Theor Appl Genet (1997) 94 : 788–795

E. V. Metakovsky · G. Branlard · V. M. Chernakov
V. P. Upelniek · R. Redaelli · N. E. Pogna

Recombination mapping of some chromosome 1A-, 1B-, 1D- and 6B-controlled gliadins and low-molecular-weight glutenin subunits in common wheat

Received: 9 July 1996/Accepted: 15 November 1996

Abstract Inheritance of low-molecular-weight glutenin subunits (LMW GS) and gliadins was studied in the segregating progeny from several crosses between common wheat genotypes. The occurrence of a few recombinants in the F_2 grains of the cross Skorospelka Uluchshennaya × Kharkovskaya 6 could be accounted for by assuming that the short arm of chromosome 1D contains two tightly linked loci each coding for at least one gliadin plus one C-type LMW GS. These loci were found to recombine at a frequency of about 2%, and to be linked to the *Glu-D3* locus coding for B-type LMW GS. Some proteins showing biochemical characteristics of D-type or C-type LMW GS were found to be encoded by the *Gli-B1* and *Gli-B2* loci, respectively. Strongly stained B-type LMW GS in cvs Skorospelka Uluchshennaya and Richelle were assigned to the *Glu-B3* locus, but recombination between this locus and *Gli-B1* was not found. Analogously, in the cross Bezostaya 1 × Anda, no recombination was found between *Gli-A1* and *Glu-A3,* suggesting the maximum genetic distance between these loci to be 0.97% ($P = 0.05$). A B-type LMW GS in cv Kharkovskaya 6 was assigned to the *Glu-B2* locus, with about 25% recombination from the *Gli-B1* locus. The present results suggested that alleles at *Gli* loci may relate to dough quality and serve as genetic markers of certain LMW GS affecting breadmaking quality.

Key words Wheat · Gliadin · Low-molecular-weight glutenins · Recombination

Communicated by G. Wenzel

R. Redaelli
Istituto Sperimentale per la Cerealicoltura, via Mulino, 3, 20079 S. Angelo Lodigiano (Milano) Italy

E. V. Metakovsky (✉) · G. Branlard
INRA Station d'Amelioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand CEDEX 02 France

V. M. Chernakov · V. P. Upelniek
N. I. Vavilov Institute of General Genetics, Gubkin St., 3, Moscow, B-333, Russia

N. E. Pogna
Sezione di Genetica Applicata, Istituto Sperimentale per la Cerealicoltura, via Cassia 176, 001000, Roma, Italy

Introduction

There are two main groups of proteins in wheat gluten: gliadins, which are monomeric proteins having only intramolecular disulphide bonds, if any, and glutenins, which can form polymers through intermolecular disulphide bonds between protein subunits (Kasarda 1989). High-molecular-weight (HMW) glutenins are coded by the *Glu-1* loci on the long arm of chromosomes of the first homoeological group, whereas low-molecular-weight glutenin subunits (LMW GS) are controlled by the *Glu-3* loci on the short arm of these chromosomes. The main gliadin-coding loci, *Gli-1* and *Gli-2*, are located on the short arms of the chromosomes of the first and sixth groups, respectively (Payne et al. 1984 a, b).

It is generally accepted that wheat dough quality is mainly determined by the glutenin fraction of gluten (Payne et al. 1981; Gupta and MacRitchie 1994; Gupta et al. 1995) and may be strongly influenced by the allelic state of their controlling genes (Payne et al. 1984a,1987; Gupta et al. 1989, 1991). Therefore, the identification of alleles at the glutenin coding loci has acquired important practical value.

Allelic variants of the *Glu-1* loci are rather easily detected by means of the SDS-electrophoretic procedure (Payne and Lawrence 1983). In contrast, analysis of LMW GS is difficult, because of their similarity in molecular weight to some gliadins, albumins and globulins, so that all these proteins may overlap in SDS electrophoretic patterns. Many glutenin preparations seem to be contaminated by gliadins (Kasarda 1989). Several procedures were

developed to avoid or decrease these contaminations and to obtain the best resolution of the LMW GS (Gupta and Shepherd 1990; Graybosch and Morris 1990; Gupta and MacRitchie 1991; Khelifi and Branlard 1991; Singh et al. 1991; Redaelli et al. 1995). Nevertheless, the complexity of the reliable definition of the LMW GS in electrophoretic patterns has resulted in a rather weak knowledge of their genetics.

Tight genetic linkages were shown between *Glu-3* and *Gli-1* so that, in common wheat, a few recombinants were found in the *Glu-B3*/*Gli-B1* complex only (Payne et al. 1984b, 1986; Singh and Shepherd 1988). Some LMW GS, especially the so called C-type and D-types, are similar to gliadins in their biochemical characteristics (Jackson et al. 1983; Payne et al. 1988) and even in their amino-acid sequences (Tao and Kasarda 1989; Lew et al. 1992; Masci et al. 1993). These LMW GS presumably resulted from gliadin-coding genes which, due to a mutation, acquired an odd number of cystein residues, could form intermolecular disulphide bonds, and therefore might be included in the glutenin fraction. Moreover, it was suggested that only B-type LMW GS are encoded by *Glu-3* loci, while gliadin-like LMW GS are, in fact, "former gliadins" and are controlled by the *Gli-1* and *Gli-2* complex loci (Lew et al. 1992). The relationship between *Gli-2* alleles and LMW GS patterns is not well studied, although there are some indications for the existence of *Gli-2*-controlled LMW GS (Gupta and Shepherd 1993; Pogna et al. 1995). Moreover, the *Gli-B3* locus, which is located between *Glu-B1* and *Gli-B1*, is claimed to control, in different allelic states, either gliadin, or D-type LMW GS (Payne et al. 1988). In tetraploid wheat, the *Glu-B2* locus encoding for B-type LMW GS was shown to be tightly linked to the *Gli-B3* locus (Liu and Shepherd 1995).

To better understand the genetics of LMW glutenins, we carried out a parallel inheritance study of different components in typical LMW GS patterns and of known gliadin alleles using segregating progenies of several genotypes.

Materials and methods

F_2 progenies from the following crosses of common wheat cultivars (abbreviations in brackets) were studied: Bezostaya 1 (B1) × Anda, Bezostaya 1 × Richelle (Rch), Tselinogradka (Ts) × Kazakhstanskaya 3 (K3), Skorospelka Uluchshennaya (SU) × Kharkovskaya 6 (Kh6), Kazakhstanskaya 126 (K126) × Saratovskaya 36 (S36). Glutenins were extracted from single seeds according to Singh et al. (1991) with modifications (Redaelli et al. 1995). One-dimensional SDS electrophoresis of glutenins was performed as described by Redaelli et al. (1995). For analysis of gliadins, acid (aluminium-lactate, pH 3.1) electrophoresis (APAGE) was used (Metakovsky and Novoselskaya 1991). To determine the molecular weight of particular gliadin components, two-dimensional separation of gliadins (APAGE × SDS-electrophoresis) was employed (Metakovsky et al. 1984). The pellet of extraction of prolamin by 70% ethanol, or single-seed flour, were sometimes used for the analysis of total protein according to Laemmli (1970). One-dimensional SDS electrophoresis of total protein was performed as described by Dachkevitch et al. (1993). The greatest expected genetic distance between two loci in the case of the absence of recombination between them was calculated using Hanson's (1959) formulae.

Results

Chromosome 1A-controlled LMW GS

B-zones of the SDS electrophoretic patterns of glutenins of B1 and Anda differed from each other in the presence of the components 3 and 4 (Fig. 1). Genes coding for these LMW GS were found to be allelic and segregated in F_2 grains of the cross B1 × Anda together with the corresponding *Gli-A1* alleles revealed by APAGE in these cultivars (*Gli-A1b* and *Gli-A1f*, respectively). No one recombinant was detected amongst the 153 F_2 grains studied, not even when gene dosage in heterozygous F_2 grains was considered. The maximum genetic distance between *Gli-A1* and *Glu-A3* calculated from this data was 0.97% ($P = 0.05$).

Apparently, the same two LMW GS segregated together with the *Gli-A1* alleles in the cross Ts (*Gli-A1f*) × K3 (*Gli-A1b*) (Fig. 2, bands 4 and 3, respectively).

No reliable polymorphism for chromosome 1A-controlled gliadins was found in other crosses studied here.

Chromosome 1B-controlled gliadins and LMW GS

The biotypes of cv Ts differ from each other in the presence or the absence of some ω-gliadins that are not controlled by *Gli-1* or *Gli-2* (Metakovsky et al. 1986). Comparison of the biotypes showed one of these "selfish" gliadins to have an apparent molecular weight of 47500. This polypeptide occurred in the SDS patterns of grain protein extracted under unreduced conditions (mercaptoethanol was omitted during the extraction procedure) (Fig. 3, band 5), and was absent in the

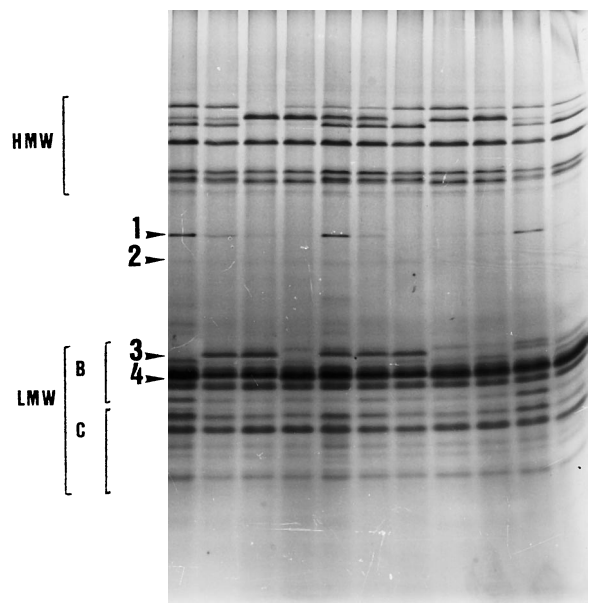

Fig. 1 Glutenin patterns of single F_2 grains from the cross Bezostaya 1 × Anda. *Bands 1 and 2* are *Gli-B1b*-controlled gliadins (*Gli-B1b* present in both parents); *bands 3 and 4* are LMW GSs controlled by *Glu-A3* of Bezostaya 1 and Anda, respectively. High-molecular-weight (HMW) and low-molecular-weight (LMW) GS are marked; B and C groups of LMW GS are also shown in the patterns

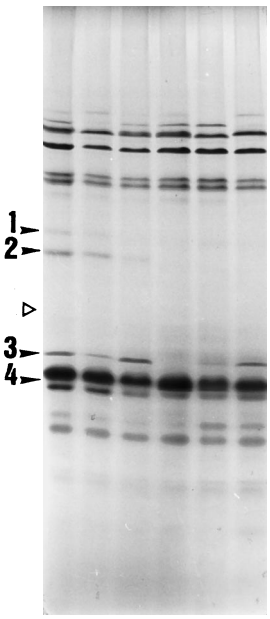

Fig. 2 Glutenin patterns of single F_2 grains from the cross Tselinogradka × Kazakhstanska ya 3. *Bands 1 and 2* are *Gli-B1e*-controlled proteins; *bands 3 and 4* are LMW GSs controlled by *Glu-A3* of Kazakhstanskaya 3 and Tselinogradka, respectively. An *open arrowhead* shows the expected position of the *Gli-B3*-controlled gliadin of Tselinogradka

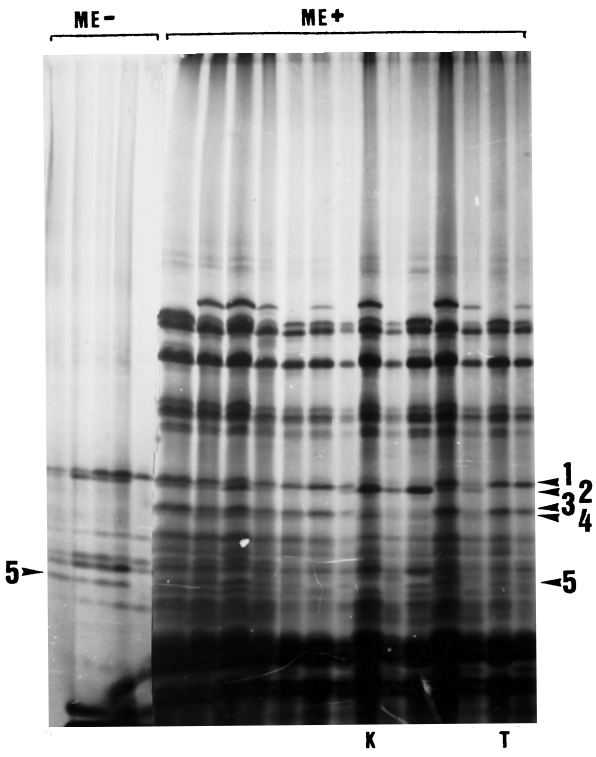

Fig. 3 SDS electrophoretic patterns of total grain proteins extracted from single grains of Tselinogradka (*T*), Kazakhstanskaya 3 (*K*) and F_2 grains of their cross. *Bands 1 and 3*, and *2 and 4*, are *Gli-B1e*-, and *Gli-B1b*-controlled proteins, respectively; *band 5* is the *Gli-B3*-controlled gliadin of Tselinogradka. *ME −* and *ME +* are unreduced and reduced conditions, respectively

glutenin patterns (Fig. 2). Gene controlling this protein recombined with *Gli-B1* with a frequency of 23.7 ± 2.8%. All these features are characteristic of a gliadin component encoded by the *Gli-B3* locus.

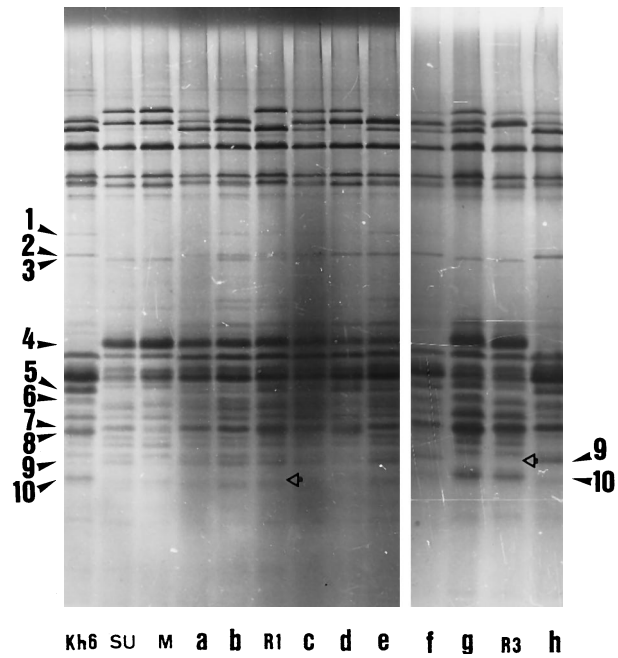

Fig. 4 Glutenin patterns of Skorospelka Uluchshennaya (*SU*), its spontaneous mutant lacking all *Gli-D1*-controlled gliadins (*M*), Kharkovskaya 6 (*Kh6*) and some F_2 grains of the cross SU × Kh6 including two recombinants at the *Gli-D1* complex locus (*R1* and *R3*). *Bands 1*, *2 and*, probably, *3* are *Gli-B1*-controlled; *band 4* is *Glu-B3*-controlled; *band 5* is *Glu-B2*-controlled; *band 6* is *Gli-B2*-controlled; *bands 7, 8, 9 and 10* are controlled by *Gli-D1* (or the *Glu-D3*/*Gli-D1* complex locus). *Open arrows* show bands lacking in the recombinant grains

Cv S36 had an APAGE band with apparently the same electrophoretic mobility as the *Gli-B3*-encoded gliadin of Ts. Indeed, in the cross K126 × S36, a gene encoding this band was found to recombine with *Gli-B1* with a frequency of 23.8 ± 7.5% (data not shown). Therefore, it is likely that this band is also controlled by *Gli-B3*. *Gli-B3*-controlled gliadin found in Ts and S36 is probably the same protein described as B30 by Galili and Feldman (1984), as component 3 by Metakovsky et al. (1986), and as gliadin N6 by Dachkevitch et al. (1993).

The B-zone of the LMW glutenin electrophoretic pattern of cv Kh6 includes component 5 (apparent molecular weight of 37500) that is absent in cv SU (Fig. 4). Analysis of F_2 grains from the cross of these cultivars (Table 1) showed the gene controlling this component to be located on the short arm of chromosome 1B at a distance of 24.8 ± 4.5% from *Gli-B1*. Taking into account that component 5 is a B-type LMW GS this protein is likely to be controlled by the *Glu-B2* locus described recently in tetraploid wheat (Liu and Shepherd 1995) thereby confirming the existence of this locus in common wheat also.

One chromosome 1B-controlled band (d4) appears in the SDS electrophoretic pattern of gliadins only when a 50% propanol

Table 1 Distribution of grains in different phenotypic classes in the cross Skorospelka Uluchshennaya × Kharkovskaya 6

Number	Penotypic classes			Number of grains
	Gli-B1e	*Gli-B1m*	LMW-GS 37 500	
1	+	+	+	52
2	+	−	+	30
3	−	+	−	12
4	+	+	−	13
5	−	+	+	13
6	+	−	−	0

extract of wheat flour is reduced before the electrophoretic separation (Branlard et al. 1993). It was suggested that this protein could be considered as a LMW GS, or an ω-gliadin associated with glutenins via S-S bonds. Our analysis of the gliadin allele compositions of 68 common wheat cultivars that had been used earlier, in particular for the identification of band d4 (Khelifi and Branlard 1992), showed that this band was always present in cultivars having *Gli-B1f*, *Gli-B1g*, or *Gli-B1e* alleles, and was absent in cultivars with other alleles at *Gli-B1* (data not shown). Each of these three alleles controls two ω-gliadins (Metakovsky 1991), the fast-moving of these two having an apparent molecular weight of 55 500 (Fig. 5a, spot 2), identical to that of band d4. To examine further peculiarities of this protein, F_2 grains of the cross between Ts and K3 were studied. These cultivars contain alleles *Gli-B1e* and *Gli-B1b*, respectively.

A parallel analysis of the same F_2 grains in APAGE and SDS gels allowed us to identify both *Gli-B1e*-controlled ω-gliadins in the SDS patterns. It was found that the faster ω-gliadin controlled by this allele was apparently absent in the SDS electrophoretic pattern of unreduced gliadins (Fig. 3, band 3), as was found earlier for band d4 (Branlard et al. 1993). It was also found that this protein appeared as a stronger band in the SDS electrophoretic pattern of glutenin (Figs. 2 and 4, band 2). The outstanding feature of this protein, however, is that having some characteristics of a glutenin subunit, it also occurs in the APAGE fractionation of unreduced alcohol-soluble proteins. An identity of the protein analysed in APAGE and glutenin patterns is further stressed by the absence of this component (band 2, Fig. 6) in the glutenin patterns of the spontaneous mutants lacking, in particular, the fast-moving ω-gliadin controlled by *Gli-B1e* (lanes b and f), but not the slower-moving one (lanes d and i). We conclude that this protein could be a D-type LMW GS controlled by *Gli-B1* (or by a tightly linked locus) which appears in the APAGE pattern when it is in a monomeric form.

In contrast, the two *Gli-B1b*-encoded ω-gliadins have the same relative intensities in both APAGE (Metakovsky 1991) and glutenin patterns (Fig. 1). Obviously, the two ω-gliadins controlled by this allele were not completely washed away during the glutenin purification procedure employed.

The electrophoretic pattern of glutenin of SU includes bands 3 and 4 which are absent in cv Kh6 (Fig. 4). In the F_2 grains of the cross of these two

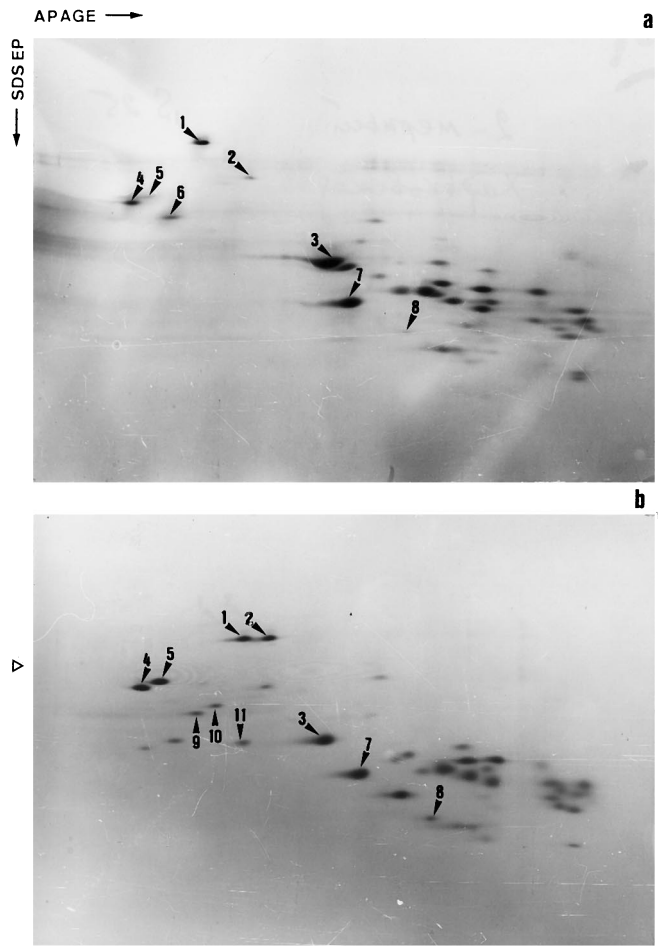

Fig. 5 Two-dimensional (APAGE/SDS PAGE) patterns of the cultivar Kharkovskaya 6 (**a**) and Skorospelka Uluchshennaya (**b**). *Spots 1,2 and 3* are *Gli-B1e*-controlled; *spots 4, 5, 6, 7 and 8* are *Gli-D1*-controlled; *spots 9 and 10* are *Gli-B5*-controlled; *spot 11* is *Gli-A3*-controlled. *Spot 2* in Kh6 is a LMW GS, or a d4 component; *spot 6* is an unusual gliadin (see the text). An *open arrowhead* shows the expected position of the LMW GS (band 3, Fig. 4)

cultivars, the gene for the very strong band 4 (B-type LMW GS) segregated together with allele *Gli-B1m* as revealed by APAGE in cv SU (Fig. 7) so that no one recombinant between them was detected amongst the 120 F_2 grains studied. Obviously, glutenin band 4 of SU is controlled by the *Glu-B3* locus. The faint band 3 of SU apparently segregated together with band 4. Band 3 is similar in molecular weight and staining intensity to LMW GS controlled by *Gli-B1e* (Fig. 4, band 2). Probably, band 3 is a D-type LMW GS encoded by the *Gli-B1* locus. However, a protein with the molecular weight of band 3 is not present in the two-dimensional pattern of gliadins of cv SU (Fig. 5b).

The glutenin pattern of cv Rch includes a strong LMW GS very similar to band 4 of SU. It is inherited together with *Gli-B1*h of Rch, without any one case of recombination in the 91 F_2 grains from the cross B1 × Rch analysed (data not shown). This band is a

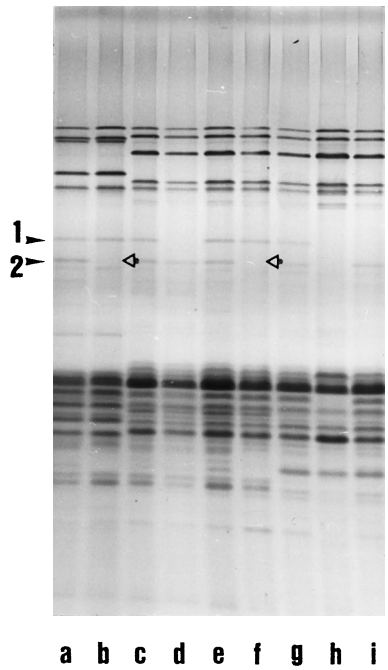

Fig. 6 Glutenin patterns of (*lanes a, b*) cv Leningradka, (*c, e, g*) different biotypes of cv Tselinnaya Ubileinaya and (*d, f, h, i*) their spontaneous mutants. The mutants lacking the fast-moving *Gli-B1*e-controlled ω-gliadin (*b, f*), the slow-moving *Gli-B1e*-controlled ω-gliadin (*d, i*), or all *Gli-B1e*-controlled gliadins (*h*) are shown. *Bands 1 and 2* the same as on Figs. 2 and 4. *Open arrows* show the absence of band 2 in the mutants

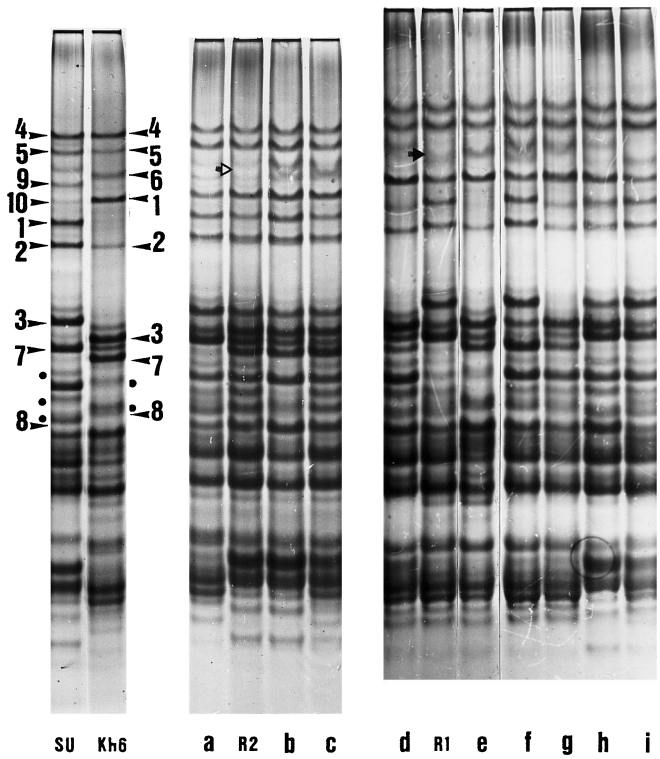

Fig. 7 APAGE patterns of the cultivar Skorospelka Uluchshennaya (*SU*), Kharkovskaya 6 (*Kh6*) and some F_2 grains of their cross including recombinants at *Gli-D1* (*R1 and R2*). The numbering of bands corresponds to that on Fig. 5. *Band 2* of Kh6 is LMW GS, or a d4 band (see the text). *Band 6* of Kh6 is an unusual gliadin (see the text). *Dots* show *Gli-B2*-controlled gliadins which were used for the identification of genotypes of the F_2 grains at *Gli-B2*. *Open and solid arrows* in the recombinant grains show the absence and presence, respectively, of the *Gli-D1* controlled bands

B-type LMW GS and, therefore, is most likely encoded by the *Glu-B3* locus. Taken together, the data from the crosses SU × Kh6 and B1 × Rch show that the distance between *Gli-B1* and *Glu-B3* does not exceed 1.42% ($P = 0.05$).

SU has two minor ω-gliadins controlled by *Gli-B5* (Pogna et al. 1993) in its APAGE and two-dimensional patterns (Fig. 5b and 7, components 9 and 10). In our work, no one recombinant was found between *Gli-B5* and *Gli-B1* (components 1, 2 and 3) in a study of 254 F_2 grains of the cross SU × Kh6. From this data, the maximum genetic distance between *Gli-B1* and *Gli-B5* is 1.18% ($P = 0.05$).

Chromosome 1D-controlled gliadins and LMW GS

SU is the sole common wheat cultivar having allele *Gli-D1*c (Metakovsky 1991) which controls the strong γ-gliadin band 7 in the APAGE pattern (Fig. 7), whereas cv Kh6 has allele *Gli-D1i* the main characteristic of which is the presence of a particular ω-gliadin (component 6, Figs. 5a and 7) that often has a horseshoe-like form in APAGE (Fig. 7, lanes b, c, f, and g). These cultivars differ from each other in the pattern of the C group of LMW GS: SU contains bands 7 and 9, and Kh6 has bands 8 and 10 (Fig. 4). Bands 7 and 9 are controlled by the *Gli-D1* locus (or by a locus linked to *Gli-D1*) because they are absent in a spontaneous mutant lacking all the *Gli-D1*-controlled APAGE bands of SU (Fig. 4, lane M). The apparent molecular weights of all chromosome 1D-controlled electrophoretic components analysed in the cross SU × Kh6 are shown in Table 2.

As a result of the analysis of 120 F_2 progenies, it was found that, as a rule, all four electrophoretic components of each parental cultivar (see Table 2) were inherited together, as a block. Moreover, these two protein blocks were found to be allelic (data not shown). However, there were three probable recombinants. One recombinant had gliadin 6 and glutenin 8 from Kh6 in the absence of gliadin 7 and glutenin 10 from the same cultivar (Fig. 4 and Fig. 7, lane R1); whereas the second recombinant contained gliadin 7 and glutenin 10 and lacked gliadin 6 and glutenin 8 (Fig. 7, lane R2). The third recombinant lacked only glutenin 9 from cv SU (Fig. 4, lane R3). Two-dimensional separation of the gliadin of the second recombinant, together with appropriate control pattern, is shown in Fig. 8.

Table 2 Apparent molecular weights of the electrophoretic components analysed in the cross Skorospelka Uluchshennaya (SU) × Kharkovskaya 6 (Kh6)

Component	Type of electrophoresis	Cultivar	Apparent molecular weight
7	APAGE of gliadins	SU	36 500
8	APAGE of gliadins	SU	31 500
7	SDS PAGE of glutenins	SU	34 000
9	SDS PAGE of glutenins	SU	30 000
6	APAGE of gliadins	Kh6	48 500
7	APAGE of gliadins	Kh6	35 000
8	SDS PAGE of glutenins	Kh6	33 500
10	SDS PAGE of glutenins	Kh6	29 000

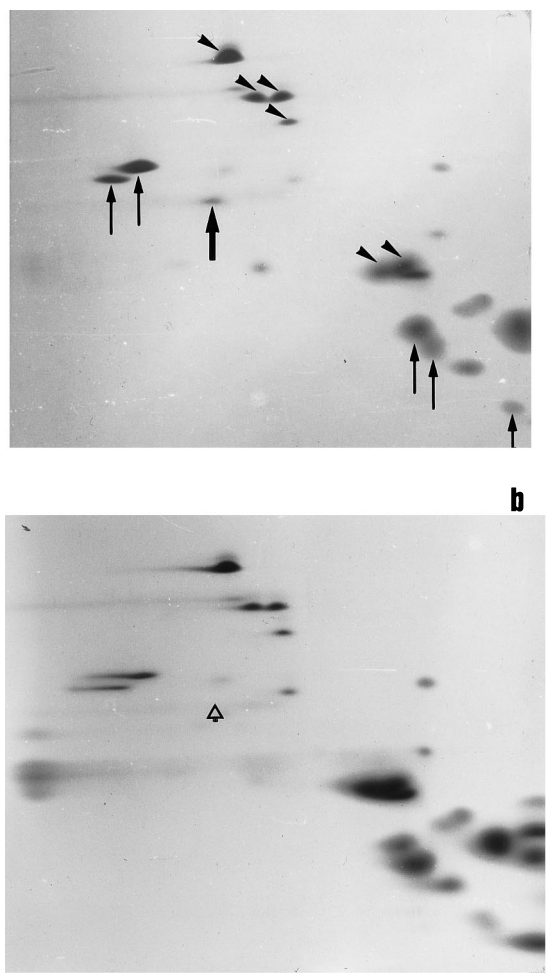

Fig. 8 Two-dimensional (APAGE/SDS PAGE) patterns of the second recombinant at the *Gli-B1/Glu-D3* complex locus found between F_2 seeds of the cross Skorospelka Uluchshennaya × Kharkovskaya 6 (**b**), and of the appropriate control F_2 grain of this cross (**a**). *Gli-D1*-, and *Gli-B1*-controlled components are shown by *vertical thin arrows*, and *arrowheads*, respectively. Component 6 which is absent in the recombinant (*open arrow*) is marked by a *thick arrow* in the control pattern

The gliadin and glutenin polypeptides analysed here showed contrasting molecular weights (Table 2) and are therefore encoded by different genes. The present results indicate a complex organization of the storage protein genes on the short arm of chromosome 1D. In cv Kh6, this chromosome contains at least two separate loci, one coding for gliadin (or D-type subunit of LMW) 6 and C-type subunit 8 with the other encoding γ-gliadin 7 and C-type subunit 10. The *Glu-D3* locus controlling B-type LMW GS should also occur nearby because it is tightly linked to *Gli-D1* (Singh and Shepherd 1988).

Chromosome 6B-controlled gliadins and LMW GS

Inheritance of LMW GS 6 (apparent molecular weight of 37 000) of cv Kh6 (Fig. 4) was analysed in 54 F_2 grains of the cross SU × Kh6. It was found that the presence and intensity of this component strongly correlated with the presence of the *Gli-B2* allele of Kh6 as revealed by APAGE. In particular, 16 grains homozygous for this allele showed a strong band 6 (Fig. 7, lane e; Fig. 4, lanes b,g) and 13 grains homozygous for the *Gli-B2* allele from SU lacked this band (Fig. 7, lane h; Fig. 4, lanes f, h). The remaining 25 grains were heterozygotes at *Gli-B2* and showed a relatively faint band 6 (for example, Fig. 7, lanes a, R1, e; Fig. 4, lanes R1, c, R3). Segregation of band 6 was found to be independent from each of the other five main *Gli* loci (data not shown). The molecular weight of component 6 is very similar to that of some *Gli-B2*-controlled gliadins in the two-dimensional separations of Kh6 (Fig. 5a) and other cultivars (Metakovsky et al. 1984).

Discussion

The inheritance of some LMW GS were studied using segregating progenies (Payne et al. 1984a; Singh and Shepherd 1988; Khelifi and Branlard 1991, 1992) and recombinant isogenic lines (Gupta and MacRitchie 1994; Pogna et al. 1995; Redaelli et al. 1995). However, it was noticed (Gupta and Shepherd 1990; Redaelli et al. 1995) that some components of the LMW GS electrophoretic patterns have inconsistent behaviour and, therefore, are difficult to analyse. The positive identification of individual alleles of *Glu-3* remains difficult (Singh et al. 1991). To simplify a procedure for the identification of LMW glutenin alleles, it was suggested (Singh et al. 1991; Gupta et al. 1994) to use alleles at the gliadin-coding *Gli-1* loci as genetic markers for particular *Glu-3* alleles because of the tight genetic linkage of *Gli-1* and *Glu-3* (Payne et al. 1984a, b, 1986; Singh and Shepherd 1988). Alleles at the gliadin loci, *Gli-1* and *Gli-2*, can be more easily revealed by means of the APAGE procedure (Metakovsky 1991).

The recent findings of Tao and Kasarda (1989) and Lew et al. (1992) suggest that some LMW GS are, in fact, slightly modified gliadins encoded by the *Gli* loci. One might suggest, that, in this case, genes controlling gliadins and C-type and D-type LMW GS are interspersed inside the *Gli*-1 locus. Our results on chromosome 1B- and 1D-controlled gliadins and LMW GS confirm this suggestion. In addition, we have found one LMW GS controlled by *Gli-B2* (or by a locus tightly linked to *Gli-B2*).

An ω-gliadin with a molecular weight of 55 500 was shown to belong to a block of gliadin components controlled by the *Gli-B1e* allele as fractionated by APAGE (Metakovsky 1991). This protein has the same molecular weight as band d4 described by Branlard et al. (1993). Band d4 was found to occur in SDS-PAGE fractionation of an alcohol extract of wheat flour only when proteins are reduced before electrophoresis (Branlard et al. 1993; this work). Further, in some mutant seeds analysed in the present paper, the *Gli-B1e*-encoded gliadin and band d4 disappeared simultaneously from the APAGE pattern of gliadins and the SDS PAGE pattern of glutenins, respectively. To explain these results, one must suggest that either there are two different tightly linked genes, the first controlling an ω-gliadin, and the second encoding for a D-type subunit of the same size, or, more likely, that some D-type LMW GS such as band d4 can be seen in the APAGE pattern of alcohol-soluble proteins. This unusual ω-gliadin is controlled by several *Gli-B1* alleles that occur in the world-common wheat germ plasm (Metakovsky 1991). According to Khelifi and Branlard (1992), band d4 may relate to dough quality. Polypeptides similar to band d4 are encoded by *Gli-B1m* (for an example, band 3 in Fig. 4) and related alleles, but they do not occur in the APAGE fractionation of gliadins. In contrast, allele *Gli-B1b* seems not to encode any LMW GS of this type. This difference may be responsible for the known positive influence of *Gli-B1b* on dough quality (Sozinov and Poperelya 1980; Metakovsky et al. 1996). Band d4, and proteins similar to it, could be originated from gliadins by acquiring one extra cysteine residue and therefore become the chain-terminating-type proteins acting to limit the growing of glutenin polymers and thereby decreasing dough quality (Kasarda 1989). This mechanism may be also responsible for the difference of *Gli-2* alleles in relation to dough quality: alleles at a particular *Gli-2* locus may differ, for example, in the number of encoded LMW GS of the chain-terminating type.

Genes at the *Glu-A3* and *Glu-B3* loci code for B-type LMW GS with molecular weights (about 40 000–42 000) similar to those of the major γ-gliadins controlled by *Gli-A1* and *Gli-B1* (for an example, component 4, Fig. 4). However, these genes are different from those coding for gliadins as suggested by the fact that cultivars with the same allele at *Gli-A1* (or *Gli-B1*) may have contrasting B-type subunit compositions (unpublished results).

In general, our results confirm the idea that some LMW GS are encoded by the *Gli* loci (Lew et al. 1992). Genes coding for gliadins or LMW GS may be interspersed along a chromosome region. This complicates the genetic analysis of LMW GS which relate to quality, as well as the nomenclature of storage proteins in wheat. However, it clarifies the role of *Gli* alleles in infuencing dough quality and indicates that *Gli* alleles may indeed serve as markers for some quality related LMW glutenins.

References

Branlard G, Dardevet M, Nieto-Taladiz MT, Khelifi D (1993) Allelic diversity of the omega gliadins as revealed by SDS-PAGE: their possible implication in quality variation. In: Gluten proteins 1993. Proc Vth Int Wheat Gluten Workshop, Detmold, Germany; Ass Cereal Res, pp 234–243

Dachkevitch T, Redaelli R, Biancardi AM, Metakovsky EV, Pogna NE (1993) Genetics of gliadins coded by the group-1 chromosomes in the high-quality bread wheat cultivar Neepawa. Theor Appl Genet 86 : 389–399

Galili G, Feldman M (1984) Mapping of glutenin and gliadin genes located on chromosome 1B of common wheat. Mol Gen Genet 193 : 293–298

Graybosch RA, Morris R (1990) An improved SDS-PAGE method for the analysis of wheat endosperm storage proteins. J Cereal Sci 11 : 201–212

Gupta RB, MacRitchie F (1991) A rapid one-step one-dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. J Cereal Sci 14 : 105–109

Gupta RB, MacRitchie F (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1* of common wheats. II.Biochemical basis of the allelic effects on dough properties. J Cereal Sci 19 : 19–29

Gupta RB, Shepherd KW (1990) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. 1.Variation and genetic control of the subunits in hexaploid wheats. Theor Appl Genet 80 : 65–74

Gupta RB, Shepherd KW (1993) Production of multiple wheat-rye 1RS translocation stocks and genetic analysis of LMW subunits of glutenin and gliadins in wheats using these stocks. Theor Appl Genet 85 : 719–728

Gupta RB, Singh NK, Shepherd KW (1989) The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theor Appl Genet 77 : 57–64

Gupta RB, Bekes F, Wrigley CW (1991) Prediction of physical dough properties from glutenin subunit composition in bread wheats: correlation studies. Cereal Chem 68 : 328–333

Gupta RB, Metakovsky EV, Wrigley CW (1994) The relationship between LMW – glutenin subunit and gliadin alleles in Australian wheat cultivars. In: Gluten proteins 1993, Proc Vth Int Wheat Gluten Workshop, Detmold, Germany; Ass Cereal Res, pp 539–548

Gupta RB, Popineau Y, Lefebvre J, Cornec M, Lawrence GJ, MacRitchie F (1995) Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low Mr or high Mr glutenin subunits. J Cereal Sci 21 : 103–116

Hanson WD (1959) Minimum family sizes for the planning of genetic experiments. Agron J 51 : 711–715

Jackson EA, Holt LM, Payne PI (1983) Characterization of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. Theor Appl Genet 66 : 29–37

Kasarda DD (1989) Glutenin structure in relation to wheat quality. In: Pomeranz Y (ed) Wheat is unique. AACC, St Paul, Minnesota, pp 299–302

Khelifi D, Branlard G (1991) A new two-step electrophoresis method for analysing gliadin polypeptides and high- and low-molecular-weight subunits of glutenin of wheat. J Cereal Sci 13 : 41–47

Khelifi D, Branlard G (1992) The effects of HMW and LMW subunits of glutenin and gliadins on the technological quality of progeny from four crosses between poor breadmaking quality and strong wheat cultivars. J Cereal Sci 16 : 195–209

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 : 680–685

Lew EJL, Kuzmicky DD, Kasarda DD (1992) Characterization of low-molecular-weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino-acid sequencing. Cereal Chem 69 : 508–515

Liu CY, Shepherd KW (1995) Inheritance of B subunits of glutenin and ω- and γ-gliadins in tetraploid wheats. Theor Appl Genet 90 : 1149–1157

Masci S, Lafiandra D, Porceddu E, Lew EJL, Tao HP, Kasarda DD (1993) D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal Chem 70 : 581–585

Metakovsky EV (1991) Gliadin allele identification in common wheat. II.Catalogue of gliadin alleles in common wheat. J Genet Breed 45 : 325–344

Metakovsky EV, Novoselskaya AYu (1991) Gliadin allele identification in common wheat. 1.Methodological aspects of the analysis of gliadin pattern by one-dimensional polyacrylamide-gel electrophoresis. J Genet Breed 45 : 317–324

Metakovsky EV, Novoselskaya AYu, Sozinov AA (1984) Genetic analysis of gliadin components in winter wheat using two-dimensional polyacrylamide-gel electrophoresis. Theor Appl Genet 69 : 31–37

Metakovsky EV, Akhmedov MG, Sozinov AA (1986) Genetic analysis of gliadin-encoding genes reveals gene clusters as well as single remote genes. Theor Appl Genet 73 : 278–285

Metakovsky EV, Annicchiarico P, Boggini G, Pogna NE (1997) Relationship between gliadin alleles and dough strength in Italian bread wheat cultivars. J Cereal Sci (in press)

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11 : 29–35

Payne PI, Corfield KG, Holt LM, Blackman JA (1981) Correlations of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J Sci Food Agric 32 : 51–60

Payne PI, Holt LM, Jackson EA, Law CN (1984a) Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Phil Trans R Soc Lond, Ser B304 : 359–371

Payne PI, Jackson EA, Holt LM, Law CN (1984b) Genetic linkage between endosperm storage protein genes on each of the short arms of chromosomes 1A and 1B in wheat. Theor Appl Genet 67 : 235–243

Payne PI, Roberts MS, Holt LM (1986) Location of genes controlling the D group of LMW glutenin subunits on chromosome 1D of bread wheat. Genet Res 47 : 175–179

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agric 40 : 51–65

Payne PI, Holt LM, Lister PG (1988) *Gli-A3* and *Gli-B3*, two newly designated loci coding for omega-type gliadins and D-subunits of glutenin. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp Cambridge, 1988, vol 2, pp 999–1002

Pogna NE, Metakovsky EV, Redaelli R, Raineri F, Dachkevitch T (1993) Recombination mapping of *Gli-5*, a new gliadin-coding locus on chromosomes 1A and 1B in common wheat. Theor Appl Genet 87 : 113–121

Pogna NE, Redaelli R, Vaccino P, Biancardi AM, Peruffo ADB, Curioni A, Metakovsky EV, Pagliaricci S (1995) Production and genetic characterization of near-isogenic lines in the bread-wheat cultivar Alpe. Theor Appl Genet 90 : 650–658

Redaelli R Morel MH, Autran JC, Pogna NE (1995) Genetic analysis of low-Mr glutenin subunits fractionated by two-dimensional electrophoresis (A-PAGE × SDS-PAGE). J Cereal Sci 21 : 5–13

Singh NK, Shepherd KW (1988) Linkage mapping of genes controlling endosperm storage proteins in wheat. 1.Genes on the short arms of group-1 chromosomes. Theor Appl Genet 75 : 628–641

Singh NK, Shepherd KW, Cornish GB (1991) A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J Cereal Sci 14 : 203–208

Sozinov AA, Poperelya FA (1980) Genetic classification of prolamines and its use for plant breeding. Ann Technol Agric 29 : 229–245

Tao HP, Kasarda DD (1989) Two-dimensional gel sequencing of LMW-glutenin subunits. J Exp Bot 40 : 1015–1020