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Production and genetic characterization of near-isogenic lines in the bread-wheat cultivar Alpe

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Abstract Two biotypes of the bread-wheat cultivar Alpe were shown to possess contrasting alleles at each of the glutenin (*Glu-B1, Glu-D1, Glu-B3* and *Glu-D3)* and gliadin (*Gli-B1* and *Gli-D1)* loci on chromosomes 1B and 1D. Fourteen near-isogenic lines (NILs) were produced by crossing these biotypes and used to determine the genetic control of both low-molecular-weight (LMW) glutenin subunits and gliadins by means of one-dimensional or two-dimensional electrophoresis. Genes coding for the B, C and D groups of LMW subunits were found to be inherited in clusters tightly linked with those controlling gliadins. Southern-blot analysis of total genomic DNAs hybridized to a γ-gliadin-specific cDNA clone revealed that seven NILs lack both the *Gli-D1* and *Glu-D3* loci on chromosome 1D. Segregation data indicated that these "null" alleles are normally inherited. Comparison of the "null" NILs with those possessing allele *b* at the *Glu-D3* locus showed one B subunit, seven C subunits and two D subunits, as fractionated by two-dimensional A-PAGE×SDS-PAGE, to be encoded by this allele. Alleles *b* and *k* at *Glu-B3* were found to code for two C subunits plus eight and six B subunits respectively, whereas alleles *b* and *k* at *Gli-B1* each controlled the synthesis of two β-gliadins, one γ- and two ω-gliadins. The novel *Gli-B5* locus coding for two ω-gliadins was shown to recombine with the *Gli-B1* locus on chromosome 1B. The two-dimensional map of glutenin subunits showed α-gliadins encoded at the *Gli-A2* locus on chromosome 6A. The use of Alpe NILs in the study of the individual and combined effects of glutenin subunits on dough properties is discussed.

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Introduction

Both the quantity and quality of endosperm proteins are major factors responsible for the baking properties of bread wheat (Finney and Barmore 1948; Orth and Bushuk 1972) and durum wheat (Boggini and Pogna 1989). There is some evidence of major genes for increased protein content (Avivi 1978; Law et al. 1978). However this character seems to be largely determined by the growing conditions and is thus mainly a matter of agronomic practices. By contrast, protein quality, that is the basic sizes and structures of the different endosperm polypeptides and their aggregative behaviour in forming the cohesive mass of gluten, is mainly due to the genetic architecture of each wheat cultivar. The storage-protein gliadins and glutenins represent about 80% of the total protein in the grain and are generally considered to contribute to the viscosity-extensibility and the elasticity of gluten respectively (Bietz et al. 1973; Flavell et al. 1984). Genes coding for most of the ω- and γ-gliadins are tightly clustered at three homoeologous loci, *Gli-A1, Gli-B1* and *Gli-D1*, on the short arm of chromosomes 1A, 1B and 1D respectively (Payne 1987). Minor ω-gliadins are encoded by additional, dispersed genes at the *Gli-A3* (Sobko 1984; Metakovsky et al. 1986; Payne et al. 1988),*Gli-B3* (Galili and Feldman 1984; Jackson et al. 1985), *Gli-A4* (Redaelli et al. 1992), and *Gli-5* loci (Pogna et al. 1993) on the short arms of the group-1 chromosomes; α- and β-gliadins are encoded by genes tightly clustered at a single locus on each of the group-6 chromosomes (Payne 1987).

Two major classes of glutenin polypeptides have been identified in wheat endosperm: the high-molecular-weight (HMW) and the low-molecular-weight (LMW) subunits, both classes occurring in flour as cross-linked proteins resulting from inter-polypeptide disulphide linkages. The genes coding for HMW subunits are located on the long

arms of chromosomes 1A, 1B and 1D at the *Glu-A1, Glu-B1* and *Glu-D1* loci respectively (Payne 1987), whereas the genes coding for LMW subunits occur on the short arms of group-1 chromosomes at the *Glu-A3, Glu-B3* and *Glu-D3* loci (Singh and Shepherd 1988) which are tightly linked to the *Gli-1* locus (Singh and Shepherd 1988; Pogna et al. 1990). In contrast to gliadins and HMW subunits, which are easily resolved by A-PAGE or SDS-PAGE, the LMW subunits (also known as the B and C subunits) have proved much more difficult to analyze by one-dimensional SDS-PAGE under reducing conditions because of overlap with the gliadins. However, different techniques have been developed to obtain gliadin-free LMW subunits before fractionation by one-dimensional (Gupta and MacRitchie 1991; Singh et al. 1991; Morel 1994) or two-dimensional electrophoresis (Redaelli et al. 1994).

The work of Payne et al. (1980) provided evidence for a strong association between the presence of certain alleles coding for HMW subunits and bread-making quality. Similarly, LMW subunits have been found to have a pronounced effect on dough viscoelastic properties in both bread wheat (Payne et al. 1987; Gupta and Sheperd 1988; Gupta et al. 1989) and durum wheat (Boggini and Pogna 1989; Pogna et al. 1990). One important finding has been that the effects of individual *Glu-3* alleles are largely additive to those of the *Glu-1* alleles (Gupta et al. 1989; Pogna et al. 1990), interaction between these loci having significant effects as well (Gupta et al. 1994). The study of the additive and interaction behaviour of glutenin subunits can provide relevant information in predicting the dough quality of progeny in breeding programs but requires special genetic materials, such as recombinant inbred lines, or near-isogenic lines, in order to obtain unbiased results.

The main objective of the present work was to develop a set of near-isogenic lines (NILs) showing allelic variation at the *Glu-1, Gli-1* and *Glu-3* loci in the high-quality bread-wheat cv Alpe. These NILs presented an opportunity to study the genetic control of gliadins and LMW subunits as a fundamental step towards their use in the analysis of the individual and combined effects of glutenin subunits on dough rheology in a common genetic background.

Materials and methods

Plant material

In previous studies (Pogna et al. 1982, 1989) two biotypes ($1I^-$ and 2II), having contrasting glutenin and gliadin patterns, were isolated by A-PAGE and SDS-PAGE fractionations of single seeds in the bread-wheat cultivar Alpe. F_1 seeds were produced by crossing these biotypes and then planted to provide F_2 seeds for studying the inheritance of storage proteins, and for isolating near-isogenic lines (NILs).

Gliadin extraction and electrophoresis

Gliadins were extracted from single seeds with 70% (v/v) ethanol and fractionated by A-PAGE and two-dimensional SDS-PAGE as described previously (Pogna et al. 1990; Dachkevitch et al. 1993).

Glutenin extraction and electrophoresis

The reduced total proteins from individual seeds were extracted and fractionated by SDS-PAGE according to Pogna et al. (1990) using 15% acrylamide separating-gels, pH 8.4. To reveal the LMW subunits, single grains were extracted three times with 1.5 ml of 50% (v/v) n-propanol for 30 min at 60°C. After centrifugation the pellet was incubated for 30 min at 60°C in 150 µl of a solution containing 50% (v/v) n-propanol, 80 m*M* Tris-HCl, pH 8.5 and 20 m*M* dithiothreitol. After centrifugation the supernatant was transferred into a new tube and diluted with the same volume of a solution containing 50% (v/v) n-propanol, 80 m*M* Tris-HCl, pH 8.5 and 40 m*M* 4-vinylpyridine. Alkylation was performed overnight at 20°C. LMW subunits were fractionated by one-dimensional SDS-PAGE as described above, and by two-dimensional A-PAGE×SDS-PAGE. After the first dimension (A-PAGE) according to Morel (1994) the gels were cut into single strips and incubated for 30 min in 62.5 m*M* Tris-HCl, pH 6.8 containing 2% (w/v) SDS and 40% (w/v) glycerol. The strips were loaded on a SDS-PAGE gel, prepared as described above, for the second dimension.

Southern-blot analysis

DNA was extracted from 7-day-old seedlings according to a modified CTAB procedure (Murray and Thompson 1980). DNA digestion with the four-cutter enzyme *Rsa*I, gel electrophoresis, blotting and hybridization with the K32 probe were carried out as described by Gebhardt et al. (1989). The probe used was a cDNA clone recognizing γ-gliadin sequences (Bartels et al. 1986) kindly provided by R.Thompson (Max-Planck Institute, Köln).

Results

Biochemical and genetic analysis of cv Alpe

The high-quality bread-wheat cultivar Alpe was registered in 1974 and has been grown in Italy for 18 years. It was selected from a cross between the Italian cv N. Strampelli and the Russian cv Bezostaya 1. In two previous studies the gliadin and HMW glutenin subunit composition of cv Alpe was determined by A-PAGE and SDS-PAGE fractionations of individual seeds respectively (Pogna et al. 1982, 1989). From these analyses cv Alpe was found to consist of four lines (or biotypes) with respect to storage-protein composition, all morphological and agronomical characters being identical amongst the biotypes. According to the gliadin allele nomenclature of Metakovsky (1991) biotypes 2II and $1I^-$ share the same alleles at the *Gli-A1* and *Gli-2* loci (Table 1) but differ from each other in the allele compositions at *Gli-B1* (allele *k* vs allele *b*, respectively) and *Gli-D1* (*b* vs "null"). The A-PAGE (Fig. 1) and A-PAGE×SDS-PAGE (Fig. 2, A and B) patterns of these biotypes showed that alleles *k* and *b* at *Gli-B1* each code for two ω-gliadins, one major γ-gliadin and two β-gliadins, whereas allele *b* at *Gli-D1* codes for five ω- and two γ-gliadins. Biotype $1I^-$ (Fig. 2B) is rather unique in lacking all the *Gli-D1*-encoded gliadins.

There are distinctive differences in the HMW glutenin subunit composition amongst the two biotypes (Fig. 3). Biotype 2II contains subunits 2* (allele *Glu-A1b*), 7+9 (*Glu-*

Table 1 Allele compositions at *Gli-1*, *Gli-5*, *Glu-1* and *Glu-3* of 14 near-isogenic lines in the bread-wheat cultivar Alpe[a]

Genotype	Gliadin locus			HMW-GS locus		LMW-GS locus	
	Gli-B1	*Gli-B5*	*Gli-D1*	*Glu-B1*	*Glu-D1*	*Glu-B3*	*Glu-D3*
1I	*b*	Null	*b*	*u*	*d*	*b*	*b*
1I$^-$	*b*	Null	Null	*u*	*d*	*b*	Null
1II	*b*	Null	*b*	*c*	*d*	*b*	*b*
1II$^-$	*b*	Null	Null	*c*	*d*	*b*	Null
2I	*k*	*a*	*b*	*u*	*a*	*k*	*b*
2I$^-$	*k*	*a*	Null	*u*	*a*	*k*	Null
2II	*k*	*a*	*b*	*c*	*a*	*k*	*b*
2II$^-$	*k*	*a*	Null	*c*	*a*	*k*	Null
3I	*b*	*a*	*b*	*u*	*a*	*b*	*b*
3II	*b*	*a*	*b*	*c*	*a*	*b*	*b*
3II$^-$	*b*	*a*	Null	*c*	*a*	*b*	Null
4I	*k*	*a*	*b*	*u*	*d*	*k*	*b*
4I$^-$	*k*	*a*	Null	*u*	*d*	*k*	Null
4II$^-$	*k*	*a*	Null	*c*	*d*	*k*	Null
Parental cvs							
N. Strampelli	*k*	*a*	*b*	*u*	*a*	*k*	*b*
Bezostaya 1	*b*	Null	*b*	*c*	*d*	*b*	*b*

[a] All the NILs contain alleles *Gli-A1b*, *Gli-A2b*, *Gli-B2y*, *Gli-D2b*, *Glu-A1b* (HMW subunit 2*)and *Glu-A3b*. Alleles *c* and *u* at *Glu-B1* code for HMW subunits 7+9 and 7*+8 respectively; alleles *a* and *d* at *Glu-D1* code for the HMW subunit pairs 2+12 and 5+10 respectively

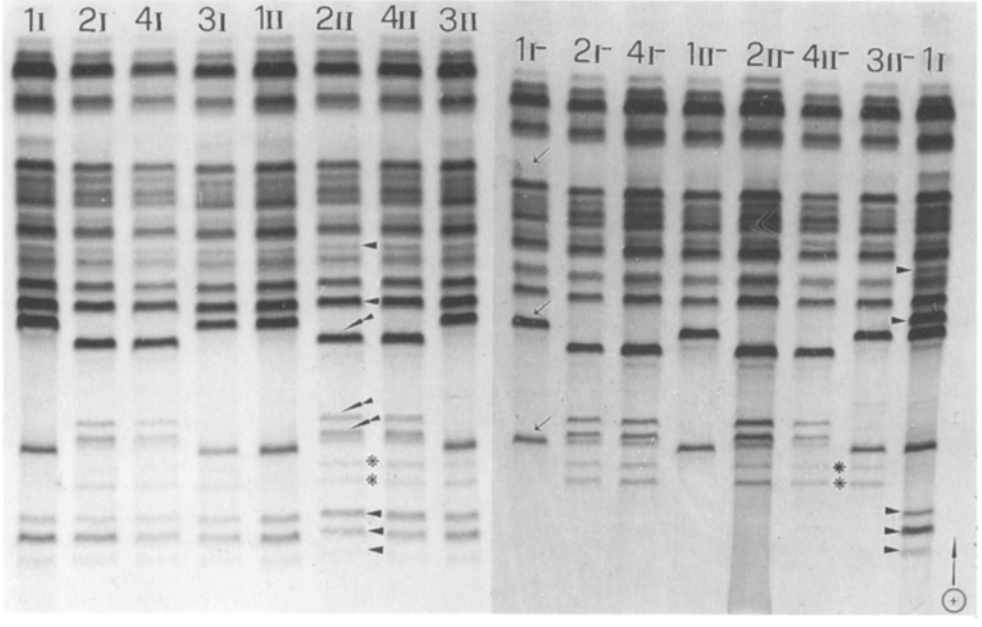
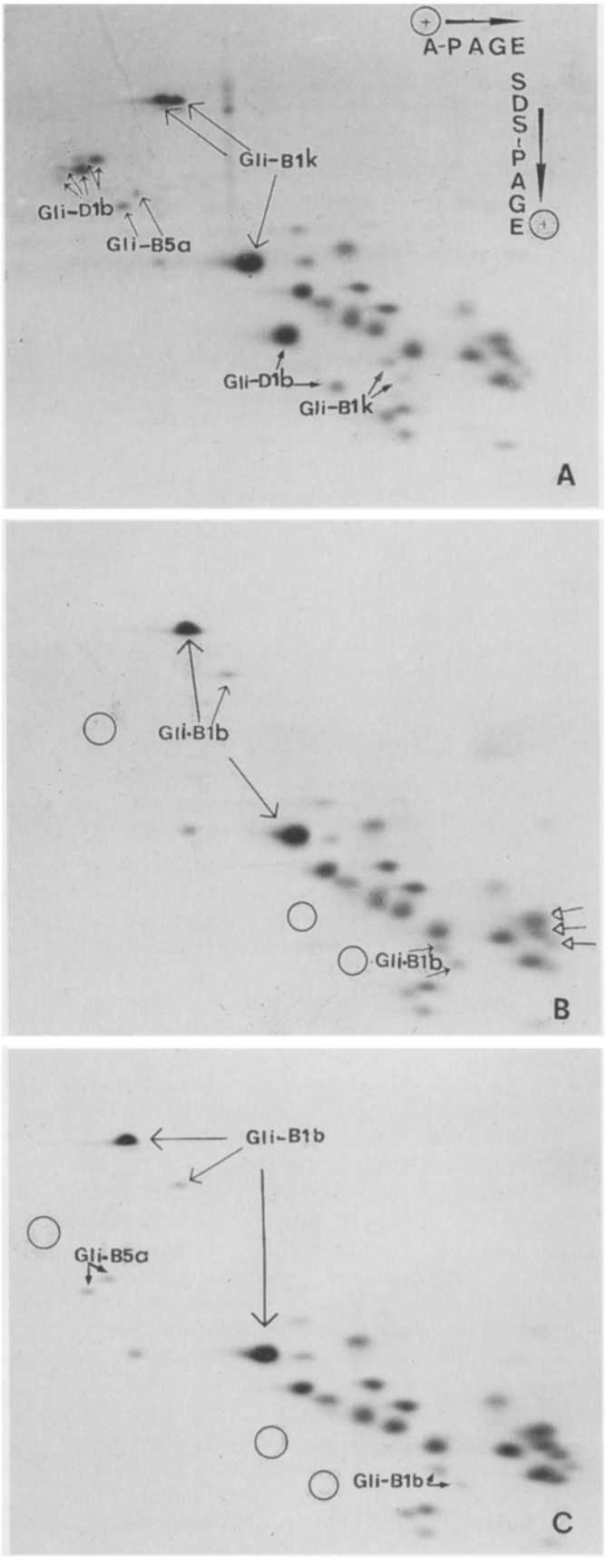


Fig. 1 A-PAGE fractionations of gliadins from 14 NILs obtained from the cross between biotype 2II and biotype 1I$^-$ in the bread-wheat cv Alpe. The gliadin bands encoded by alleles *Gli-B1k* (*double arrowheads*), *Gli-D1b* (*arrowheads*), *Gli-B1b* (*arrows*) and *Gli-B5a* (*asterisks*) are indicated

B1c) and 2+12 (*Glu-D1a*) whereas biotype 1I$^-$ has subunits 2*, 7*+8 (*Glu-B1u)* and 5+10 (*Glu-D1d*) (see also Table 1). The storage-protein compositions of these biotypes are in accordance with those of their parental cultivars, alleles *Gli-B1k, Gli-B2y, Glu-B1u* and *Glu-D1a* being inherited from cv N. Strampelli, all other alleles from cv Bezostaya 1.

Southern-blot analysis of biotype 1I$^-$

The K32 probe recognizing the *Gli-1* sequences (Bartels et al. 1986) was hybridized to DNA extracted from several wheat genotypes, and digested with the restriction enzyme *Rsa*I (Fig. 4). The fragments attributed to the *Gli-D1* locus (Vaccino et al. 1993) were found to be absent in biotype



$1I^-$ (lane 7), suggesting the occurrence of a partial chromosome deletion on the short arm of chromosome 1D. However, cytological observations on root-tip chromosomes at metaphase revealed a normal chromosome 1D pair in biotype $1I^-$ (data not shown).

Inheritance of gliadins and HMW subunits of glutenin

A total of 274 seeds from the 2II×$1I^-$ cross were screened by A-PAGE and SDS-PAGE to reveal gliadins and HMW glutenin subunits. Segregation data showed that about 25% of the progeny were homozygous for the null allele at the *Gli-D1* locus (Table 2). Moreover the gliadin bands assigned to each of the *Gli-D1b*, *Gli-B1b* and *Gli-B1k* alleles in Figs. 1 and 2 were inherited as a unit indicating that the genes controlling them are tightly linked. In a previous study (Pogna et al. 1993) allele *a* at the novel gliadin locus *Gli-B5,* which is closely linked to the *Gli-B1* locus, was found to code for a pair of ω-gliadins. These gliadins also occur in the parental genotype 2II and are marked in Figs. 1 and 2. Two recombinant phenotypes represented by F_2 seeds containing allele *Gli-B1k* in the absence of *Gli-B5a*, or the converse (*Gli-B5a* without *Gli-B1k*), were found in the progeny analysed here (Table 2). Using the method of maximum likelihood (Allard 1956), the recombination value between *Gli-B1* and *Gli-B5* was calculated to be 1.5±0.6%. As expected, no linkage was found between alleles coding for gliadins and those coding for HMW glutenin subunits at each *Glu-1* locus.

Isolation of near-isogenic lines (NILs) and inheritance of LMW subunits of glutenin

The parental biotypes 2II and $1I^-$ contain contrasting alleles at the *Gli-B1*, *Gli-D1*, *Gli-B5*, *Glu-B1* and *Glu-D1* loci and, therefore, 32 different homozygous genotypes (or NILs) are expected in their progeny. Twelve homozygous combinations were found in the F_2 generation and were multiplied for five self-pollinated generations, whereas the genotypes 3II and $3II^-$ (Table 1) were isolated from F_3 seeds and multiplied for four generations to provide NILs. Some of the remaining genotypes are currently being multiplied or screened and are not considered here. Observations on the plant morphology and agronomical characteristics of the 14 NILs were made on replicated plots in the field. These NILs were found to be indistinguishable from each other in terms of growth behaviour, plant morphology, heading time and albumin/globulin composition as de-

Fig. 2 Two-dimensional A-PAGE×SDS-PAGE fractionations of gliadins from biotype 2II (**A**), biotype $1I^-$ (**B**) and line $3II^-$ (**C**). *Open-headed arrows* indicate *Gli-A2*-encoded gliadins. *Circles*=map positions of gliadins encoded by allele *Gli-D1b*

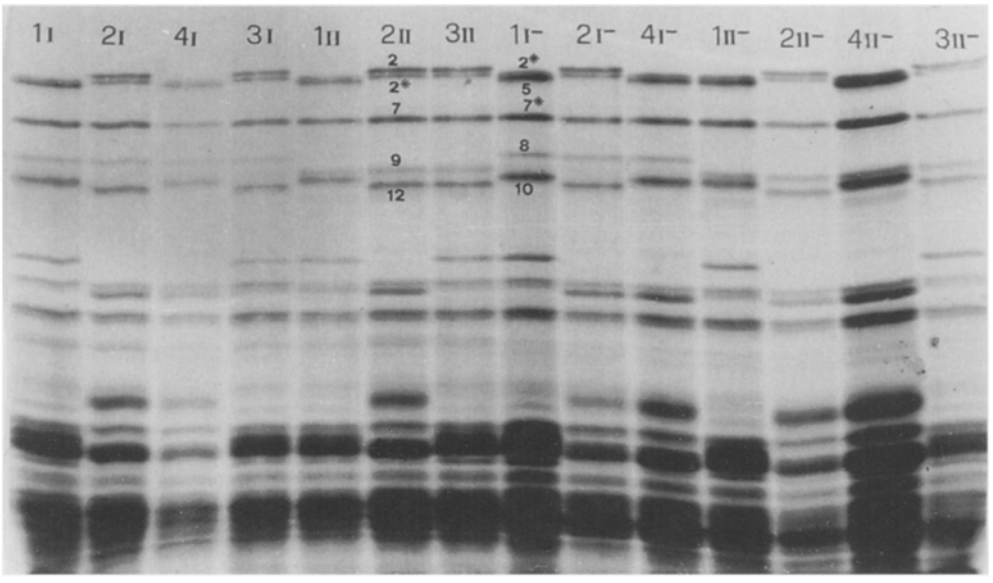


Fig. 3 SDS-PAGE fractionation of total protein from 14 NILs obtained from the cross between biotype 2II and biotype 1I⁻ in the bread-wheat cv Alpe. The HMW subunits of glutenin are *numbered*

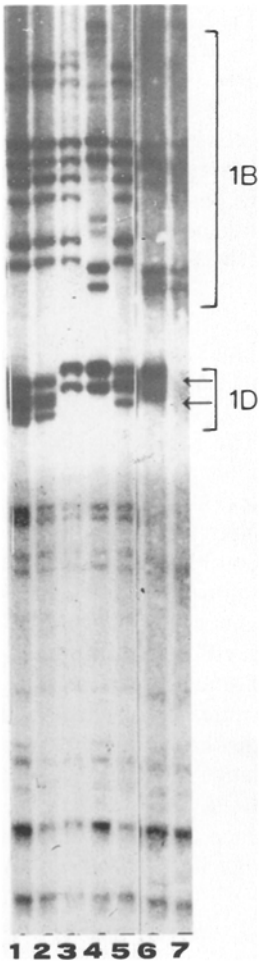


Fig. 4 RFLP patterns of DNAs from bread-wheat cultivars after digestion with *Rsa*I and hybridization to clone K32. *Lanes 1 to 7*: cvs Granarolo, Irnerio, Kosutza, Saliente, Oderzo, Alpe biotype 2II and Alpe biotype 1I⁻. The restriction fragments of the *Gli-B1* and *Gli-D1* loci are indicated. *Arrowheads* show two fragments absent in Alpe biotype 1I⁻

termined by SDS-PAGE (data not shown); additionally, no significant differences were found for yield, seed weight and hectoliter weight (data not shown). By contrast, the lines contained different combinations of alleles at the gliadin (*Gli-B1, Gli-B5* and *Gli-D1*) and HMW subunit (*Glu-B1* and *Glu-D1*) loci (Figs. 1 and 3, Table 1). In particular, the A-PAGE gliadin patterns (Fig. 1) revealed unambiguously that lines 3I, 3II and 3II⁻ are recombinant genotypes possessing the ω-gliadin pair encoded by allele *Gli-B5a* along with the gliadin block encoded by allele *Gli-B1b* (see also Fig. 2C).

Reduced and alkylated glutenins from the 14 NILs and their parental cultivars N. Strampelli and Bezostaya 1 were fractionated by one-dimensional SDS-PAGE (Fig. 5). By this type of analysis, the HMW subunits of glutenin were easily recognized; moreover, there was a close correspondence between the patterns of gliadins and those of LMW subunits. Because of this tight linkage, the *Glu-3* alleles of the NILs were designated with the same letters as the *Gli-1* alleles (Table 1). In particular, two bands in each of the B and C groups of LMW subunits were attributed to allele *Glu-B3k* (Fig. 5, arrows), and two C subunits to allele *Glu-B3b* (double arrows). Moreover, allele *Glu-D3b* was found to code for three C subunits and two D subunits (arrowheads). In addition to these LMW subunits, a few faint bands, marked by ▲ in the SDS-PAGE patterns of Fig. 5, were found in NILs possessing certain gliadin alleles at *Gli-B1* or *Gli-D1*. However, these bands also occurred in the SDS-PAGE fractionations of unreduced gliadins (data not shown) and, therefore, could be ω-gliadins which have not been removed by the washings with n-propanol during the preparation of the glutenin extracts.

Several LMW subunits in the SDS-PAGE patterns were not attributed to any specific *Glu-3* locus because of the overlapping between polypeptides encoded by different alleles. Therefore, reduced and alkylated glutenins were fractionated by a novel two-dimensional A-PAGE×SDS-PAGE method (Fig. 6). All the HMW subunits and most of the LMW subunits could be easily resolved and attributed to specific alleles by this type of analysis. For example, comparison of lines 1I and 1I⁻ (Fig. 6, A and B) showed one major LMW subunit in the B group, six in the C group

Table 2 F_2 segregation data for the progeny from the cross 2II×1I⁻

Allele		Number of progeny				χ^2 (1:1:2)	χ^2 (3:3:9:1)	Conclusion	R±S.E. (%)
A	*B*	*A–*	*–B*	*AB*	–				
Gli-D1b	*Gli-D1null*	66	64	144	0	ns[a]		Allelism	
Gli-B1k	*Gli-B1b*	63	75	136	0	ns		Allelism	
Gli-B1k	*Gli-B5a*	1	3	205	65		***	Linkage	1.5±0.6
Gli-B1k	*Glu-B1c*	47	54	158	15		ns	No linkage	
Gli-D1b	*Glu-D1a*	56	53	147	18		ns	No linkage	

[a] ns, not significant; *** significant at P=0.001

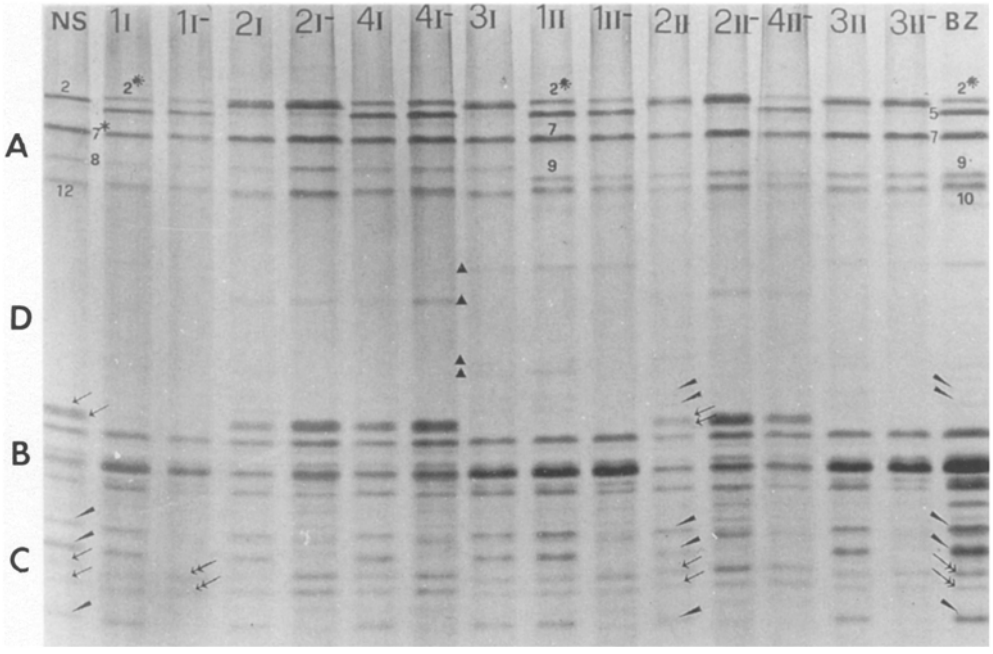


Fig. 5 SDS-PAGE fractionation of alkylated glutenin subunits from cvs N. Strampelli (*NS*) and Bezostaya 1 (*BZ*), and from 14 NILs in the bread-wheat cv Alpe. The LMW subunits encoded by alleles *Glu-B3k* (*arrows*), *Glu-B3b* (*double arrows*) and *Glu-D3b* (*arrowheads*) are indicated. The HMW subunits are *numbered*. The bands marked by ▲ also occur in the SDS-PAGE patterns of gliadins

and two in the D group to be encoded by allele *Glu-D3b* (arrows). The LMW subunits encoded by alleles *b* and *k* at the *Glu-B3* locus could be identified by comparing line 1I or 1I⁻ with line 4I (Fig. 6D). Six B subunits and two C subunits were attributed to allele *Glu-B3k* (double arrowheads), and two C subunits plus eight strong B subunits were assigned to allele *Glu-B3b* (arrowheads).

The *Gli-B1/Gli-B5* recombinant lines 3I, 3II and 3II⁻ were all shown to contain the LMW subunits encoded by allele *Glu-B3b* (compare A and C in Fig. 6), providing further support for the gene order *Glu-B3–Gli-B1–Gli-B5* on the short arm of chromosome 1B.

Several C subunits and some B subunits could not be assigned to any *Glu-3* locus because they occurred in all the NILs analysed. However, some subunits, as marked by ⋙ in Fig. 6C, were attributed to allele *Glu-A3b* by the A-PAGE×SDS-PAGE analysis of glutenins from several wheat cultivars (data not shown). Moreover, when Figs. 6B and 2B were compared to each other it was clear that the three *Gli-A2*-encoded α-gliadins marked by open-headed arrows in Fig. 2B also occurred in the C group of LMW subunits of glutenin (Fig. 6B, open-headed arrows). Finally, several minor spots in the D zone of the two-dimensional map of LMW subunits occupied identical positions in the A-PAGE×SDS-PAGE fractionations of gliadins (Fig. 2). These could be ω-gliadins and are shown in brackets in Fig. 6.

Discussion

The three groups of storage-protein monomers, gliadins, HMW glutenin subunits and LMW glutenin subunits, occurred as discrete entities when fractionated by two-dimensional A-PAGE×SDS-PAGE. Analysis of NILs in cultivar Alpe by this electrophoretic procedure has enabled several gliadins and glutenin subunits to be assigned to certain alleles at the *Gli-1* or *Glu-3* loci. In particular, alleles *b* and *k* at the *Gli-B1* locus were each shown to code for a pair of β-gliadins plus three components in the γ- and ω-groups of gliadins. The two β-gliadins encoded by allele *Gli-B1k*

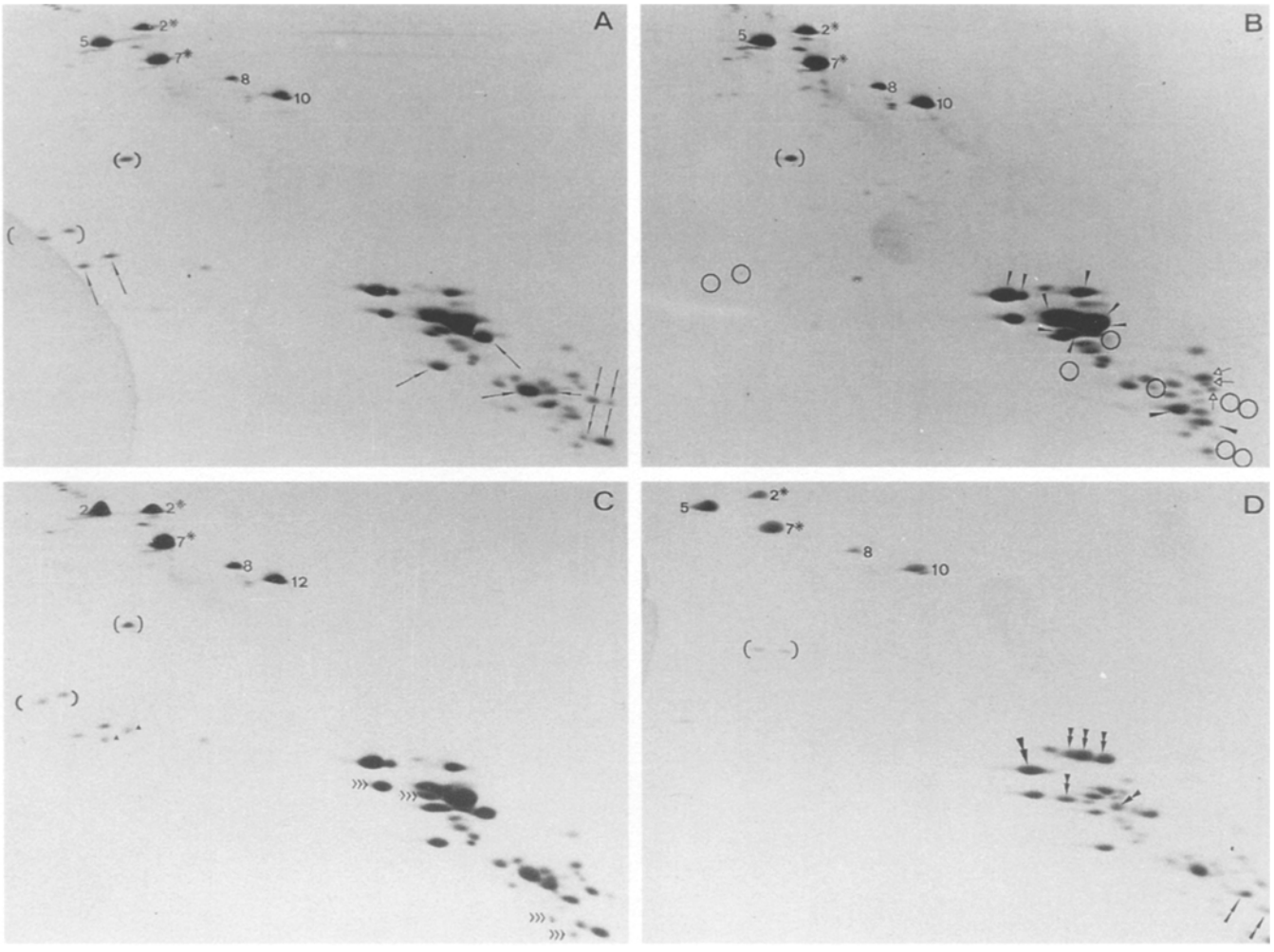


Fig. 6 Two-dimensional A-PAGE×SDS-PAGE fractionation of alkylated glutenin subunits from line 1I (**A**), line 1I$^-$ (**B**), line 3I (**C**) and line 4I (**D**). The HMW subunits are *numbered*. The LMW subunits encoded by alleles *Glu-D3b* (*arrows*), *Glu-B3b* (*arrowheads*), *Glu-B3k* (*double arrowheads*) and *Glu-A3b* (>>>) are indicated. *Circles*=map positions of LMW subunits encoded by allele *Glu-D3b* in line 1I$^-$. Gliadins are shown in *brackets*. The faint spots marked by ▲ in (**C**) are ω-gliadins encoded by allele *Gli-B5a*. *Open-headed arrows* in (**B**) indicate *Gli-A2*-encoded gliadins

have not been previously detected due to their overlap with some other β-gliadins (*Gli-B2* or *Gli-D2* bands) in the one-dimensional A-PAGE fractionations (Metakovsky 1991). It remains to be seen whether these pairs of *Gli-B1*-encoded gliadins possess α/β-gliadin type sequences which are typical of *Gli-2*-encoded gliadins (Okita et al. 1985).

The single-grain analysis of the F_2 progeny from the cross between biotype 2II and biotype 1I$^-$ confirmed the occurrence of a pair of ω-gliadins encoded by the *Gli-B5* locus. This locus was found to have 1.5% recombination with *Gli-B1* as compared to the value of 1.4% obtained previously (Pogna et al. 1993). The gene order *centromere–Glu-B3–Gli-B1–Gli-B5*, as deduced from the linkage mapping results by Singh and Shepherd (1988) and Pogna et al. (1993), is consistent with the allele compositions at *Gli-B1* and *Glu-B3* of the recombinant lines 3I, 3II and 3 II$^-$ obtained here.

Using a two-step one-dimensional SDS-PAGE procedure Gupta and Shepherd (1990) detected several alleles at each of the LMW glutenin subunit loci, *Glu-A3*, *Glu-B3* and *Glu-D3*. Recently (Singh et al. 1991), a particular extraction procedure has been developed to obtain gliadin-free glutenin subunits before fractionation by one-dimensional SDS-PAGE or A-PAGE (Singh et al. 1991; Morel 1994) and two-dimensional A-PAGE×SDS-PAGE (Redaelli et al. 1994). Using the latter procedure for the fractionation of LMW glutenin subunits in the NILs of cv Alpe, one B subunit, seven C subunits and two D subunits were assigned to the *Glu-D3* locus, and two C subunits plus six to eight B subunits to the *Glu-B3* locus. The number of B and C subunits that have been assigned to these loci is much greater than those obtained previously using one- or two-dimensional methods (Gupta and Shepherd 1990; Jackson et al. 1983). It is interesting to note that alleles *b* and *k* at the *Glu-B3* locus code for most of the B subunits, only one or two polypeptides in this group being encoded by the homoeoallelic loci on chromosomes 1A and 1D. However,

some B subunits could not be assigned to any *Glu-3* locus and this seems to be in contrast with the allocation of all the B subunits in several cultivars to the short arms of group-1 chromosomes (Gupta and Shepherd 1990, 1993). On the other hand, Ruiz and Carrillo (1993) have provided evidence of one B subunit encoded by the *Gli-B3* locus in durum wheat, suggesting that two separate loci on the short arm of chromosome 1B are involved in the synthesis of B subunits in this species. Thus further efforts to allocate B subunit genes by recombination mapping in bread wheat will be worth continuing.

Seven C subunits plus one major B subunit were found to be encoded by the *Glu-D3* locus as determined by the two-dimensional fractionations of NILs lacking this locus. Furthermore, the same locus was shown to control the synthesis of two subunits in the D zone of the SDS-PAGE pattern of glutenins. In the two-dimensional A-PAGE×SDS-PAGE map this subunit pair occurred in the ω-gliadin zone, suggesting a close homology in structure between D subunits and *Gli-D1*-encoded ω-gliadins. This conclusion is further supported by the occasional occurrence of this subunit pair in the A-PAGE×SDS-PAGE fractionations of unreduced, ethanol-soluble proteins (data not shown). Our results confirm the earlier findings of Masci et al. (1991, 1993) that D subunits appear to originate from a mutation of ω-gliadin genes such that at least one cysteine codon was produced. One interesting finding of the present study was that some *Gli-A2*-encoded α-gliadins occurred in the two-dimensional patterns of glutenin subunits. In this context it is worth noting that the C subunits have been shown to possess α- or γ-type sequences and an odd number of cysteine residues, at least one of these residues being available for inter-polypeptide disulphide linkages to form cross-linked proteins (Kasarda et al. 1988; Tao and Kasarda 1989).

The B and C subunits were found to be inherited in tightly linked clusters with no intra-locus recombination, confirming earlier reports using one-dimensional SDS-PAGE (Gupta and Shepherd 1993); the close linkage between the *Gli-1* and the *Glu-3* loci observed here is also in agreement with previous observations (Payne et al. 1984; Singh and Shepherd 1988; Pogna et al. 1990).

Several alleles at the *Glu-3* locus have been ranked with respect to their effect on dough resistance and extensibility (Gupta et al. 1989, 1991). In particular, allele *Glu-B3b* was shown to increase dough strength as compared to allele *c* (Gupta et al. 1991), confirming earlier reports on Italian bread-wheat cultivars by Pogna et al. (1982) and Dal Belin Peruffo et al. (1985). These authors found consistent relationships between the presence of γ-gliadin 43.5 and strong gluten properties, and between the presence of allelic γ-gliadin 40 and gluten weakness, these contrasting effects being attributed to allelic variation of LMW glutenin subunits linked to γ-gliadins 43.5 and 40. It has been recently assessed that most 43.5-type Italian cultivars possess allele *Glu-B3b* whereas 40-type varieties contain either the *Glu-B3c* or the *Glu-B3k* allele (unpublished data). In the present study, alleles *b* and *k* have been shown to produce different amounts of the B-type subunits (compare A and D in Fig. 6). This variation in quantity could affect both the size distribution and the quantity of the polymeric proteins and, thus, gluten strength (Gupta and MacRitchie 1994).

The effects of individual glutenin loci on gluten viscoelastic properties have been found to be largely cumulative (Payne et al. 1987; Gupta et al. 1989; Pogna et al. 1990). However, interaction between different alleles at the *Glu-1* and *Glu-3* loci was also shown to affect dough strength significantly (Gupta et al. 1994). In order to study the individual and combined effects (cumulative and epistatic) of glutenin alleles, special genotypes such as recombinant inbred lines, substitution lines and near-isogenic lines must be compared. The present NILs in cultivar Alpe are currently being used for this purpose.

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