gli-B1 locus									
Number	Size, (bp)	*d*	*b*	*s*	*n, q*	*e, g*	*c*	*f*	*a*	*k, m*	*l*[a]
*Taq*I											
3	1035	+	–	–	–	+	+	+	+	–	–
4	1010	+	–	–	–	+	+	+	+	–	–
15	632	–	+	+	+	–	–	–	–	–	+
16	621	–	+	+	+	–	–	–	–	–	–
17	609	+	+	+	+	+	+	+	+	+	–
18	600	–	–	–	–	–	–	–	–	+	–
19	596	+	+	+	+	+	+	+	+	+	–
20	588	–	–	–	–	–	–	–	–	+	–
21	585	–	–	–	–	–	–	–	+	–	–
22	583	–	–	–	–	+	+	+	–	–	–
23	576	–	–	–	–	–	–	–	+	–	–
24	573	–	–	–	–	+	+	+	–	–	–
28	486	–	–	–	–	–	–	–	+	–	–
29	480	–	–	–	–	–	–	–	+	–	–
30	469	–	–	–	+	+	+	+	–	–	–
31	463	–	–	+	+	+	+	+	–	–	–
32	459	+	+	–	–	–	–	–	–	–	–
33	457	–	–	+	–	+	+	–	–	+	–
34	454	+	+	–	–	–	–	–	–	–	–
35	449	–	–	–	–	+	+	+	–	+	–
36	442	–	–	–	–	–	–	+	–	–	–
*Hae*III											
4	686	+	+	+	+	–	+	–	–	+	–
5	674	+	+	+	+	–	+	–	–	+	–
7	626	–	+	+	+	–	–	–	–	–	–
10	582	–	–	–	–	+	+	+	+	–	–
11	570	–	–	–	–	+	+	+	+	–	–
16	505	+	+	+	+	+	+	+	+	+	–
17	502	–	–	–	–	–	–	–	–	+	–
18	499	–	–	+	–	–	–	–	–	–	–
20	470	+	+	+	+	–	+	–	–	–	–
28	420	–	+	–	–	–	–	–	–	–	–
*Rsa*I											
1	944	–	+	+	+	–	–	–	–	–	–
2	923	–	+	+	+	–	–	–	–	–	–
3	912	+	–	–	–	–	–	–	–	–	–
4	900	–	–	–	–	+	+	+	+	–	–
5	888	+	–	–	–	–	–	–	–	–	–
6	870	–	–	–	–	+	+	+	+	–	–
7	804	–	–	–	+	–	–	–	–	–	–
8	800	+	+	+	–	+	+	–	+	+	–
9	796	–	–	–	–	–	–	+	–	–	–
10	791	–	–	–	+	–	–	–	–	–	–
11	787	+	+	+	–	+	+	–	+	+	–
12	780	–	–	–	–	–	–	+	–	–	–
13	772	+	–	–	–	+	+	+	+	+	–
14	756	+	–	–	–	+	+	+	+	+	–
15	736	–	–	–	–	–	–	–	+	–	+
16	732	+	–	–	–	–	–	–	–	–	–
17	727	–	–	+	–	–	–	–	–	+	–
18	718	–	–	–	–	+	+	+	–	–	–
19	707	–	–	–	–	–	–	–	–	+	–
20	701	–	–	–	–	+	+	+	–	–	–
21	698	–	+	+	–	–	–	–	–	–	–
22	693	–	–	–	+	–	–	–	–	–	–
23	680	–	+	+	–	–	–	–	–	–	–
24	677	–	–	–	+	–	–	–	–	–	–

[a] Bands 10–14, 25–27, 37–39 in *Taq*I, bands 3, 13–15, 19, 22–24, 29–32 in *Hae*III and several fragments >1 kb in *Rsa*I derived from chromosome 1RS of rye in wheat 1B/1R translocation cultivars (see Fig. 3)

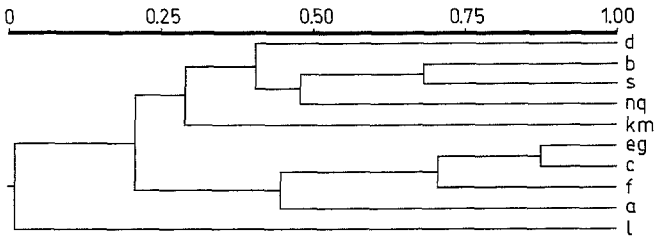


Fig. 4 Relationships among alleles at the *Gli-B1* locus based on cluster analysis of RFLP data

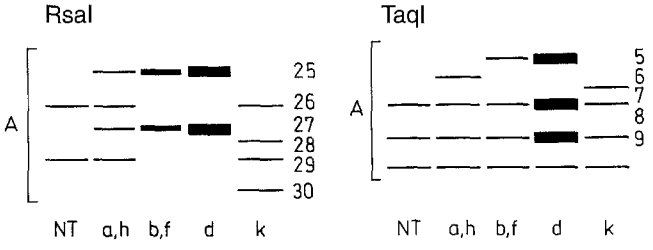


Fig. 5 Schematic diagrams of *Gli-D1* fragments in the "zone A" of RFLP patterns obtained with the K32 probe (*NT*: RFLP patterns of N1DT1B line of cv 'Chinese Spring')

strongly indicate an amplification event for the *Gli-D1d* allele, so that the amount of synthesized protein was approximately proportional to the number of encoding DNA sequences. However, not all *Gli-D1*-derived bands were amplified in *Gli-D1d*: *Hae*III fragments 9 and 27, which are specific for *Gli-D1b* and *Gli-D1f*, did not show any increase in intensity in *Gli-D1d*.

A particular group of "zone A" bands occurred only in cultivars having *Gli-D1k* (Fig. 2B, lanes 4, 7, 11–13; 2C, lanes 3, 6, 10–12, 22, 23; Fig. 5). In *Hae*III, this allele differs from others in lacking band 9. Taking into account that *Gli-D1k* lacks the fast-moving γ-gliadin (Fig. 1, double arrowhead), our findings suggest that most of the DNA sequence for this γ-gliadin was not lost, but became silent. In addition, analysis of *Hae*III patterns showed that *Gli-D1k* related probably to *Gli-D1a*: both lack band 27 and share specific bands 12 and 14 (Fig. 2A, lanes 7 and 8).

Four fragments were missing from *Hae*III RFLP patterns in the N1DT1B line and were therefore assigned to the *Gli-D1a* allele in 'Chinese Spring' (Fig. 3, lanes 4 and 7). However, only 1 and 2 bands completely disappeared from "zone A" of the *Taq*I and *Rsa*I patterns, respectively (Fig. 3, lanes 10 and 13, and 16 and 19; Fig. 5). Nevertheless, we tentatively assigned 4 bands to *Gli-D1a* for two reasons. First, the intensity of these bands decreased in N1DT1B and increased in N1BT1D lines (Fig. 3). Second, the presence or absence of these bands correlated accurately with the presence or absence, respectively, of *Gli-D1a* in the cultivars analysed.

RFLP analysis using the pTU1 probe

*Hae*III/pTU1 patterns were less polymorphic than those of *Hae*III/K32 (Fig. 6A). *Taq*I (Fig. 6B) and *Rsa*I (Fig. 6C) digests gave about 30 and 22 polymorphic fragments, respectively, most of which, however, were smaller than 0.4 kb. Not one polymorphic fragment was identical in size in the pTU1 and K32 patterns.

The 'Chinese Spring'-type of α-gliadins (Fig. 1) lacked bands 1 and 2 in *Hae*III (Fig. 6A, lanes 7, 11), band 1 in *Taq*I (Fig. 6B, lanes 9 and 13) and bands 4 and 5 in *Rsa*I (Fig. 6C, lanes 9 and 13). In addition, these alleles showed an absence or considerable decrease in the relative intensity of several *Rsa*I fragments of small sizes (Fig. 6C, lanes 9, 13), and *Gli-A2o* differed from the other alleles of this group in having both bands 1 and 2 in the *Rsa*I digests (Fig. 6C, lane 9).

Some bands were tentatively attributed to alleles encoding 'Cheyenne'-type α-gliadins, i.e. *Gli-A2g* (bands 6, 8–10 in *Taq*I; band 7 in *Rsa*I), *Gli-A2c* (bands 3–5 in *Taq*I; 6 and 8 in *Rsa*I) and *Gli-A2b* and *Gli-A2e* (data not shown). No unumbigous RFLP pattern was obtained for alleles at the *Gli-B2* or *Gli-D2* loci.

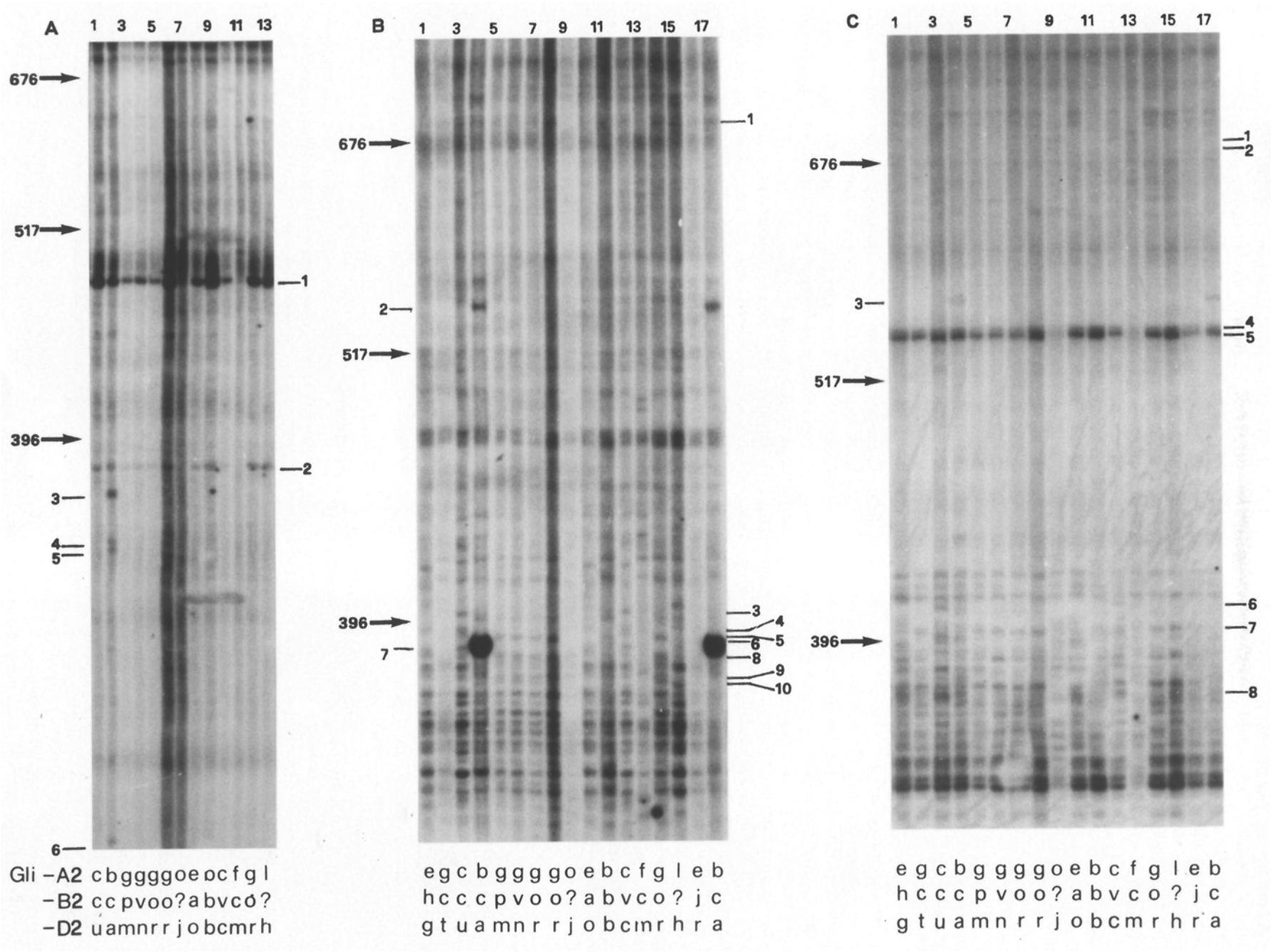
We were surprised to discover that some fragments occurred only in genotypes having the 1B/1R translocation: bands 3–6 in *Hae*III (Fig. 6A, lane 2), bands 2 and 7 in *Taq*I and band 3 in *Rsa*I (Fig. 6B, C, lanes 4 and 17). This finding provides evidence for the presence of α-gliadin-like sequences on chromosome 1R.

Discussion

Anderson et al. (1984) described differences in Southern hybridization patterns among common wheat cultivars having distinct types of α-gliadins. Later, a good correspondence between RFLP and β-amylase alleles (Sharp et al. 1988), and RFLP and high-molecular-weight glutenin subunits (Reddy and Appels 1993), was found. Moreover, the RFLP technique was found to identify more storage protein alleles in barley than the electrophoretic analysis of proteins (Bunce et al. 1986).

The K32 probe is claimed to hybridize to most if not all of the γ-type gliadin sequences at *Gli-1* (Bartels et al. 1986; RD Thompson, personal communication). In our work, its ability to recognize alleleic differences in γ- but not ω- or α-gliadin genes, at least under the hybridization conditions used, was confirmed.

Fig. 6 RFLP patterns after *Hae*III (**A**), *Taq*I (**B**) and *Rsa*I (**C**) digestion and hybridization with the clone pTU1. **A** *lanes 1–13* 'Loreto', 'Maestra', 'Oderzo', 'Irnerio', 'Costantino', 'Aquileja', 'Chiarano', 'Salmone', 'Etruria', 'Eridano', 'Farneto', 'Arquà', 'Bolero', **B, C** *lanes 1–17* 'Mec', 'Manital', 'Loreto', 'Maestra', 'Oderzo', 'Irnerio', 'Costantino', 'Aquileja', 'Chiarano', 'Salmone', 'Etruria', 'Eridano', 'Farneto', 'Arquà', 'Bolero', 'Brasilia', 'Fiocco'. Positions of molecular markers are indicated by *arrows*


A
1 3 5 7 9 11 13
676
517
396
3
4
5
6
1
2
Gli -A2 c b g g g g o e ɒ c f g l
-B2 c c p v o o ? a b v c ó ?
-D2 u a m n r r j o b c m r h
B
1 3 5 7 9 11 13 15 17
676
2
517
396
7
1
3
4
5
6
8
9
10
e g c b g g g g o e b c f g l e b
h c c c p v o o ? a b v c o ? j c
g t u a m n r r j o b c ı m r h r a
C
1 3 5 7 9 11 13 15 17
676
3
517
396
1
2
4
5
6
7
8
e g c b g g g g o e b c f g l e b
h c c c p v o o ? a b v c o ? j c
g t u a m n r r j o b c m r h r a

Genes having γ-sequences have been shown to be located at the *Gli-1* loci (Sabelli and Shewry 1991). There are about 40 copies of γ-sequences in 'Chinese Spring' (Sabelli and Shewry 1991). However, only 3–6 γ-gliadins in total in the different genotypes are encoded by all three *Gli-1* loci, while other gliadins located in the γ-zone of the APAGE pattern are encoded by the *Gli-B2* locus (Metakovsky 1991) and have α-type sequences (Kasarda et al. 1987). In our work, 6–10 *Gli-B1* fragments appeared in *Taq*I and *Rsa*I patterns, with a total length of 3 to 6 kb in different alleles (Table 2). Only some of these fragments may belong to active γ-genes because their size does not exceed 0.85 kb (Shewry and Tatham 1990). Therefore, most K32 fragments originate from silent γ-gliadin sequences. However, a good correspondence was found between K32 RFLP patterns and gliadin alleles revealed by APAGE.

The existence of groups of distinctly organized *Gli-B1* alleles in common wheat suggests their origin from different ancestral genotypes. Genetic variation at the *Gli-B1* locus in common wheat can hardly be accounted for by divergence from one ancestral AABB individual. Similarly, polymorphism of genome A gliadins is likely to be due to a contribution from more than one ancestral genotype (Kasarda 1980, Kudryavtsev et al. 1988). The distinct organization, at the DNA level, of the two types of α-gliadin alleles at the *Gli-A2* locus is confirmed in our work.

The high level of DNA polymorphism at the *Gli-1* loci seems to be largely due to insertion/deletion. Indeed, a small deletion of about 15 bp (calculated as the difference in size between fragments 33 and 36 in *Taq*I digests) may account for the change in mobility of 1 band in both APAGE and RFLP patterns and the conversion of *Gli-B1e* into *Gli-B1f* (Fig. 2B, lanes 8 and 9). Amplification of a DNA sequence of 1.25 kb (the value obtained as the sum of sizes of fragments 25 and 27 in the *Rsa*I pattern, Fig. 5) may convert *Gli-D1b* into *Gli-D1d* (Fig. 2C, lanes 15 and 16). Duplication of 1 gene within the *Glu-B1* locus has been described earlier (Lukow et al. 1992). *Gli-D1k*, a derivative of *Gli-D1a*, could result from a mutational silencing of 1 gene and the subsequent degeneration of the silent sequence (Fig. 2C, lanes 5 and 6, see "zone A").

The most frequent genetic event that causes the appearance of new gliadin alleles is probably gene conversion (Baltimore 1981), or ordinary intralocus crossing-over. Accordingly, the *Gli-B1s* allele may be a product of intralocus recombination between *Gli-B1b* and *Gli-B1k* or *Gli-B1m*. *Gli-B1c* differed from other *Gli-B1e*-like alleles in having 3 *Hae*III bands that may result, together with the *Gli-B5* sequences, from recombination. A similar event could convert *Gli-B1m* into *Gli-B1m**. In all of the above-mentioned cases, a change in the *Gli-B1* locus affected an active gene and resulted in a novel allele. In cv 'Liocorno', however, a small deletion probably occurred in a silent γ-sequence at the *Gli-B1* locus and did not display itself in APAGE (Fig. 2B, lane 11).

To our knowledge, there are no data on the presence of any α-gene sequences on chromosome 1R of rye. The absence of α-type N-terminal sequences in rye prolamines was first shown by Autran et al. (1979). Later, the absence of α-type genes in rye was confirmed (Shewry et al. 1984). Moreover, a complete homoeology and co-linearity was found between the rye 1R chromosome and wheat chromosomes 1A, 1B and 1D (Devos et al. 1993) on which no α-gliadin genes have been found so far. In our work, several DNA fragments specific for cultivars with the 1B/1R translocation were recognized by the K32 probe (Table 1), making the identification of this translocation in RFLP patterns as easy as in APAGE fractionation. In the same cultivars, however, several other fragments hybridized with the pTU1 probe; among these, band 7 in the *Taq*I pattern was extremely strong (Fig. 6B). This probe represents a typical α-gene (D'Ovidio et al. 1992). Although homology of non-repetitive sequences of α- and γ-gliadin genes may reach 60–70% (Harberd et al. 1985; Okita et al. 1985; Rafalski 1986), not one of the assumed 1B/1R fragments recognized by pTU1 hybridized to K32. Consequently, we suggest that chromosome arm 1RS contains some untranslated sequences that are homologous to sequences at the *Gli-A2* locus. A considerable amount of these α-sequences on chromosome 1RS occurs as repeats about 380 bp in size (fragment 7 in *Taq*I digest).

Gliadin alleles are claimed to be efficient genetic markers for various wheat traits, including dough quality (Sozinov and Poperelya 1980), but a direct influence of gliadins on dough strength seems to be improbable (Gupta and McRitchie 1994). It is the low-molecular-weight glutenin encoded by the *Glu-3* genes that mainly affect the quality (Gupta et al. 1994); the apparent involvement of some gliadin alleles can therefore be explained as a result of the tight linkage between *Gli-1* and *Glu-3* loci (Payne et al. 1984; Singh and Shepherd 1988). The correspondence among the alleles at *Gli-1* and *Glu-3* at the DNA level is currently under way in our laboratory.

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