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Chromosome 1B-encoded Gliadins and Glutenin Subunits in Durum Wheat: Genetics and Relationship to Gluten Strength

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The progenies of crosses between Berillo and four durum wheat cultivars were analysed for storage protein composition (by four different electrophoresis procedures), genetic segregation and gluten quality (by SDS sedimentation test and Viscoelastograph). The crosses enabled the segregation patterns of alleles at *Gli-B1*, *Glu-B3* and *Glu-B1* on chromosome 1B, and at *Gli-A2* on chromosome 6A to be determined. The gene order on chromosome 1B was deduced to be *Glu-B1*–centromere–*Glu-B3*–*Gli-B1*, with 47% recombination between *Glu-B1* and *Glu-B3*, and 2% between *Glu-B3* and *Gli-B1*. Genes coding for γ-gliadins at *Gli-B1* were distal to ω-gliadin genes with respect to the centromere. Analyses of the progeny (F_4 grains) from single F_2 plants, indicated that gliadins γ-42 and γ-45 are only genetic markers of quality, whereas allelic variation for low molecular weight (LMW) glutenin subunits encoded at the *Glu-B3* locus is primarily responsible for differences in SDS sedimentation volume and gluten viscoelastic properties. High molecular weight (HMW) glutenin subunits 7+8 also gave larger SDS sedimentation volumes and higher gluten elastic recoveries than subunits 6+8 and 20. The positive effects of the so-called LMW-2 glutenin subunits and HMW subunits 7+8 were additive, with LMW-2 being the most important proteins for pasta-making quality as evaluated by SDS-sedimentation and gluten viscoelasticity (both parameters related to firmness of cooked pasta). Two alleles at *Gli-A2* coding for α-gliadins were also found to have different effects on gluten firmness.

Introduction

In a previous study[1], gluten extracted from the Italian durum wheat cultivar Berillo, which contains γ-gliadin 42 associated with the so-called LMW-2 subunits of glutenin, was shown to have high elastic recovery and gluten firmness similar to cultivars with γ-gliadin 45 and LMW-2 glutenin subunits. This result suggested that the strong association observed between γ-gliadin 45 and gluten strength[2–4] is not functional and that variation in the quantity and type of low molecular weight (LMW) glutenin subunits can strongly influence gluten viscoelastic properties.

This variation is due to allelic differences at the complex locus *Glu-B3*[5], which is

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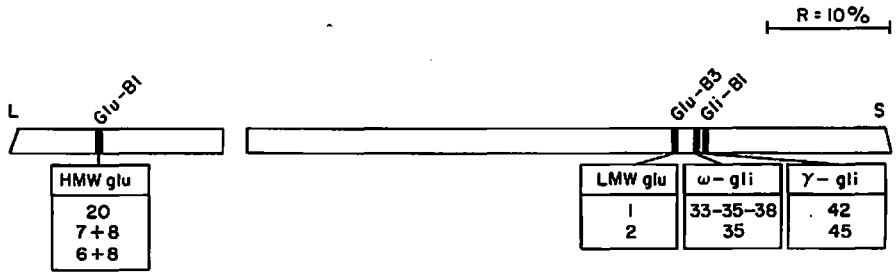

FIGURE 1. Genetic map of chromosome 1B showing the three storage-protein loci and their distances in terms of the proportion (%) of recombination. The alleles at *Glu-B3* and *Gli-B1* are also described. Abbreviations: R = recombination, glu = glutenin, gli = gliadin, S = short arm, L = long arm.

located very near to another complex locus *Gli-B1* on the short arm of chromosome 1B (see Fig. 1). Allelic γ-gliadins 42 and 45 are encoded at the *Gli-B1* locus[6], which also controls the synthesis of ω-gliadins. In commercial cultivars γ-gliadin 45 is associated with ω-gliadin 35 and the LMW glutenin subunit triplet referred to as LMW-2[7], whereas γ-gliadin 42 is genetically linked to ω-gliadin triplet 33–35–38 and LMW glutenin subunit quadruplet LMW-1. The Berillo genotype (γ-42, ω-35 and LMW-2) is thought to be the result of a rare recombination event within the *Gli-B1* locus.

In bread wheat, analysis of both random lines from many crosses and cultivars from different wheat-growing areas of the world[8–11] has shown that the different alleles at the *Glu-1* locus, coding for high molecular weight (HMW) glutenin subunits[12], have contrasting effects on SDS-sedimentation volume and dough viscoelastic properties. In the case of durum wheat, examination of commercial cultivars and breeding lines gave conflicting results with regard to the relationship between allelic variation in HMW glutenin subunits encoded at the *Glu-B1* locus (chromosome 1B) and gluten viscoelasticity and strength[4,13,14].

The study reported here was undertaken to determine the effects of both LMW and HMW glutenin subunits on gluten properties in random lines from crosses between cultivar Berillo and four Italian durum cultivars. At the same time these lines were analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS–PAGE) or lactic acid (A–PAGE) to determine the genetic linkage between *Gli-B1*, *Glu-B1* and *Glu-B3*, and the orientation of *Gli-B1* and *Glu-B3*, with respect to the centromere.

Experimental

Production of random lines

Crosses were made by standard procedures in the glasshouse between the durum wheat cultivar Berillo and four Italian cultivars, Creso, Valforte, Trinakria and Latino. Cultivar Berillo contains γ-gliadin 42 and ω-gliadin 35 (*Gli-B1* locus), LMW-2 glutenin subunits (*Glu-B3*) and HMW glutenin subunit 20 (*Glu-B1*). Creso (HMW glutenin subunits 6+8), Valforte (HMW 7+8) and

Trinakria (HMW 20) all possess γ-gliadin 45, ω-gliadin 35 and LMW-2 subunits, whereas Latino contains γ-gliadin 42 and ω-gliadins 33, 35, 38 (*Gli-B1*), LMW-1 subunits (*Glu-B3*), and HMW subunits 7+8 (*Glu-B1*). HMW glutenin subunits were classified according to Payne and Lawrence[15]. Two Berillo biotypes with different alleles at the *Gli-A2* locus (Fig. 2) were used in the crosses. More than 140 F_2 grains from each cross were grown in the glasshouse in large pots to obtain as much F_3 grain from each line as possible.

Electrophoretic analyses

Gliadins were extracted from flour (60 mg, obtained from eight crushed grains of each F_2 spike, i.e. F_3 seeds) for 1 h at room temperature with aqueous 35% (v/v) ethanol, 30% (w/v) glycerol, 0·03% (w/v) pyronine G (250 µl). After centrifugation for 5 min at 20000 × *g*, a portion (25 µl) of the clarified supernatant was fractionated at pH 3·1 in a 7·5% acrylamide gel (A–PAGE) and stained as previously described[16]. Another portion (75 µl) of supernatant was transferred to a fresh tube for further analyses.

To reveal the LMW and HMW glutenin subunits, the residual flour-ethanol mixture was mixed with 1·25 ml of an extraction buffer [water (1·0 ml) plus 2-mercaptoethanol (0·1 ml) plus a stock solution containing 0·2 M Tris–HCl, pH 6·8, 7% (w/v) SDS, 30% (v/v) glycerol (0·4 ml)]. The samples were incubated at room temperature for 1·5–2 h, at 80 °C for 30 min and then centrifuged. Proteins were fractionated by SDS–PAGE according to Laemmli[17] with minor modifications[18]. Ethanol-extracted proteins were fractionated in two dimensions using two procedures. In both procedures, separation in the first dimension was at pH 3·1 (A–PAGE). The second dimension was either SDS–PAGE using 10% gels as described by Payne *et al.*[19] or electrophoresis at pH 9·2 (B–PAGE) as described previously[20].

Multiplication of seeds for SDS sedimentation and rheological tests

Forty seeds from each F_2 spike (F_3 seeds) were sown in a single row, 8 cm apart within the row and 25 cm between rows; seeds were sown in November 1987 at Catania (Sicily, Italy) and Montpellier (France). The plants from each row were harvested by hand and threshed. After genotypic classification based on the storage protein alleles present at the *Glu-B1*, *Glu-B3*, *Gli-B1* and *Gli-A2* loci, the F_4 seed samples from Catania and Montpellier were used for SDS-sedimentation tests and viscoelastic measurements, respectively. Progeny with the same genotype were treated as replicates in the analyses of variance (ANOVA).

SDS-sedimentation test and viscoelastic test

Grain from each row was milled at 2 g/s grinding rate using a Udy Cyclone Mill (Tecator AB, Sweden), fitted with a 0·5 mm sieve. The whole wheat meal samples were then used for the SDS-sedimentation test developed for durum wheat[21]. Measurements of gluten firmness and elastic recovery were carried out with gluten from purified semolina (Brabender Junior mill) according to Damidaux *et al.*[2] After extraction, wet gluten (1 g) was placed in a moulding cell and immersed for 90 s in boiling water. Gluten elastic recovery and gluten firmness were determined on the resulting gluten disc using a Viscoelastograph (Tripette Renaud – Chopin, France).

Results

Electrophoretic analyses of the parental cultivars

The cultivar Berillo was found to comprise two main biotypes with respect to α-gliadin composition. Both biotypes contained the *Gli-B1* encoded gliadins ω-35 and γ-42 as well as a faint band γ-41 arrowed in Fig. 2(A) (lanes 1 and 2). Berillo biotype 1 [Fig. 2(A),

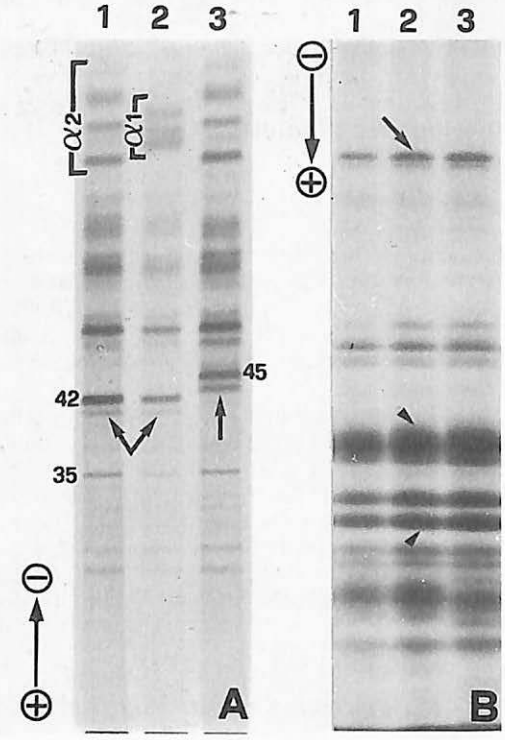

FIGURE 2. A–PAGE of gliadins (A) and SDS–PAGE of total proteins (B) extracted from (1) biotype 2, (2) biotype 1 and (3) biotype 3 of the durum wheat cultivar Berillo. In (A) γ-gliadin 41 is indicated by diagonal arrows and γ-gliadin 43 by a vertical arrow. In (B) HMW subunit 20 is arrowed and LMW-2 subunits of glutenin are marked by arrowheads. α-1 and α-2 are two allelic groups of α-gliadins.

lane 2] also contained a group of three strong α-gliadins with similar electrophoretic mobilities (α-1 gliadins), whereas Berillo biotype 2 [Fig. 2(A), lane 1] showed six α-gliadins with a wider range of electrophoretic mobilities (α-2 gliadins). A few seeds (Berillo biotype 3) were also found to possess γ-gliadin 45, ω-gliadin 35, α-2 gliadins and a 1B- encoded gliadin 43 arrowed in Fig. 2(A), lane 3. All three biotypes contained the same storage protein alleles at the *Gli-A1, Gli-B2, Glu-B3, Glu-A1* and *Glu-B1* loci [Fig. 2(B)].

In two dimensional fractionation (A–PAGE in the first dimension and SDS–PAGE in the second), the α-1 gliadin components from Berillo 1 appeared as eight spots with similar molecular weights [Fig. 3(A)], whereas the gliadin pattern of the parental cultivar Latino contained γ-gliadins 41 and 42, ω-gliadins 33,35 and 38, and α-1 gliadins [Fig. 3(C)]. Two dimensional fractionation of a 1:1 mixture of gliadin extracts from Berillo 1 and Latino [Fig. 3(B)] showed that γ-41 and γ-42 gliadins in Berillo 1 have the same electrophoretic mobilities and molecular weights as their counterparts in the regular γ-

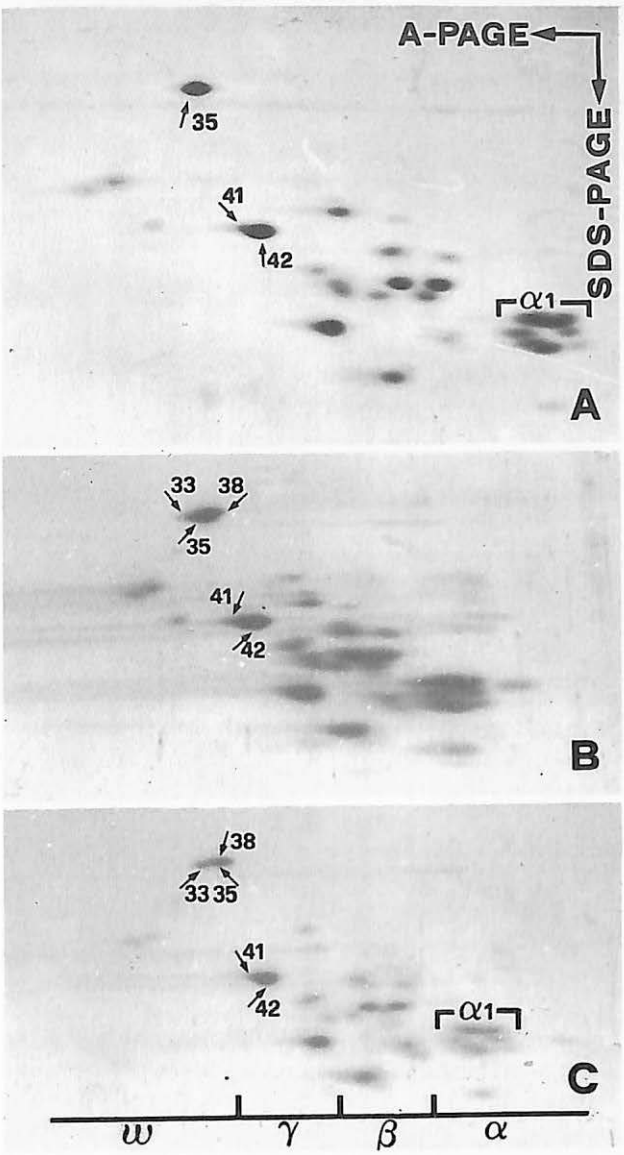

FIGURE 3. Fractionation, by A–PAGE in the first dimension and SDS–PAGE in the second, of gliadins from (A) Berillo 1, (B) 1:1 mixture of Berillo 1 and Latino and (C) Latino; ω- and γ-gliadins encoded at *Gli-B1* are arrowed. The α-1 group of gliadins encoded at *Gli-A2* is also shown.

42 type cultivar Latino and that ω-35 has the same apparent molecular weight as the ω-gliadin triplet 33–35–38.

Gliadin proteins of the parental cultivars Trinakria, Latino, Creso and Berillo 2 were also fractionated by two dimensional electrophoresis using A–PAGE in the first

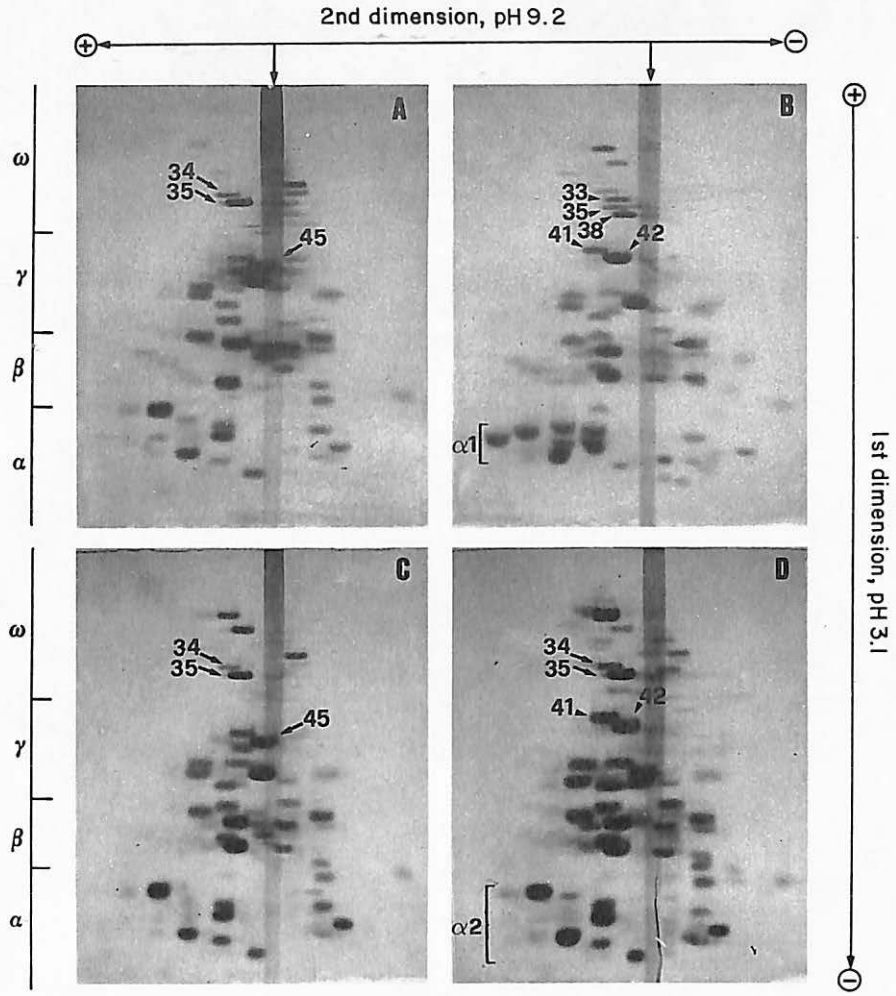

FIGURE 4. Fractionation, by A–PAGE in the first dimension and B–PAGE in the second, of gliadins from (A) Trinakria, (B) Latino, (C) Creso and (D) Berillo 2. Arrows and arrowheads indicate *Gli-B1* encoded gliadins found in type-45 and type-42 cultivars respectively; α-1 and α-2 gliadin groups are also shown.

dimension and B–PAGE in the second [Fig. 4(A), (B), (C) and (D) respectively]. Berillo 2 contained six prominent α-gliadin polypeptides (α-2 gliadins), which were also present in Trinakria and Creso [Fig. 4(A) and (C)]. This fractionation technique also showed a faint band, ω-34, which was hardly detectable by one dimensional A–PAGE separation [Fig. 4(A) (C) and (D)]. ω-Gliadins 34 and 35, and γ-gliadins 41 and 42 in Berillo 2 had the same electrophoretic mobilities as their counterparts in Trinakria, Latino and Creso.

TABLE I. Endosperm proteins analysed for linkage in three crosses involving the cultivar Berillo

				Protein type[a]	
	Parent A	Parent B	Locus	Parent A	Parent B
(1)	Berillo 2	Latino	*Gli-B1*	ω-35	ω–33–35–38
			Glu-B3	LMW-2	LMW-1
			Glu-B1	HMW 20	HMW 7+8
			Gli-A2	α-2	α-1
(2)	Berillo 1	Valforte	*Gli-B1*	γ-42	γ-45
			Glu-B1	HMW 20	HMW 7+8
(3)	Berillo 2	Creso	*Gli-B1*	γ-42	γ-45
			Glu-B1	HMW 20	HMW 6+8

[a] α-, γ- and ω-gliadins, high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits.

Genetics of storage proteins

(1) *Berillo 2 × Latino*. The parents in this cross differ from each other with respect to alleles at the *Gli-B1, Glu-B3, Glu-B1* and *Gli-A2* loci (Table I). Typical gliadin patterns obtained from seed progeny of single F_2 plants are shown in Fig. 5; the segregation of gliadins ω-35, ω-33-35-38, α-1 and α-2 can be followed unambiguously. For example, the pattern of lane 8 was obtained from the progeny of an F_2 plant heterozygous for α-1 and α-2 gliadins and homozygous for ω-gliadin triplet 33–35–38, whereas the pattern of lane 2 was obtained from the progeny of an F_2 plant homozygous for α-1 gliadins and heterozygous for ω-gliadins. In order to identify progeny heterozygous for alleles at *Gli-B1* the relative intensity of ω-gliadin 35 was checked carefully. In the parental cultivar 'Latino' (lane 10) and in progeny homozygous for ω–33–35–38, band 35 was fainter than band 33, whereas in the heterozygotes (lanes 1 to 4) band 35 was stronger than band 33 because of overlap with ω-gliadin 35 from Berillo. No segregation was found for γ-gliadins 42 and 41. The two parents have different LMW and HMW subunits of glutenin, and, therefore, their progeny were analysed by SDS–PAGE. In Fig. 6 the HMW subunit 20 from Berillo 2 is indicated by diagonal arrows and the Latino subunits 7+8 by vertical arrows; LMW-2 subunits from Berillo 2 are marked with arrowheads on the right-hand side and LMW-1 subunits from Latino are arrowed on the left-hand side. The patterns shown in lanes 3, 5 and 11 are for F_2 plants heterozygous for both LMW and HMW subunits. The combined results for the 153 progeny of this cross showed that the ratios of the phenotypic classes for ω-gliadins, α-gliadins, HMW and LMW glutenin subunits agreed well with the expected 1:1:2 if two codominant alleles at single loci controlled the synthesis of these proteins (Table II). As expected no significant linkage was found between genes coding for ω-gliadins, α-gliadins and HMW subunits, their segregation data being close to the expected 27:9:9:3:3:3:1 ratio. Furthermore, no linkage was demonstrated between *Glu-B1* and *Glu-B3*, at which are encoded the HMW and LMW subunits of glutenin, respectively. In contrast, the linkage between *Glu-B3* and *Gli-B1* was highly significant. In fact, apart from six progeny, only three phenotypic classes were observed: (1) LMW-2 subunits and ω-gliadin 35 from

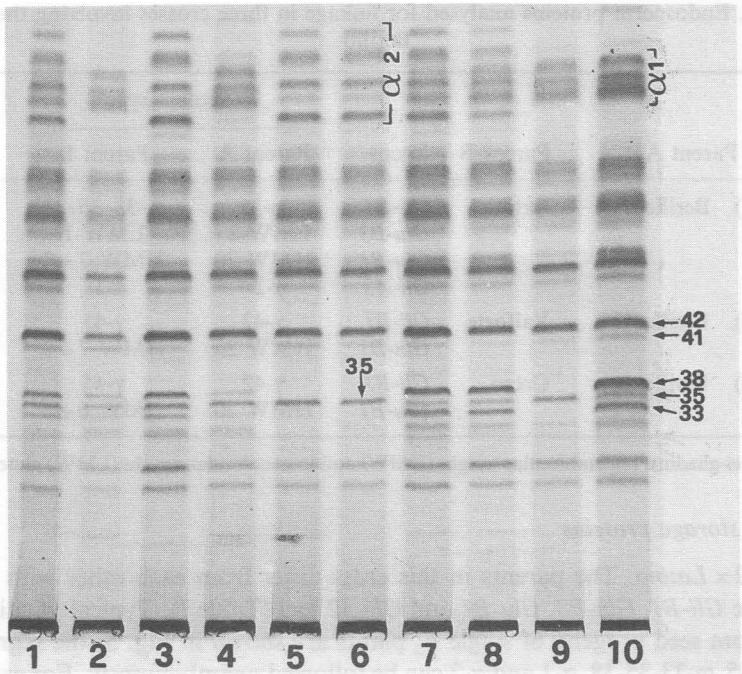

FIGURE 5. A–PAGE of gliadins of the progeny of single F_2 plants of the cross Berillo 2 (lane 6) × Latino (lane 10). The *Gli-B1* encoded gliadins found in type-42 cultivars are arrowed on the right-hand side; ω-gliadin 35 from Berillo and the gliadins groups α-1 and α-2 are also shown.

Berillo 2, (2) LMW-1 and ω–33–35–38 from Latino, and (3) double phenotypes with all these proteins. Among the six exceptional progeny, four individuals were homozygous for ω-35 and heterozygous for LMW subunits, and two were heterozygous for ω-gliadins and homozygous for either LMW-1 or LMW-2 (Table III). These progeny come from infrequent recombination between *Gli-B1* and *Glu-B3*, indicating that these loci are closely linked on the short arm of chromosome 1B. Using the method of maximum likelihood[22], the recombination value was calculated to be $2{\cdot}0 \pm 0{\cdot}8$ %, which corresponds to a map distance of $2{\cdot}0 \pm 0{\cdot}8$ cM as estimated by the Kosambi function[23]. Since these loci occur on the short arm of chromosome 1B and the *Glu-B1* locus is on the long arm, there are two possible orders for gene location. Either *Gli-B1* is proximal or distal to *Glu-B3* with respect to the centromere. The most likely order can be predicted from the *Glu-B1* allele composition of the recombinants for *Gli-B1* and *Glu-B3*. If *Gli-B1* is located between *Glu-B1* and *Glu-B3* then genotypes 1 and 4 of Table III are double recombinants, and these occur in three of the six *Glu-B3/Gli-B1* recombinant progeny. Furthermore, genotypes 2 and 3 could involve double recombinants. If *Gli-B1* is distal then the possible double recombinants are genotypes 2 and 3, which also occur in three progeny; however these latter genotypes are more likely to arise following combination

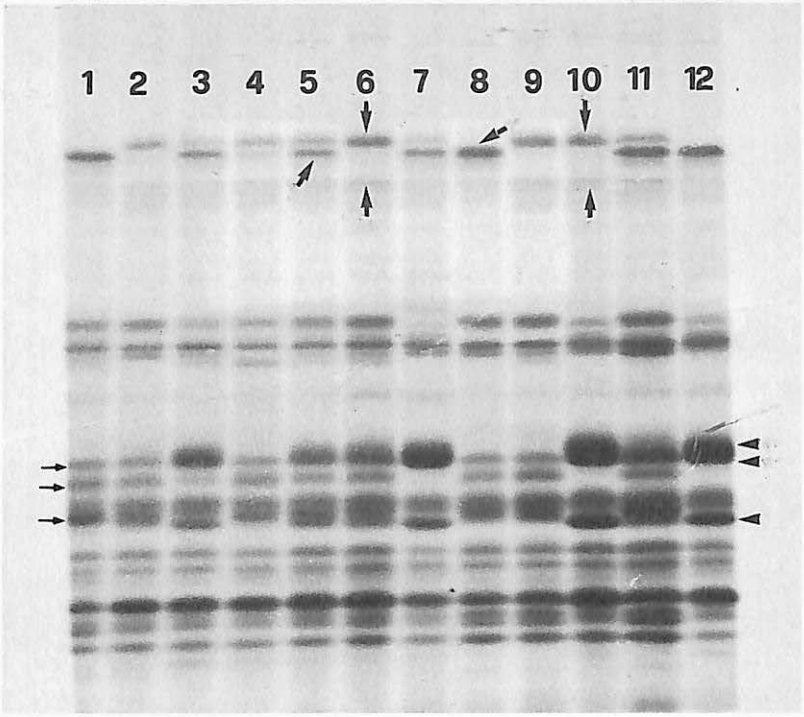

FIGURE 6. SDS–PAGE of total proteins of the progeny of single F_2 plants from the cross Berillo 2 × Latino. HMW glutenin subunit 20 from Berillo is indicated by a diagonal arrow, and HMW subunits 7 + 8 from Latino by vertical arrows. The LMW-2 subunits of glutenin from Berillo 2 and the LMW-1 subunits from Latino are indicated by horizontal arrowheads and arrows respectively.

of a *Gli-B1/Glu-B3* single-crossover chromosome with either a non-crossover chromosome (genotype 2) or a *Glu-B3/Glu-B1* single-crossover chromosome (genotype 3). Therefore, although statistically not proven, the most probable locus order is *Glu-B1–Glu-B3–Gli-B1*. The recombination values were calculated to be 47·0 % between *Glu-B1* and *Glu-B3*, 2·0 % between *Glu-B3* and *Gli-B1*, and 49·0 % between *Glu-B1* and *Gli-B1*.

(2) *Berillo 1 × Valforte*. The parents of this cross differ from each other with respect to alleles at *Gli-B1* and *Glu-B1* (Table I). Figure 7(A) is a photograph of typical A–PAGE patterns of gliadins extracted from F_3 seed. All progeny contained ω-gliadin 35, and they segregated for γ-gliadins 42 and 45. Moreover, γ-gliadins 41 and 43 were always inherited with γ-42 and γ-45 respectively. The segregation of alleles at *Glu-B1* was scored by SDS–PAGE as shown in Fig. 8(A), where HMW subunit 20 is indicated by diagonal arrows and subunits 7 + 8 by horizontal arrows. As expected, all progeny contained the LMW-2 subunits of glutenin. The frequencies of the different genotypes amongst the 185

TABLE II. F_2 segregation data for progeny from the cross Berillo 2 × Latino deduced from electrophoretic analysis of F_3 seed

Protein type[a]		Number of progeny							
a	b	a–	–b	ab	—	χ^2(1:1:2)	χ^2(3:3:9:1)	Conclusion	R(%)±S.D.
ω-35	ω–33–35–38	45	42	66	0	3·0 N.S.	—	Allelism	—
α-2	α-1	42	37	74	0	0·4 N.S.	—	Allelism	—
HMW 20	HMW 7+8	39	42	72	0	0·6 N.S.	—	Allelism	—
LMW-2	LMW-1	42	43	68	0	1·9 N.S.	—	Allelism	—
ω-35	α-2	27	32	84	10	—	0·6 N.S.	No linkage	—
ω-35	HMW 20	29	29	82	13	—	1·4 N.S.	No linkage	49±4·0
ω-35	LMW-2	1	0	110	42	—	172·1 ***	Linkage	2±0·8
α-2	HMW 20	31	26	85	11	—	0·7 N.S.	No linkage	—
LMW-2	HMW 20	28	29	82	14	—	2·3 N.S.	No linkage	47±4·0

*** Significant at $P < 0.001$; N.S., not significant.
[a] α-gliadins, ω-gliadins, HMW glutenin subunits, LMW glutenin subunits.

TABLE III. Frequency of recombinants for *Glu-B1* and *Glu-B3* in F_2 progeny of the cross Berillo 2 (B) × Latino (L) as determined by electrophoretic analysis of F_3 seed

Genotype	*Gli-B1*		*Glu-B3*		*Glu-B1*		No. of progeny
	Protein[a]	Allele	Protein[b]	Allele	Protein[c]	Allele	
1	35	B	2/1	BL	7+8	L	2
2	35	B	2/1	BL	20/7+8	BL	2
3	35/33–35–38	BL	2	B	20/7+8	BL	1
4	33/33–35–38	BL	1	L	7+8	L	1
Total							6

[a] ω-gliadins.
[b] LMW glutenin subunits.
[c] HMW glutenin subunits.

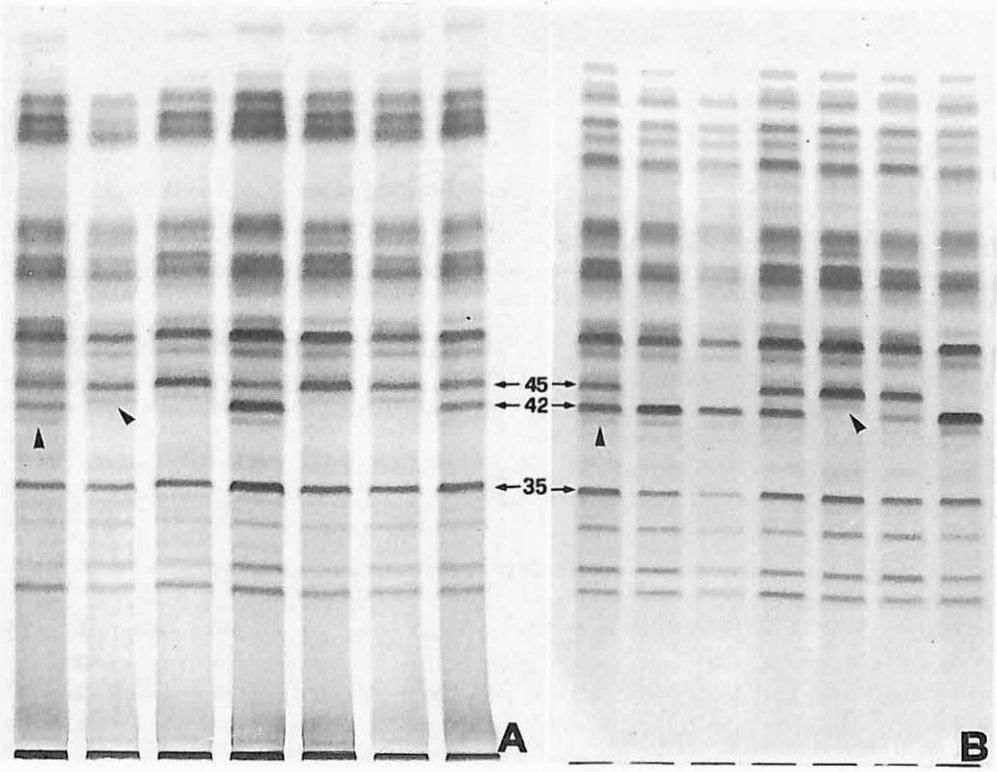

FIGURE 7. A–PAGE of gliadins of the progeny of single F_2 plants from the crosses (A) Berillo 1 × Valforte and (B) Berillo 2 × Creso. γ- Gliadins 41 and 43 are indicated by vertical and diagonal arrowheads respectively; ω- gliadin 35 and γ-gliadins 42 and 45 are also shown.

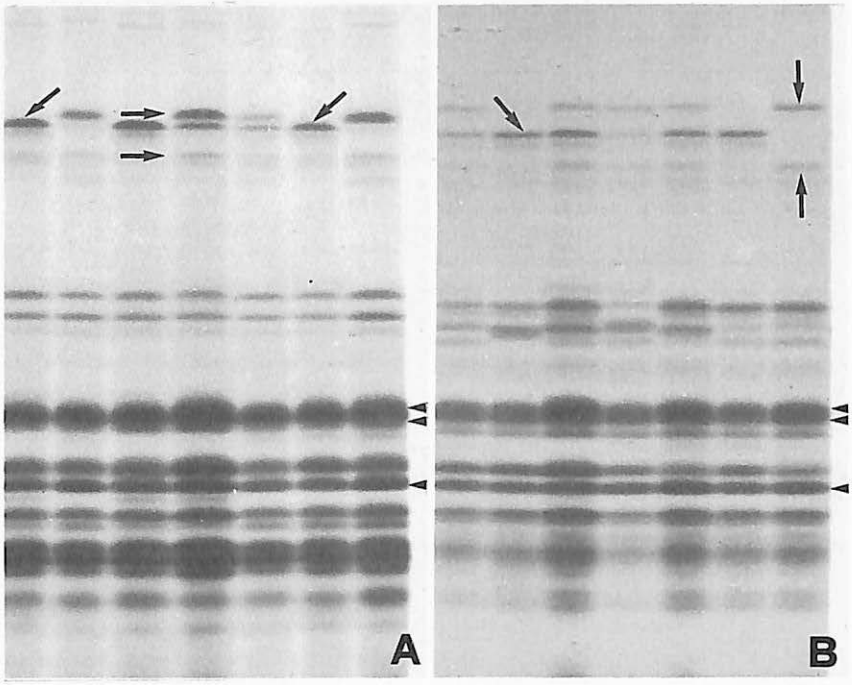

FIGURE 8. SDS–PAGE of total proteins of the progeny of single F_2 plants of the crosses (A) Berillo 1 × Valforte and (B) Berillo 2 × Creso. The HMW glutenin subunit 20 from Berillo is indicated by a diagonal arrow, HMW subunits 7+8 from Valforte by horizontal arrows and HMW subunits 6+8 from Creso by vertical arrows. The LMW-2 subunits of glutenin are marked by arrowheads.

progeny scored are shown in Table IV. The segregation data for γ-gliadins and HMW subunits were close to the expected 1:1:2 ratio, and the proportion of recombination between *Glu-B1* and *Gli-B1* was 49·0 ± 3·6 %.

(3) *Berillo 2 × Creso*. The parents of this cross have different HMW subunits and γ-gliadins (Table I). In total 140 progeny were analysed by A–PAGE and SDS–PAGE, and the electrophoresis patterns of some of them are shown in Figs 7(B) and 8(B) respectively. As in the previous cross, all progeny contained ω-gliadin 35 and LMW-2 subunits, and segregated for γ-gliadin 42/45 and HMW subunits 20/6+8.

The frequencies of the three phenotypes for HMW glutenin subunits were close to the expected 1:1:2, whereas a significant departure from this segregation ratio was found for γ-gliadins 42 and 45 (Table IV). This was due to reduced transmission of the allele coding for γ-gliadin 45 at *Gli-B1*. Linkage between *Glu-B1* and *Gli-B1* was insignificant, although the recombination proportion (45·0 %) was lower than the previous results. To study in more detail the transmission frequency of this locus on Creso chromosome 1B, progeny of the reciprocal crosses (Berillo 2 × Creso) F_1 × Latino were screened by A–PAGE. In the male transmission experiment (Table V) only 35·3 % of progeny contained γ-gliadin 45, showing that the male gametes carrying the Creso allele

TABLE IV. F_2 segregation data for progeny from the crosses Berillo 1 × Valforte and Berillo 2 × Creso deduced from electrophoretic analysis of F_3 seeds

Cross	Protein types[a]		No. of Progeny				$\chi^2(1:1:2)$	$\chi^2(3:3:9:1)$	Conclusion	R(%) ± S.D.
	a	b	a–	–b	ab	—				
Berillo 1 × Valforte	γ-42	γ-45	42	49	94	—	0·58 N.S.	—	Allelism	—
	HMW 20	HMW 7+8	51	38	96	—	2·1 N.S.	—	Allelism	—
	γ-42	HMW 20	23	34	113	15	—	5·74 N.S.	No linkage	49 ± 3·6
Berillo 2 × Creso	γ-42	γ-45	47	26	67	—	6·6 **	—	Allelism, RT[b]	—
	HMW 20	HMW 6+8	37	41	62	—	2·1 N.S.	—	Allelism	—
	γ-42	HMW 6+8	33	22	81	4	—	5·1 N.S.	No linkage	45 ± 4·2

** Significant at $P < 0{\cdot}01$; N.S., not significant.
[a] γ-gliadins and high molecular weight (HMW) glutenin subunits.
[b] RT, reduced transmission of γ-45 coding allele.

TABLE V. Transmission of the *Gli-B1* allele from Creso by male and female gametes in the progeny of the reciprocal crosses (Berillo 2 × Creso) × Latino

	No. of Progeny			
	γ-45/γ-42	γ-42	$\chi^2(1:1)$	Transmission (%)
γ-Gliadin 45 from				
Male parent	24	44	5·9*	35·3
Female parent	27	24	0·2	52·9

* Significant at $P < 0{\cdot}05$.

competed less effectively than those with the Latino allele in fertilizing the ovules. In the reciprocal cross the frequencies of transmission of both alleles were very close to the expected 1:1.

SDS-sedimentation test and rheological measurements

The effects of allelic variation at *Gli-B1, Glu-B3* and *Gli-A2* on SDS-sedimentation volume are shown in Table VI. In the cross Berillo 2 × Latino the mean sedimentation volume of progeny that possessed LMW-2 but lacked LMW-1 was significantly greater than that of progeny with LMW-1 only; progeny with both types of LMW subunits had intermediate sedimentation volumes. HMW subunits 7+8 were also associated with larger sedimentation volumes compared with HMW subunit 20; however, the difference between the two mean volumes was not as large as for LMW subunits of glutenin. Variation for α-gliadins encoded at *Gli-A2* did not show any significant influence on quality. The progeny with HMW subunits 7+8 and LMW-2 subunits had the greatest mean sedimentation volumes, whereas progeny lacking both types of glutenin subunit had the lowest mean volumes. The progeny with just one of the glutenin subunit types had intermediate values, indicating the additive effects of these subunits on sedimentation volume. Accordingly, the ANOVA showed insignificant interaction ($F < 1{\cdot}5$; $P > 0{\cdot}2$) between HMW and LMW subunits for this character. Amongst the six *Glu-B3/Gli-B1* recombinant progeny (Table III), genotype 3 a had larger sedimentation volume (60·0 ml) than those of genotype 1 (52·0 ml and 49·0 ml), genotype 2 (45·5 ml and 39·5 ml) and genotype 4 (38·8 ml).

Progeny from the cross Berillo 1 × Valforte confirmed the positive correlation between the presence of subunits 7+8 and larger sedimentation volumes, and did not show any differential effect of γ-gliadins 42 and 45 on quality. Similarly, there were no significant associations between these allelic gliadins and sedimentation volumes when progeny from the crosses Berillo 2 × Creso and Berillo 1 × Trinakria were analysed. Moreover, no significant differences in quality were observed when HMW subunits 6+8 and α-1 gliadins were compared with HMW subunit 20 and α-2 gliadins respectively.

The viscoelastic properties of gluten of progeny from three crosses involving the cultivar Berillo were studied using the Viscoelastograph (Table VII). In the cross Berillo

TABLE VI. Comparative effects of LMW glutenin subunits, HMW glutenin subunits, α- and γ-gliadins on SDS-sedimentation volumes, in the progeny of the cultivar Berillo, crossed with four other cultivars

Cross or cultivar	Proteins compared A	Proteins compared B	Number of progeny analysed	Mean sedimentation volume (ml) A–	B–	AB	*F*-test
Berillo 2 × Latino	LMW-2	LMW-1	108	56·6	30·1	41·2	**
	HMW 20	HMW 7+8	108	36·8	50·5	40·2	**
	α-2	α-1	108	41·3	43·7	43·0	N.S.
Berillo	—	—	—	49·0	—	—	—
Latino	—	—	—	—	35·5	—	—
Berillo 1 × Valforte	HMW 20	HMW 7+8	108	46·4	59·8	53·7	**
	γ-42	γ-45	108	53·1	52·7	54·3	N.S.
Valforte	—	—	—	—	64·0	—	—
Berillo 1 × Creso	HMW 20	HMW 6+8	108	47·1	50·1	46·7	N.S.
	γ-42	γ-45	108	47·9	48·5	47·5	N.S.
Creso	—	—	—	—	50·5	—	—
Berillo 1 × Trinakria	γ-42	γ-45	54	56·9	57·9	55·6	N.S.
	α-1	α-2	54	54·2	56·8	59·6	N.S.
Trinakria	—	—	—	—	66·5	—	—

** Significant at $P = 0{\cdot}01$; N.S., not significant.

2 × Latino, elastic recovery and firmness of samples homozygous for LMW-2 subunits were higher than the corresponding values of samples homozygous for LMW-1, and the differences between the means were highly significant. Samples that were heterozygous or heterogeneous for LMW-2 and LMW-1 subunits had gluten viscoelastic properties that lay between those of the above two types. In the same cross, the gluten elastic recovery was higher for HMW subunits 7+8 than subunit 20, and the difference was highly significant. As observed for the SDS-sedimentation test, the beneficial effects of HMW subunits 7+8 and LMW-2 subunits on gluten elastic recovery were additive, the presence of both types of glutenin subunit giving better elastic characteristics than the presence of each one separately. The ANOVA showed no interaction between HMW and LMW glutenin subunits for elastic recovery ($F = 1{\cdot}15$; $P > 0{\cdot}2$), confirming their additive behaviour. However, the contribution of LMW-2 subunits to variation in gluten elastic recovery was much higher than that of HMW subunits 7+8; moreover, subunits 7+8 and 20 did not show differential effects on gluten firmness. Finally, gluten firmness was higher for α-2 gliadins than α-1 gliadins, but the difference was significant at the 5% probability level only; these two gliadin types had no differential effects on elastic recovery.

As both parents of the crosses Berillo 1 × Valforte and Berillo 2 × Creso possess LMW-2 subunits, it is not surprising that their progeny had high gluten elastic recovery. Two sets of HMW subunits were compared for viscoelastic properties in progeny of

TABLE VII. Comparative effects of LMW glutenin subunits, HMW glutenin subunits, α- and γ-gliadins on gluten viscoelastic properties in the progeny of the cultivar Berillo, crossed with three other cultivars

Cross or cultivar	Proteins compared		Number of progeny analysed	Gluten elastic recovery (mm)				Gluten firmness (mm)			
	A	B		A–	–B	AB	*F*-test[a]	A–	–B	AB	*F*-test
Berillo 2 × Latino	LMW-2	LMW-1	49	1·18	0·77	1·11	**	2·28	1·96	2·13	**
	HMW 20	HMW 7+8	49	0·96	1·09	1·05	**	2·14	2·04	2·20	N.S.
	α-2	α-1	49	1·04	1·00	1·06	N.S.	2·21	2·07	2·10	*
Berillo	—	—	—	1·09	—	—	—	2·22	—	—	—
Latino	—	—	—	—	0·87	—	—	—	1·88	—	—
Berillo 1 × Valforte	HMW 20	HMW 7+8	18	1·14	1·23	1·15	*	2·05	2·23	1·96	N.S.
	γ-42	γ-45	18	1·15	1·17	1·20	N.S.	2·07	2·10	2·08	N.S.
Valforte	—	—	—	—	1·32	—	—	—	1·84	—	—
Berillo 2 × Creso	HMW 20	HMW 6+8	18	1·22	1·24	1·22	N.S.	2·18	2·33	2·35	N.S.
	γ-42	γ-45	18	1·20	1·30	1·19	*	2·38	2·19	2·29	N.S.
Creso	—	—	—	—	0·88	—	—	—	1·81	—	—

* Significant at $P < 0·05$; ** significant at $P < 0·01$; N.S., not significant.

these crosses. In accord with the results obtained in the cross Berillo × Latino, progeny with HMW subunits 7+8 had higher elastic recoveries than those with subunit 20, but on this occasion the correlation was significant at the 5% probability level only. The progeny with a mixture of subunits 7+8 and 20 were intermediate in elasticity.

The other HMW subunits compared, 20 and 6+8, showed no differential effects either on elastic recovery or firmness of gluten. Finally, inconsistent results were obtained when γ-gliadins 42 and 45 were compared for gluten viscoelastic properties. In the cross between Berillo 1 and Valforte, neither exerted a greater effect than the other on elastic recovery of gluten, whereas γ-gliadin 45 progeny from Berillo 2 × Creso had higher elastic recoveries than γ-gliadin 42 progeny, although the difference was significant at the 5% probability level only. It is worth noting that in the latter cross both the two homozygous progeny showed high elastic recovery; moreover, progeny with a mixture of γ-gliadins 42 and 45 were not intermediate in quality. In both crosses, variation in γ-gliadin alleles had no effect on gluten firmness.

Discussion

Two dimensional separations using A–PAGE in the first dimension with B–PAGE and with SDS–PAGE in the second have confirmed that γ-gliadin 42 and ω-gliadin 35 in Berillo have apparent molecular weights and electrophoretic mobilities identical to those of corresponding gliadins in type γ-42 or type γ-45 cultivars. Therefore, the Berillo genotype can be accounted for by a rare recombination event between the genes coding ω- and γ-gliadins at the *Gli-B1* locus. HMW subunits 20, 6+8 and 7+8, which are the most commonly occurring glutenin polypeptides in durum wheats grown throughout the world, have been found to be coded by alleles at a single locus. Allelism has been also demonstrated for γ-gliadins 41, 42, 43 and 45, α-gliadins 1 and 2, ω-gliadins 35 and 33–35–38, and LMW subunits 1 and 2. All these findings confirm and complement previous reports on the genetic structure of storage protein genes in common[24, 25] or durum wheat[2, 26].

LMW subunit genes have been found to be linked to genes coding for ω-gliadins. The estimated proportion of recombination and map distance in cM was 2·0±0·8. This is consistent with the results of Singh and Shepherd[5, 36], who observed 1·7% recombination between *Glu-B3* and *Gli-B1* in common wheat.

In previous work[1], we proposed that the recombinant genotype of Berillo could be accounted for by a crossing over between genes in two possible orders, e.g. LMW-glu/ω-gli/γ-gli or γ-gli/LMW-glu/ω-gli. Because no recombination was found between γ- and ω-gliadins in the many hundreds of segregating progeny of crosses between type γ-45 and type γ-42 cultivars[2, 27], the gene order LMW-glu/ω-gli/γ-gli appears to be the most likely. The present results also suggests that *Glu-B3* is located between *Glu-B1* and *Gli-B1*, supporting the recent work of Sing and Shepherd[36] in common wheat.

The estimated proportion of recombination between *Glu-B1* and *Gli-B1* was 49·0±2·7, as obtained by the pooled linkage data for the crosses Berillo 2 × Latino and Berillo 1 × Valforte. There are several published estimates of recombination frequencies between these two loci in common wheat. For example, Lawrence and Shepherd[28] obtained a recombination frequency of 48·8%, which is equal to our value. Lower estimates of 43·3 and 30·8% were obtained by Payne *et al.*[12] and Snape *et al.*[29]

respectively. These differences are not unexpected because polymorphism in nucleotide sequences between homologous chromosomes in hybrids of wheat cultivars may reduce the likelihood of crossing over[30], so that recombination frequency depends on the relatedness of the parents. In this context it is worth noting that Berillo, Valforte and Latino are closely related genetically, with one or more common parents in their pedigrees. The gene map for chromosome 1B, incorporating the results of this work, is shown in Fig. 1.

The distorted segregation for γ-gliadins in the progeny Berillo 2 × Creso could be accounted for by the presence of a genetic factor linked to *Gli-B1* that reduces the frequency of transmission of γ-gliadin 45 by male gametes. The nature of this factor warrants further investigation.

Allelic variation at *Glu-B3* and *Glu-B1* has a major effect on gluten quality as measured by the SDS-sedimentation test and the Viscoelastograph. In the cross Berillo 2 × Latino, the progeny that contained LMW-2 subunits had higher sedimentation volume, elastic recovery and gluten firmness than the progeny that contained LMW-1 subunits; the difference between means was significant at the $P < 0{\cdot}01$ level. Furthermore, the *LMW-2* -type progeny from the other three crosses analysed all showed high mean values of elastic recovery and firmness (over 1·1 and 2·0 respectively) as in the γ-gliadin 45 type cultivars examined so far. The close genetic linkage between LMW-2 subunits of glutenin and ω-gliadin 35 raises the question as to whether the observed effects on quality are due to the glutenin subunits or the gliadin band. In this context it is worth noting that the ω-gliadins 35 and 33–35–38 map at very similar positions in the gliadin two-dimensional electrophoretic fractionations (Figs 3 and 4,) indicating that they have very similar charges at low or high pHs, and very similar molecular weights. Furthermore, ω-gliadin 35 makes up a minor amount of fractions that have no apparent functional properties[37]. Our conclusion from these observations is that LMW-2 subunits rather than ω-gliadin 35 are responsible for the improved gluten properties. This statement is supported here by the relative performance of the four *Glu-B3/Gli-B1* recombinant genotypes (Table III) for sedimentation volume. The results given in this paper confirm that γ-gliadins are only genetic markers of quality, and that allelic variation for LMW subunits of glutenin results in gluten having different viscoelastic properties. Similarly, LMW subunits coded for by genes at the *Gli-A1* locus in common wheat were found to be primarily responsible for the differences in SDS sedimentation volume, with the genetically linked ω- and γ-gliadins having only minor effects on quality[31]. It was also shown[32] that common wheat cultivars with γ-gliadin 43·5 have stronger gluten than those with γ-gliadin 40. As in durum wheat, these allelic gliadins encoded at *Gli-B1* are linked to two different groups of LMW glutenin subunits[33].

The finding that HMW subunits 7+8 are associated with large SDS sedimentation volumes and high elastic recoveries compared with allelic subunits 6+8 or 20 is consistent with previous reports for common wheat[34, 11]; subunits 7+8 are also strongly correlated with the bread-making quality of durum wheat cultivars[4]. Allelic variation at *Glu-B1* had a smaller effect on gluten quality than variation at *Glu-B3*; however, the effects at each locus were additive, so that genotypes containing LMW-2 and HMW 7+8 had the best gluten properties. These findings are in accordance with those of Payne

et al.[31] and Gupta *et al.*[38], who showed the additive effects of allelic variation at the *Glu-A1* and *Glu-A3* loci on dough quality in common wheat. The latter authors also found that superior dough properties were associated with variation at *Glu-A3* rather than variation at *Glu-A1*.

Finally, the association between α-2 gliadins and high gluten firmness is consistent with a recent study[35] showing that durum cultivars with the 'α-73' group of gliadins (α-2 gliadins in our nomenclature) have higher gluten firmness that those containing 'α-76' group of gliadins (α-1 gliadins).

The results of the present study have important implications for wheat breeding and molecular studies on structural and regulating genes affecting gluten quality in durum wheat.

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