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Linkage mapping of '25-kDa globulin' genes on homoeologous group-1 chromosomes of bread and durum wheat

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Abstract Acid polyacrylamide-gel electrophoresis (A-PAGE) of ethanol-soluble proteins from the endosperm of bread and durum wheats reveals some bands encoded by genes on the homoeologous group-1 chromosomes with higher mobility than the α-gliadins. The isolation of these proteins showed that they were the previously described '25-kDa globulins' encoded by genes at the *Glo-A1*, *Glo-B1*, and *Glo-D1* loci. The variability found among a collection of 51 bread and 81 durum wheats was very low: two allelic variants at *Glo-A1* and no variants at *Glo-B1* in each of the two species, and two allelic variants at *Glo-D1* in bread wheat. Inheritance studies of '25-kDa globulin' genes on group-1 chromosomes of bread and durum wheat were carried out on the F_2 progeny from four crosses, two in bread wheat and two in durum wheat. The linkage mapping of the 1A '25-kDa globulin' genes of bread wheat was done based on four prolamin loci: *Glu-A1*, *Glu-A3*, *Gli-A1* and *Gli-A3*. The percentages of recombination and the distances found allowed a re-evaluation of the linkage map of endosperm protein loci on this chromosome. The *Glo-A1* locus was found to be located at the distal end of the short arm of 1A chromosome, at a distance of 5.23 ± 1.99 cM from *Gli-A1*, 6.85 ± 2.22 cM from *Glu-A3*, 22.64 ± 3.62 cM from *Gli-A3*, and at a recombination percentage of 49.30 ± 4.40 from *Glu-A1*. A similar distance between *Gli-A1* and *Glo-A1* (4.82 ± 1.75 and 6.66 ± 2.26 cM) was found in durum wheat. The distance between *Gli-D1* and *Glo-D1* on chromosome 1D was 2.86 ± 1.39 cM.

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Introduction

The salt-soluble fraction of the wheat endosperm proteins (albumins and globulins) represents 15–25% of the total protein and includes the α-amylase/trypsin inhibitor family (see Carbonero et al. 1993 for a review), the high-molecular-weight (HMW) albumins, and other non-storage protein groups (Payne et al. 1985). The genes coding for the major salt-soluble proteins have been located on the short arms of chromosome groups 3, 4, 5, 6 and 7 (Fra-Mon et al. 1984). Only the genes coding for two groups of globulin-type proteins have been assigned to group-1 chromosomes: triticins (Singh and Shepherd 1985) and '25-kDa globulins' (Gómez et al 1988). The triticins are controlled by genes at the *Tri-A1* and the *Tri-D1* loci, located on the short arms of the 1A and 1D chromosomes, closely linked with the centromere (Singh and Shepherd 1988). The '25-kDa globulins' are encoded by genes at the *Glo-1* loci, on the short arms of group-1 chromosomes (Gómez et al. 1988).

The '25-kDa globulins' show particular solubility properties among the wheat salt-soluble proteins; they can be extracted with aqueous ethanol and with chloroform-methanol mixtures, but not with water (Gómez et al. 1988). This protein family has been also detected in other *Triticeae* species (Gómez et al. 1988, 1991) and represents the major low-molecular-weight (LMW) proteins of the salt-extractable fraction from *Triticum monococcum* (Gómez et al. 1991). Their amino-acid compositions and the amino-terminal amino-acid sequence of one of their components have been reported (Gómez et al. 1988, 1991).

The prolamins (glutenins and gliadins) comprise most of the endosperm proteins of the wheat kernel. The major prolamins are encoded by genes on the homoeologous group-1 chromosomes. The HMW glu-

tenin subunits are encoded by genes at the *Glu-1* loci on the long arms, whereas LMW glutenin subunits are encoded by genes at the *Glu-3* loci on the short arms of the same chromosomes (see Payne 1987 for review). Genes coding for β-, γ- and ω-gliadins have been mapped on the short arms, at the *Gli-1* loci, very tightly linked with the *Glu-3* loci (Payne 1987; Singh and Shepherd 1988). Some minor ω-gliadin genes have been detected and mapped on the short arms of chromosomes 1A and 1B: namely, *Gli-B3* (Galili and Feldman 1984; Jackson et al. 1985; Payne et al. 1988), *Gli-A3* (Sobko 1984; Metakovsky et al. 1986; Payne et al. 1988), *Gli-A4* (Metakovsky et al. 1986; Redaelli et al. 1992), and *Gli-A5* and *Gli-B5* (Metakovsky et al. 1986; Pogna et al. 1993).

The aim of the present study was to analyze the variability and the genetics of the '25-kDa globulins' in bread and durum wheat. The linkage mapping of the genes coding for these proteins was undertaken using prolamin loci as markers.

Materials and methods

Plant material

The compensating nulli-tetrasomic (NT) lines of *Triticum aestivum* cv 'Chinese Spring' (CS) and the D-genome disomic substitution lines of *Triticum durum* cv 'Langdon' (Lnd) were used to identify the '25-Da globulins' encoded by genes on the homoeologous group-1 chromosomes in these cultivars. Flour from the bread wheat cv 'Ariana 8' was used to partially purify these proteins. Variability was studied in a collection of 51 bread and 81 durum wheats. The genetical analysis was done in four sets of F_2 progenies. Two of them derived from crosses between bread wheats, 'Cajeme 71' 'Ariana 8' and 'Cajeme 71'/'Darius', and the other two from crosses between durum wheats, 'Alaga'/'Peñafiel' and 'Alaga'/'Blatfort'

Electrophoresis

Single embryo-less seeds were crushed and extracted with 70% ethanol (v/v) (1:5 w/v, 1 h, room temperature). After centrifugation, the extract was mixed with glycerol containing methyl green. The residue was subjected to the sequential extraction procedure of Singh et al. (1991) to obtain unreduced and reduced (HMW and LMW glutenin subunits) proteins.

The ethanol-extracted proteins were fractionated by polyacrylamide-gel electrophoresis, pH 3.1 (A-PAGE), according to Lafiandra and Kasarda (1985) with minor modifications. Gel plates were longer (24 cm) and the electrophoresis was stopped when the first dye front reached the bottom (1 h 20 min approximately). Sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) for unreduced proteins was performed as described previously (Nieto-Taladriz et al. 1994).

Two types of two-dimensional electrophoresis procedures were used. The first type was A-PAGE × SDS-PAGE, performed according to Payne et al. (1984), except that the first dimension was carried out as described above. The second type was isoelectrofocusing (IEF; pH 6–8) in the first dimension and starch-gel electrophoresis (SGE), pH 3.2, in the second dimension, performed as described by Gómez et al. (1988).

Isolation of '25-kDa globulins'

Flour from the bread wheat cv 'Ariana 8' was de-lipidated with acetone (1:5 w/v; 1 h; 4°C) and extracted with 70% aqueous ethanol (1:5 w/v; 1 h, room temperature). The extract was roto-evaporated to half its volume, dialyzed against 0.1 M acetic acid (48 h; 4°C) and lyophilized.

The protein extract was fractionated by gel filtration on Sephadex G-100 (2.5 × 90 cm column; 25 ml/h) using 0.1 M aluminium lactate 3 M urea as an elution buffer. Fractions from each peak were pooled, dialyzed against 0.1 M acetic acid and lyophilized.

The fraction containing the proteins under study was further fractionated by HPLC on a Vydac C4 column (particle size 10 µm; 250 × 22 mm; 1.5 ml/min) using a 30–45% B linear gradient in 200 min [A: 0.1% trifluoroacetic acid (TFA) in water; B: 0.1% TFA in acetonitrile].

Genetic analysis

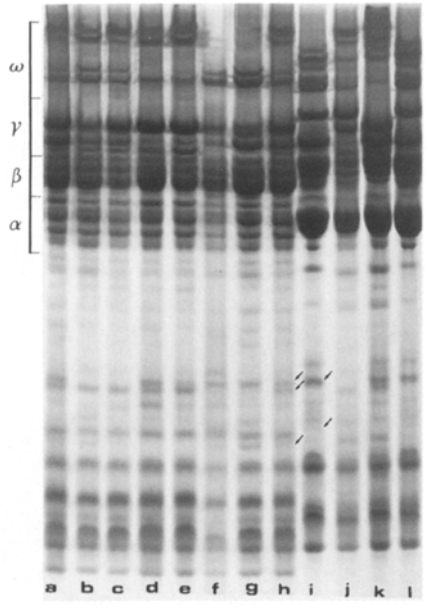
The recombination frequency between genes was calculated using the method of maximum likelihood (Allard 1956). The genetic distances in centi Morgan (cM) units were obtained using the Kosambi function (Kosambi 1944).

Results

Detection and isolation of '25-kDa globulins'

The A-PAGE pattern of the alcohol-soluble proteins of CS showed three protein bands, with a higher mobility than the α-gliands (Fig. 1h, arrows), encoded by genes at the homoeologous group-1 chromosomes, as revealed by the NT lines (Fig. 1b–g). The slowest moving band was coded for by the 1A chromosome, the intermediate one by the 1D chromosome, and the fastest moving band, fainter than the two others, by the 1B chromosome. In all the NT lines a dosage effect was detectable. The 'Langdon' substitution lines (Fig. 1j, k) showed that this cultivar (Fig. 1i) contains one 1A-encoded band

Fig. 1 A-PAGE of ethanol-soluble proteins fom CS (*a*, *h*), CS NT1A-1B (*b*), CS NT1A-1D (*c*), CS NT1B-1A (*d*), CS NT1B-1D (*e*), CS NTID-1A (*f*) CS NT1D-1B (*g*), Lnd (*i*, *l*), Lnd 1D(1A) (*j*) and Lnd 1D(1B) (*k*) Bands under study are *arrowed*



which has the same mobility as the 1A-encoded band of CS, and a faint faster-moving band encoded by the 1B chromosome. As expected, in both lines the 1D-encoded band of CS was present.

The identification of the bands under study was carried out in the bread wheat cv 'Ariana 8', which showed the same electrophoretic pattern in these proteins as CS (Fig. 2b). They had a Mr around 25 000, as shown by the A-PAGE × SDS-PAGE map (Fig. 3), and could also be extracted with 0.5 M NaCl and chloroform-methanol (2:1 v/v) mixtures, but not with water (data not shown).

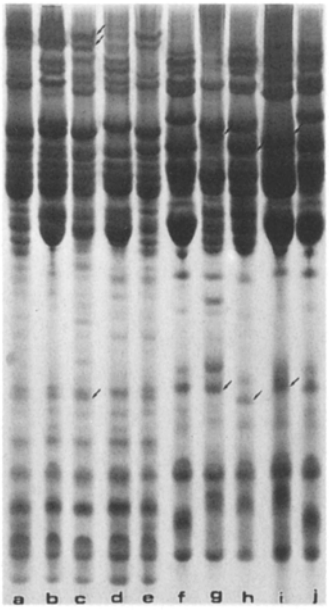
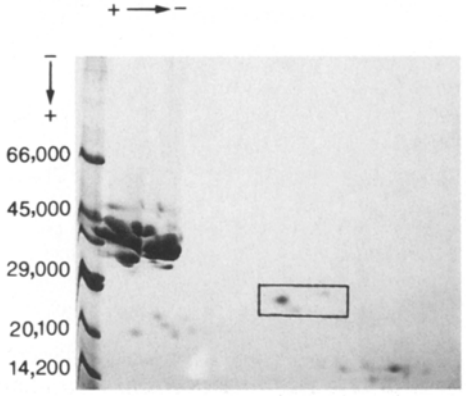


Fig. 2 A-PAGE of ethanol-soluble proteins from the bread wheats CS (*a*, *e*), 'Ariana 8' (*b*), 'Cajeme 71' (*c*), 'Darius' (*d*) and the durum wheats Lnd (*f*, *j*), 'Peñafiel' (*g*), 'Alaga' (*h*), Blatfort (*i*). Bands under study are *arrowed*

Fig. 3 Two-dimensional protein map (A-PAGE × SDS-PAGE) of the ethanol-soluble proteins from the bread wheat cv 'Ariana 8'. The proteins under study are inside the *square*. MW markers appear at the left of the figure



In order to ascertain the identity of these proteins, the ethanol extract from 'Ariana 8' was fractionated by gel filtration on Sephadex G-100 under dissociating conditions (Fig. 4A). The putative '25-kDa globulin' bands appeared only in fraction 6 (Fig. 4B) which corresponded to proteins with an apparent molecular size of 25 kDa. This fraction (F6) was further fractionated by RP-HPLC (Fig. 4 C). The bands under study were found as the main components of a broad peak which eluted near the end of the gradient (Fig. 4 C, D). Analysis by two-dimensional electrophoresis (IEF × SGE) of ethanol extracts from kernels of cvs CS and 'Ariana 8', as well as of the appropriate RP-HPLC fractions (Fig. 5), showed that both wheat cultivars have the same '25-kDa globulin' variants (overlapping of spots in Fig. 5 C), and that the bands under study corresponded to the major components of this protein group.

Polymorphism of the '25-kDa' globulins

The polymorphism of the '25-kDa globulins' as determined by A-PAGE was analyzed in a collection of 51 bread wheat and 81 durum wheat cultivars. Among the bread wheats, only three electrophoretic patterns were detected: the CS-type (Fig. 2a), the 'Cajeme 71'-type (Fig. 2c), and 'Darius'-type (Fig. 2d). Among the durum wheats, only two electrophoretic patterns were detected: the Lnd-type (Fig. 2f) and 'Alaga'-type (Fig. 2 h).

Linkage mapping of globulin genes on the 1A chromosome of bread wheat

The genetic mapping of the 1A globulin genes was performed based on the prolamin loci located on this chromosome. Therefore, the endosperm proteins of the F_2 progeny from the 'Cajeme 71'/'Ariana 8' cross were analyzed. The loci studied, and the phenotypic segretation data obtained at each of them, are summarized in Table 1.

Figure 6A shows the '25-kDa globulin' patterns and of some F_2 grains. At the *Glo-A1* locus 'Ariana 8' has the same allelic variant as CS, and one band (Fig. 6Ae, arrow) was used as marker of this locus. 'Cajeme 71' has a band of slightly faster mobility (Fig. 6Ad, arrow) which, in the F_2 progeny, was found to be allelic to that of 'Ariana 8'.

Figure 6B shows the HMW and LMW glutenin patterns of the parents and of some F_2 grains. At the *Glu-A1* locus 'Ariana 8' (e) possesses the *Glu-A1b* allele (band 2*, arrowhead) and 'Cajeme 71' (d) the *Glu-A1a* allele (band 1, arrowhead). At the *Glu-A3* locus 'Ariana 8' had one B-zone protein band (arrow) with the same mobility as the 1A-encoded LMW glutenin subunit of 'Courtôt' (Nieto-Taladriz et al. 1994) and was, therefore, considered to be the same variant. 'Cajeme 71' has a band (arrow) which proved to be allelic to that of 'Ariana 8'.

Two ω-gliadin bands of 'Cajeme 71' (Fig. 6Ad, arrowheads), tightly linked in the progeny to the LMW

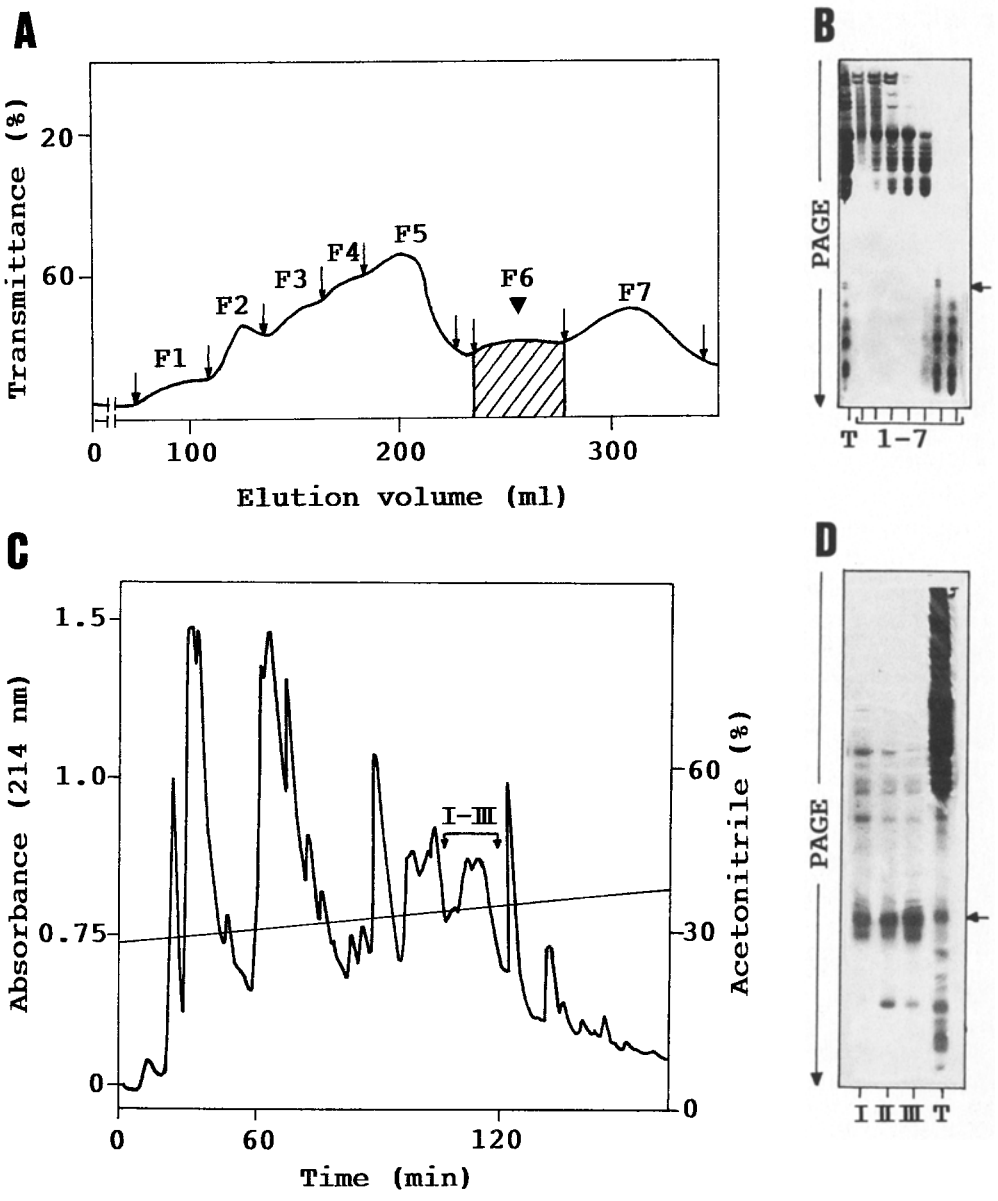


Fig. 4A–D **A** Gel-filtration on Sephadex G-100 of the ethanol extract from bread wheat cv 'Ariana 8'. **B** A-PAGE of the ethanol extract (*T*) and of the gel-filtration fractions F1–F7 (*1–7*) shown in **A**. **C** RP-HPLC profile of the gel-filtration fraction F6 indicated in **A**. The elution peak of the proteins under study is marked (*I–III*). **D** A-PAGE of the ethanol extract (*T*) and of the RP-HPLC *fractions I–III* The position of the proteins under study is indicated by an *arrow* in **B** and **C**

glutenin subunit described above, were used as markers of the *Gli-A1* locus. In the F_2 progeny none of the 'Ariana 8' gliadin bands (Fig. 6Ae) could be considered as an alternative to these ω-gliadins of 'Cajeme 71'.

The unreduced protein pattern of 'Cajeme 71' showed one band (Fig. 6Cd, arrow) which was found to be linked with the LMW glutenin subunit and the ω-gliadins described above. 'Ariana 8' possessed two bands (Fig. 6Ce, arrows) as an alternative to the one of 'Cajeme 71'. These allelic variants were assigned to the *Gli-A3* locus (Nieto-Taladriz and Carrillo 1996).

Segregation data at each of the *Glo-A1*, *Glu-A1*, *Glu-A3* and *Gli-A3* loci (Table 1) agreed with the expected 1:2:1 ratio for two co-dominant alleles. At *Gli-A1* the segregation data (Table 1) agreed with the expected 3:1 ratio for a single allele at a locus.

Table 1 summarizes the joint segregation of the F_2 phenotypes found for the specific pairs of loci considered. Table 2 includes the recombination percentages and genetic distances found between the five loci considered. The *Glu-A1* locus was not significantly linked with any of the other protein loci analyzed. Recombination has been detected between the *Glu-A3* and the *Gli-A1* loci, and the distance obtained between them was 1.50 ± 1.07 cM. The *Glo-A1* locus was shown to be located at a distance of 5.23 ± 1.99 cM from *Gli-A1*, 6.85 ± 2.22 cM from *Glu-A3*, and 22.64 ± 3.62 cM from *Gli-A3*. Otherwise, it was not significantly linked with the *Glu-A1* locus, showing a recombination percentage of 49.30 ± 4.40.

Linkage mapping of 1D-encoded globulin genes in bread wheat

The genetic mapping of the 1D globulin genes was carried out by analyzing these proteins and gliadins in

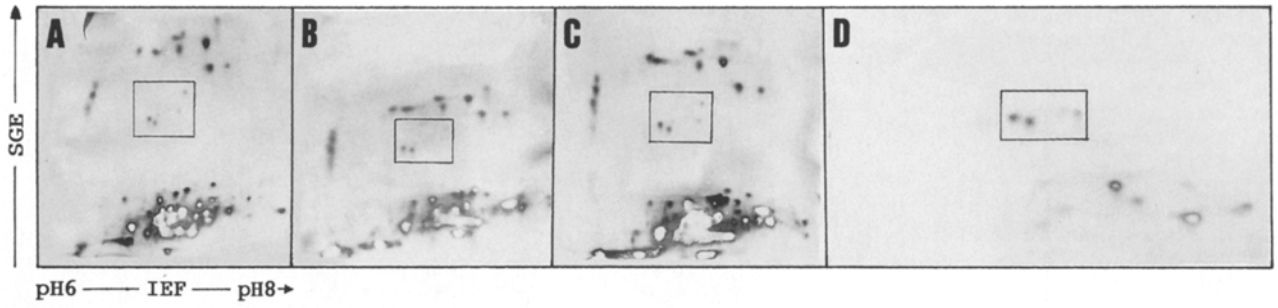


Fig. 5A–D Two-dimensional protein maps (IEF pH 6–8 × SGE pH 3.2) of the ethanol extracts from the bread wheat cvs 'Ariana 8' (**A**), CS (**B**), a mixture of 'Ariana 8' and CS (**C**), and of the RP-HPLC fraction I of Fig. 4 D. The '25-kDa globulins' are those inside the *square*

the F_2 progeny from the 'Cajeme 71'/'Darius' cross. Table 3 shows the phenotypic classes obtained at each of the loci analyzed.

At the *Glo-D1* locus 'Cajeme 71' showed the same band (Fig. 2c, arrow) as the 1D-encoded globulin of CS. 'Darius' (Fig. 2d) lacked this band and, in the F_2 progeny, no band could be considered as an alternative to the *Glo-D1* allele of 'Cajeme 71'. The gliadin pattern of 'Cajeme 71' showed two slow-moving ω-gliadin bands (Fig. 2c, arrows) which were assigned to the *Gli-D1* locus which is known to control the synthesis of the slow-moving ω-gliadins in acid gels (Sozinov and Poperelya 1980). Cv 'Darius' (Fig. 2d) had a null allele at this locus (Autran 1975). At both loci, segregation data (Table 3) agreed with the expected 3:1 ratio for a single allele at a locus. Table 3 summarizes the percentage of recombination and the genetical distance (2.86 ± 1.39 cM) between the *Glo-D1* and the *Gli-D1* loci.

Linkage mapping of 1A-encoded globulin genes in durum wheat

The F_2 progenies from two durum wheat crosses, 'Alaga'/'Peñafiel' and 'Alaga'/'Blatfort', were analyzed for globulin and gliadin composition. 'Peñafiel' and 'Blatfort' have the same 1A-encoded globulin as Lnd (Fig. 2g, i, respectively), whereas 'Alaga' showed a faster-moving band (Fig. 2h, arrow) which in both progenies proved to be an alternative to that of 'Peñafiel' and 'Blatfort'. In both crosses, segregation data (Tables 4 and 5) agreed with the expected 1:2:1 ratio for two co-dominant alleles. At the *Gli-A1* locus 'Alaga' possesses the γ-51 gliadin band, 'Peñafiel' band γ-47 and 'Blatfort' band γ-49 (Fig. 2h, g, i, respectively). In the F_2 progeny from the 'Alaga'/'Peñafiel' cross, segregation data (Table 4) agreed with the expected 1:2:1 ratio for two co-dominant alleles. Among the F_2 progeny from the 'Alaga'/'Blatfort' cross it was not possible to differentiate the homozygous from the heterozygous types when band γ-51 was present. Segregation data (Table 5) agreed with the expected 3:1 ratio for a single allele at a locus.

Tables 4 and 5 summarize the recombination percentages and genetical distances found between the *Glo-A1*

Table 1 Individual and joint segregation of phenotypes at a specific locus and pairs of loci among the F_2 progeny from the 'Cajeme 71'/'Ariana 8' cross

Loci	Phenotype	*Gli-A3*[a]			*Glu-A3*[a]			*Gli-A1*[a]		*Glo-A1*[a]			Total
		A	A/C	C	A	A/C	C	–	C	A	A/C	C	
Glu-A1	A	7	19	6	8	21	3	9	23	9	17	6	32
	A/C	21	25	18	16	33	15	16	48	19	29	16	64
	C	4	17	12	10	12	11	9	24	11	13	9	33
Gli-A3	A				19	13	0	20	12	22	10	0	32
	A/C				15	41	5	14	47	17	35	9	61
	C				0	12	24	0	36	0	14	22	36
Glu-A3	A							33	1	33	1	0	34
	A/C							1	65	6	54	6	66
	C							0	29	0	4	25	29
Gli-A1	–									33	1	0	34
	C									6	58	31	95
Total	32	61	36	34	66	29	34	95	35	59	31	129	

[a]A: 'Ariana 8'-type; C: 'Cajeme 71'-type, A/C: heterozygous; –: absence of band

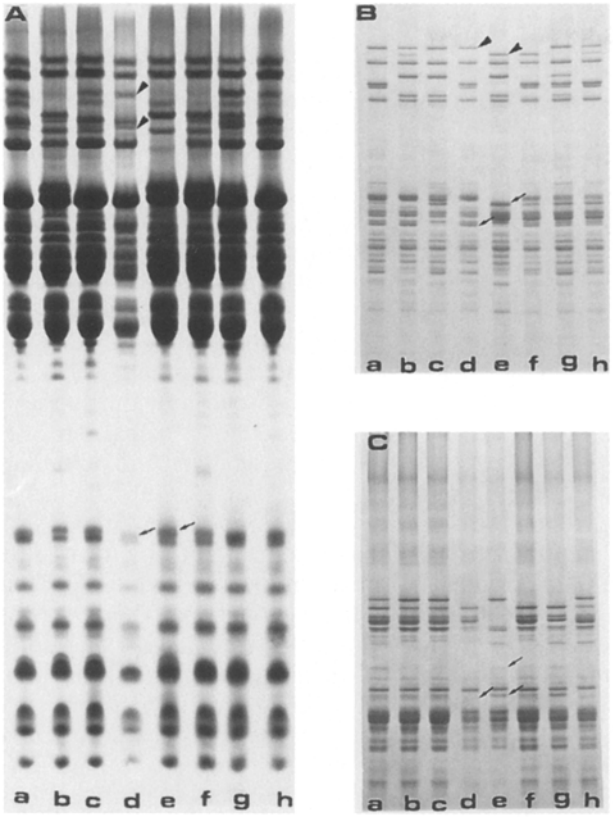


Fig. 6A–C A-PAGE of 70% ethanol soluble proteins (**A**), SDS-PAGE of reduced (**B**) and unreduced (**C**) prolamins obtained by the sequential extraction procedure of Singh et al. (1991) of the parents 'Cajeme 71' (*d*) and 'Ariana 8' (*e*) and some F_2 grains (*a–c* and *f–h*). Bands used as markers of the alleles at the *Gli-A1* (**A**: *arrowheads*), *Glo-A1* (**A**: *arrows*), *Glu-A1* (**B**: *arrowheads*), *Glu-A3* (**B**: *arrows*) and *Gli-A3* (**C**: *arrows*) loci are indicated

and the *Gli-A1* loci in the two durum wheat crosses analyzed. These loci were at a distance of 4.82 ± 1.75 cM in the 'Alaga'/'Peñafiel' cross and 6.66 ± 2.26 cM in the 'Alaga'/'Blatfort' cross.

Table 2 χ^2 for independent segregation, percentages of recombination (R), and map distances (cM) for each pair-wise combination of loci among the F_2 progeny from the 'Cajeme 71'/'Ariana 8' cross

Pair of loci	X^2	R ± SD(%)	cM ± SD
Glu-A1–Gli-A3	8.53 ns[(a)]	44.35 ± 4.37	
Glu-A1–Glu-A3	7.37 ns[a]	45.28 ± 4.38	
Glu-A1–Gli-A1	0.36 ns[b]	49.62 ± 4 40	
Glu-A1–Glo-A1	8.26 ns[a]	49.30 ± 4 40	
Gli-A3–Glu-A3	74.26***[a]	19.13 ± 3.46	20.15 ± 3.48
Gli-A3–Gli-A1	37.98***[b]	21.03 ± 3.59	22.42 ± 3.61
Gli-A3–Glo-A1	62.30***[a]	21 21 ± 3 60	22.64 ± 3.62
Glu-A3–Gli-A1	128.32***[b]	1.50 ± 1 07	1.50 ± 1.07
Glu-A3–Glo-A1	109.55***[a]	6.81 ± 2.22	6.85 ± 2 22
Gli-A1–Glo-A1	119 58***[b]	5.06 ± 1.93	5.08 ± 1.93

ns: Not signficant, ***: significant at the 0 1% level of probability
[a] Expected 1·2:1·2:4:2:1:2:1 ratio
[b] Expected 3:6:3:1:2:1 ratio

Table 3 Segregation of phenotypes at the *Gli-D1* and *Glo-D1* loci in the F_2 progeny from the 'Cajeme 71'/'Darius' cross. χ^2 (9:3·3:1) for independent segregation = 118 86 ($P < 0.1\%$). Percentage of recombination, R = 2.86 ± 1.39%. Distance = 2.86 ± 1.39 cM

Locus	Phenotype	*Glo-D1*[a]		Total
		C	–	
Gli-D1	C	106	3	109
	–	1	33	34
Total		107	36	143

[a] C: 'Cajeme 71' -type. –: absence of band

Table 4 Segregation of phenotypes at the *Gli-A1* and *Glo-A1* loci in the F_2 progeny from the 'Alaga'/'Peñafiel' cross. χ^2 (1:2:1·2:4·2:1:2:1) for independent segregation = 224.74 ($P < 0.1\%$). Percentage of recombination R = 4 81 ± 1.75%. Distance = 4.82 ± 1.75 cM

Locus	Phenotype	*Glo-A1*[a]			Total
		A	A/P	P	
Gli-A1	A	32	7	0	39
	A/P	1	67	1	69
	P	0	5	36	41
Total		33	79	37	149

[a] A: 'Alaga' -type; P; 'Peñafiel' -type; A/P heterozygous

Table 5 Segregation of phenotypes at *Gli-A1* and *Glo-A1* loci in the F_2 progeny from the 'Alaga'/'Blatfort' cross. χ^2 (3:6:3:1·2:1) for independent segregation = 83.91 ($P < 0.1\%$) Percentage of recombination R = 6.62 ± 2.26. Distance = 6.66 ± 2.26 cM

Locus	Phenotype	*Glo-A1*[a]			Total
		A	A B	B	
Gli-A1	A	30	57	0	87
	–	0	8	26	34
Total		30	65	26	121

[a] A: 'Alaga' -type; B: 'Blatfort' -type; A/B: heterozygous. –. absence of band

Discussion

The '25-kDa globulins' are a group of endosperm proteins encoded by genes that have been assigned to the short arms of the homoeologous group-1 chromosomes of wheat (Gómez et al. 1988). Results obtained in the present study allowed us to easily detect these proteins in one-dimensional electrophoresis and to analyse their variability in both bread and durum wheat. Furthermore, the genes coding for these proteins were mapped on the 1A and 1D chromosomes of bread wheat and on chromosome 1A of durum wheat, based on the use of prolamin loci as markers.

The A-PAGE patterns of the ethanol-extracted proteins revealed some bands, encoded by genes on the homoeologous group-1 chromosomes of wheat, with a higher electrophoretic mobility than the α-gliadins. Their genetic control, extractability properties and Mr suggested that they should be the *Glo-1*-encoded '25-kDa globulins' studied by Gómez et al. (1988) in two-dimensional electrophoresis. The isolation of the proteins detected in A-PAGE confirmed that they were those globulins, and that 'Ariana 8' possesses the same '25-kDa globulins' as CS.

The variability at *Glo-1* found among a collection of bread and durum wheats was very low, with three and two different patterns, respectively. Two allelic variants were detected at the *Glo-A1* locus in bread wheat and two in durum wheat. One band, used as a marker of the *Glo-A1* locus, had the same mobility in A-PAGE in both species, and probably corresponds to the same allele. At the *Glo-D1* locus, the CS allele and a null allele were found. No variants have been detected at the *Glo-B1* locus.

The linkage mapping of the gene(s) coding for '25-kDa globulins' on the 1A chromosome was performed based on an analysis of the prolamin genes on this chromosome. Four prolamin loci were used: *Glu-A1*, *Glu-A3*, *Gli-A1* and *Gli-A3*. The results obtained allow a re-evaluation of the genetic linkage map of the endosperm protein genes on the 1A chromosome of bread wheat. The *Gli-A3* locus was found at a distance of 22.42 ± 3.61 cM from the *Gli-A1* locus. This distance is shorter than that given by Sobko (1984) and Metakovsky et al. (1986) (31% and 36%, respectively), but similar to that found by Galili and Feldman (1984) and Jackson et al. (1985) between *Gli-B3* and *Gli-B1* (22 cM and 28 cM, respectively). On the other hand, *Gli-A3* was not linked with the *Glu-A1* locus (R = 44.35%). This distance has not been reported previously, but different results have been obtained at the 1B chromosome. Galili and Feldman (1984) found a recombination percentage of 23.5 between *Gli-B3* and *Glu-B1*, whereas Jackson et al. (1985) found a distance of 17 cM between them and Ruiz and Carrillo (1993) obtained a distance of 31.1 cM in durum wheat. Moreover, the recombinants found between the *Gli-A1* and the *Glu-A3* loci allowed us to establish the distance between these loci to be 1.5 cM, similar to that obtained in durum wheat (Ruiz and Carrillo 1993). The percentages of recombination and the genetic distances obtained between *Glu-A3* and the other loci considered are reported here for the first time in bread wheat and confirm its position, between *Gli-A1* and *Gli-A3*.

The recombination percentages and the distances found between the five endosperm protein loci studied allowed us to establish the following order of loci on the 1A chomosome: *Glu-A1* – centromere – *Gli-A3*–*Glu-A3*–*Gli-A1*–*Glo-A1*. Thus, *Glo-A1* is located at the distal end of the short arm of the 1A chromosome, at a distance of 5.23 ± 1.99 cM from the *Gli-A1* locus. In addition, the *Glo-D1* locus has been mapped at 2.86 ± 1.39 cM from the *Gli-D1* locus on the 1D chromosome. This distance is slightly shorter than that found between *Gli-A1* and *Glo-A1*, which might be due to a deletion of a small chromosome segment containing the *Gli-D1* locus of cv 'Darius'. Based on the results obtained for the 1A chromosome, and due to the homoeology between the 1A and 1D chromosomes, the *Glo-D1* locus should also be located at the distal end of the short arm of the 1D chromosome.

The results obtained in the analysis of two crosses of durum wheat allocate the *Glo-A1* locus at a similar distance from the *Gli-A1* locus (4.82 ± 1.75 cM and 6.66 ± 2.26 cM) to that obtained in bread wheat. The homoeology between loci in bread and durum wheat has been largely demonstrated, so the position of the *Glo-A1* locus should also be at the distal end of the short arm of the 1A chromosome. Although no variants have been detected at the *Glo-B1* locus in either bread or durum wheat, this locus is expected to be located at a similar distance and position to *Glo-A1* and *Glo-D1*.

Two ω-gliadin genes, *Gli-A5* and *Gli-B5* (Metakovsky et al. 1986; Pogna et al. 1993), have been found in bread wheat at a similar distance and position to the globulin genes mapped here. The relationships between the *Gli-5* and the *Glo-1* genes is not known as yet, but their encoded proteins belong to different families (ω-gliadins and globulin-type proteins, respectively) with contrasting molecular masses, and could not be considered as allelic variants. At the 1A and 1D chromosomes, only the genes coding for the triplet proteins (globulin-type proteins) have been mapped (Singh and Shepherd 1988). Their chromosomal location, between *Glu-1* and *Gli-1*, and their biochemical characteristics (Singh and Shepherd 1985) are different from the globulin-type proteins studied here.

The '25-kDa globulins' are aggregated under non-dissociating conditions and have a relatively high content of sulphur amino acids (Gómez et al. 1988). These characteristics are important determinants of the bread-making quality of wheat flour (see Lásztity 1984 for a review) and suggest that the '25-kDa globulins' may be involved in the quality properties of the doughs to make bread and pasta. Additionally, a knowledge of the chromosomal locations of the *Glo-1* loci, together with the other protein loci located on the short arm of the group-1 chromosomes, will be useful in the detection of translocations of chromosome segments in wheats.

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