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Location of genes controlling the D group of LMW glutenin subunits on chromosome 1D of bread wheat

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Summary

Over one hundred backcross-one progeny were analysed by two dimensional electrophoresis to locate the genes on the short arm of chromosome 1D which code for, or control, the D group of LMW glutenin subunits. Recombination between these genes and *Glu-D1*, encoding HMW glutenin subunits, was frequent but none could be detected between the D subunit genes and *Gli-D1*, coding for ω-gliadins, γ-gliadins and the B group of LMW glutenin subunits. These results are in contrast to those for chromosome 1B because apparently homoeologous D subunit genes occur at a different position, being equidistant between *Glu-B1* (homoeologous to *Glu-D1*) and *Gli-B1* (homoeologous to *Gli-D1*). A range of 18 genetically-diverse wheat varieties, each containing one of two allelic groups of 1D-encoded D subunits, were also analysed by two-dimensional electrophoresis. Consistent with the genetic analysis above, unbreakable linkages were found between these alleles and alleles of *Gli-D1*. The results are discussed in relation to the evolution of the distribution of prolamin genes in the wheat genome.

1. Introduction

The D group of low-molecular-weight (LMW) glutenin subunits are a small group of storage proteins in the endosperm whose genes are located on the short arms of chromosomes 1B and 1D (Jackson, Holt & Payne, 1983). In a previous paper (Jackson, Holt & Payne, 1985) it was shown that the genes for the 1B-controlled proteins occur at a newly designated locus, *Glu-B2*, and not with the rest of the storage protein genes which code for ω-gliadins, γ-gliadins and the B group of LMW glutenin subunits and occur at a common locus, *Gli-B1* (Payne *et al.* 1984*b*). *Glu-B2* was shown by recombination mapping to lie equidistant between *Gli-B1* and another locus, *Glu-B1*, which lies close to the centromere on the long arm and codes for high-molecular-weight (HMW) subunits of glutenin (Payne *et al.* 1982).

In this paper the position on the short arm of chromosome 1D of the genes controlling the D group of LMW glutenin subunits was determined by recombination mapping.

2. Methods

The stock of variety Sicco used as a parent was derived from several generations of enforced backcrossing in the glasshouse. The other varieties analysed by electrophoresis were taken from the Institute's germplasm collection. The remaining genotype, which was crossed to Sicco, was H-322, an ancient hexaploid landrace from Afghanistan, collected by the University of Reading Afghanistan Expedition in 1965.

(i) *Sodium dodecyl sulphate, polyacrylamide gel electrophoresis (SDS-PAGE)*

Total proteins were extracted from half grains and analysed by SDS-PAGE as described previously (Payne, Law & Mudd, 1980; Payne *et al.* 1981). This enabled allelic variation to be assessed at *Glu-B1*, *Gli-B1* and *Glu-D1*.

(ii) *Two-dimensional electrophoresis*

The remaining half grains from SDS-PAGE were analysed by two-dimensional electrophoresis. The method, which uses isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second (abbreviated to IEF × SDS-PAGE), was based on the method of O'Farrell (1975) as modified by Holt, Astin & Payne (1981). It was used to detect allelic variation at *Gli-B1*, *Gli-D1*, *Glu-B2*, and in the 1D-encoded, D group of LMW glutenin subunits.

3. Results

(i) *Preliminary studies*

A collection of varieties and landraces were first analysed by IEF × SDS-PAGE to search for allelic variation in the 1 D-encoded, D group of LMW glutenin subunits. As with the corresponding D subunits coded by chromosome 1 B, little variation was found. One of the first variants to be detected was a landrace from Afghanistan, H-322. It lacked the two minor acidic components which comprise the 1 D-encoded D subunits of Sicco (Fig. 1 *b*: double headed, open arrows marked S) and these were replaced by two similar components which were slightly more basic and had a slightly greater mobility in the second dimension (Fig 1 *b*: double-headed, open arrows marked H). As the ω-gliadins coded by genes on chromosome 1 D are also different to those of Sicco (Fig 1 *b*: double headed, solid arrows marked H and S), the following crosses were made:

H-322 ♀ × Sicco ♂ (Primary cross)
↓
F_1 ♀ × Sicco ♂ (Backcross)
↓
grain for analysis.

The two parents also expressed allelic variation for most of the storage-protein loci and this enabled the 1 D-encoded D subunits to be mapped to *Glu-D 1* as well as *Gli-D 1*. In addition the 1 B-encoded D subunits at locus *Glu-B 2* could also be mapped to *Glu-B 1* and *Gli-B 1*, linkage data for which has already been published using a different cross (Jackson *et al.* 1985).

The chromosome arm location of the loci under study, the proteins they code for and the electrophoretic methods used to follow the segregation of the allelic variants are summarized in Table 1.

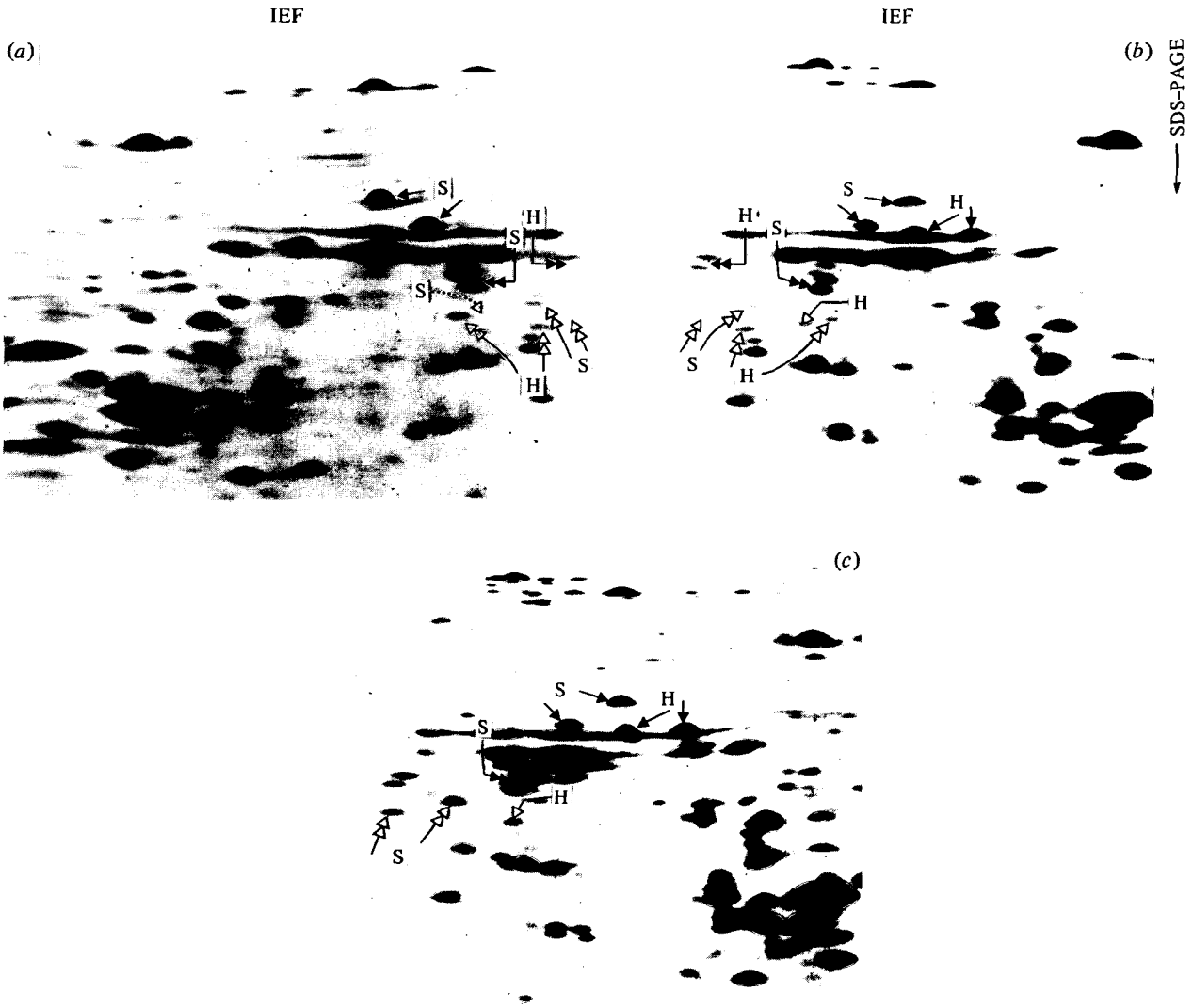

Fig. 1. Two-dimensional electrophoresis of the proteins of three progeny from the cross (H-322 × Sicco) × Sicco. For grains *b* and *c* the acidic ends of the IEF gradient are on the left and the basic ends on the right. For progeny *a*, the pH gradient is in the reverse direction; the protein patterns of *a* and *b* therefore appear as mirror images. The symbols used are described in the text.

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Table 1. *Summary of protein groups analysed and the location of their genes*

Chromosome	Locus	Chromosome arm	Proteins analysed	Technique
1 D	*Glu-D 1*	Long	HMW glutenin subunits	SDS-PAGE
1 D	*Gli-D 1*	Short	ω-gliadins[a]	IEF × SDS-PAGE
1 D	Under study	Short	D, LMW glutenin subunits	IEF × SDS-PAGE
1 B	*Glu-B 1*	Long	HMW glutenin subunits	SDS-PAGE
1 B	*Gli-B 1*	Short	ω-gliadins[a b]	SDS-PAGE IEF × SDS-PAGE
1 B	*Glu-B 2*	Short	D, LMW glutenin subunits	IEF × SDS-PAGE

[a] These loci also contain genes for γ-gliadins and the B group of LMW glutenin subunits but only ω-gliadins were made use of in this study to recognize allelic variation.
[b] Allelic variation for these proteins was recognized from both analytical techniques and served as a valuable check in the unlikely event of corresponding grain halves being accidentally interchanged.

(ii) *Recombination mapping*

Two-dimensional electrophoresis maps of three backcross 1 progeny are shown in Fig 1. Variation in ω-gliadins coded at (1) *Gli-B 1* are indicated by single headed, solid arrows and (2) *Gli-D 1* by double-headed, solid arrows. By contrast the D group of LMW glutenin subunits coded at *Glu-B 2* are indicated by single-headed, open arrows and those by the genes under test by double-headed open arrows. Because of the triploid nature of the endosperm, each locus will be expressed at three dose levels. One of these doses will always be due to the alleles of Sicco (S) coming from the backcross, male parent. The other two doses of each locus will be inherited from the F_1, female parent and be either Sicco alleles or landrace H-322 (H) alleles. The interpretation of the inheritance of the four protein groups in the three progeny shown in Fig 1 are set out in Table 2.

For each of the three progenies illustrated in Fig 1 the alleles of *Glu-B 2* and *Gli-B 1* were inherited from the same parent (Table 2), indicating that no recombination had occurred on the chromosome segment between these two loci. However in the total number of 113 progeny analysed by two-dimensional electrophoresis, recombination between *Glu-B 2* and *Gli-B 1* was common. The half grains remaining after two dimensional electrophoresis were analysed by SDS-PAGE to reveal allelic variation at *Glu-B 1* (not shown). In Table 3 the frequencies of the eight possible genotypes for the three loci on chromosome 1 B under study are given. Genotypes 3 and 7 are by far the most rare (Table 3), indicating that double recombinations have taken place between the three loci in their formation. The order of the loci on chromosome 1 B is therefore as indicated in Table 3 with *Glu-B 2* occurring proximally on the short arm, equidistant between *Gli-B 1*, which occurs near the end of the short arm in the satellited region (Payne *et al.* 1984*a*), and *Glu-B 1*, which occurs on the long arm close to the centromere (Payne *et al.* 1982). The relative position of *Glu-B 2* is therefore consistent with our previous publication (Jackson *et al.* 1985) where two different crosses were analysed.

In contrast to the results above for chromosome 1 B, no recombinations were observed on chromosome 1 D between genes coding for the D group of LMW glutenin subunits and ω-gliadin genes at *Gli-D 1* (Table 4).

(iii) *Two dimensional electrophoresis of varieties*

The chromosome 1 D-encoded ω-gliadins of landrace H-322 have distinctive mobilities and they can easily be observed by one-dimensional electrophoresis at pH 3·1 using aluminium lactate buffer. They have slightly slower mobilites than the allelic proteins from Sicco and stain much less intensively with Coomassie Brilliant Blue. The latter is the reason why in Fig 1*a*,

Table 2. *Inheritance of protein alleles from F_1, ♀ gametes in the backcross 1 generation for the three progeny shown in Fig. 1.*

Chromosomes	Locus	Protein group	Inheritance of alleles from F_1, ♀ gametes[a] Fig. 1*a*	Fig. 1*b*	Fig. 1*c*
1 B	*Gli-B 1*	ω-gliadins	S } P	H } P	H } P
1 B	*Glu-B 2*	D subunits	S }	H }	H }
1 D	*Gli-D 1*	ω-gliadins	H } P	H } P	S } P
1 D	Under study	D subunits	H }	H }	S }

[a] All alleles expressed as two doses. The third dose for all loci is always an S allele.
P, Parental: H and S are alleles inherited from landrace H-322 and Sicco respectively.

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Table 3. *Genotypic classification of the progeny for alleles at three loci on chromosome 1 B*

Genotype	Allele types *Glu-B 1*	*Glu-B 2*	*Gli-B 1*	Observed frequencies
1	S	S	S	30
2	S	S	H	11
3	S	H	S	4
4	S	H	H	10
5	H	H	H	35
6	H	H	S	10
7	H	S	H	3
8	H	S	S	10
			Total:	113

Recombination percentages:
Glu-B 1 – *Glu-B 2* = 23·9±4·01%[a]
Glu-B 2 – *Gli-B 1* = 24·8±4·06%[a]
Glu-B 1 – *Gli-B 1* = 48·7±4·70%[a]

S, Sicco alleles; H, alleles of landrace H-322.
The two parental genotypes are 1 and 5.
[a] Standard deviation.

one dose of Sicco 1 D-encoded ω-gliadins stain more strongly then two doses of their allelic counterparts from the landrace. Using the nomenclature of Zillman & Bushuk (1979) they have relative electrophoretic mobilities of 12·5 and 15·5 (see their fig. 1, slots 3–5).

Varieties were therefore screened for the presence of these two proteins by electrophoresis at pH 3·1 and seven varieties containing them (Cama, Cardinal, Fylgia, Glenlea, Kolibri, Maris Butler and Maris Dove) were analysed by two-dimensional electrophoresis. All contained the alternative pair of chromosome 1 D-encoded LMW glutenin subunits found in landrace H-322 (not shown). Eleven varieties containing 1 D-encoded ω-gliadins similar to Sicco were also analysed (Armada, Berseé, Bezostaya-1, Brigand, Cappelle-Desprez, Champlein, Chinese Spring, Condor, Heron, Highbury and Hope) and all contained the Sicco allele of the 1 D-encoded D subunits.

Table 4. *Genotypic classification of the progeny for alleles at three loci on chromosome 1 D*

Genotype	Allele types *Glu-D 1*	D subunit genes	*Gli-D 1*	Observed frequencies
1	S	S	S	23
2	S	S	H	0
3	S	H	S	0
4	S	H	H	33
5	H	H	H	24
6	H	H	S	0
7	H	S	H	0
8	H	S	S	33
			Total:	113

Recombination percentages:
Glu-D 1 and *Gli-D 1* = 58·4±4·64%[a]

S, Sicco alleles; H, landrace H-322 alleles.
The two parental genotypes are 1 and 5.
[a] Standard deviation.

4. Discussion

The proteins under study, the D subunits, form a distinctive but minor group of proteins in wheat endosperm. They are deposited in protein bodies in the developing endosperm (Payne *et al.* 1986) and are thus true storage proteins. They belong to the glutenin complex of proteins for they occur as aggregates (Jackson *et al.* 1983), presumably through disulphide bond formation, either with themselves or with other glutenin subunits. The 1 B- and 1 D-encoded group of LMW glutenin subunits have similar properties to each other; they are expressed in similar amounts, they only occur in small aggregates of glutenin, they both display little allelic variation, and they have similar isoelectric points and molecular weights. All the indications are that they are two sets of homoeologous proteins yet their genes do not occur at the same position on their respective chromosomes (Fig 2). This is the first report of such an occurrence in wheat for storage-protein genes.

Recent progress in molecular biology has established that the different groups of glutenin subunits and gliadins have regions of pronounced sequence similarity and it is now generally agreed that they were formed from the replication and subsequent divergence of a common ancestral gene (Shewry & Miflin, 1985). Assuming that this is true also for the D group of LMW glutenin subunits it is suggested that at some period in evolution after the divergence of the B-genome and D-genome diploid wheats the D subunit genes at *Gli-B 1* were transferred closer to the centromere on chromosome 1 B. There are several possible mechanisms which could account for this transfer, including chromosome inversion and gene conversion (Baltimore, 1981).

The situation on chromosome 1 A is not clear for no D subunits are produced from gene activity on this chromosome. However very recently Sobko (1984) described a locus encoding minor ω-glandins at a comparable position on chromosome 1 A to *Glu-B 2* on chromosome 1 B. Also Galili & Feldman (1984) described a locus on chromosome 1 B at a similar position to *Glu-B 2* but they described the single protein product as a gliadin rather than a D subunit of glutenin. The relationship between this locus and *Glu-B 2* will be evaluated by making crosses between relevant genotypes. It is not impossible that these three sets of proteins may represent a homoeologous series with their genes being proximal on chromosome 1 A and 1 B and distal at *Gli-D 1* on 1 D.

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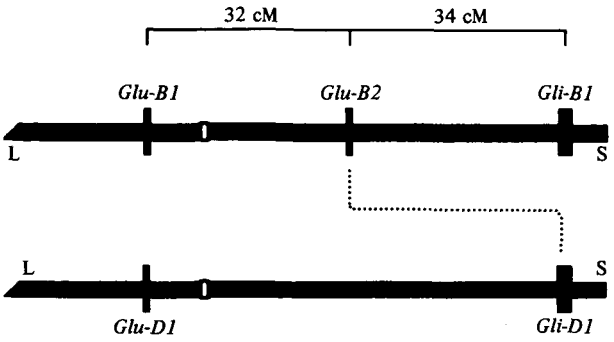

Fig. 2. Location of prolamin storage-protein genes on chromosomes 1 B and 1 D of wheat. The results include those from this paper and from Jackson *et al.* (1985). The distance between *Glu-1* and *Gli-1* has been normalized to 66 cM as in previous publications (Payne *et al.* 1982). The dotted line indicates the relative positions of the D subunit genes. L stands for long and S for short. Only part of the long arms are shown.

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