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Glu-B2, a storage protein locus controlling the D group of LMW glutenin subunits in bread wheat (*Triticum aestivum*)

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SUMMARY

Genes controlling the synthesis of the D group of low-molecular-weight (LMW) subunits of glutenin occur on the short arms of chromosomes 1 B and 1 D. Their position on chromosome 1 B, relative to the storage protein loci *Glu-B1* (long arm) and *Gli-B1* (short arm), was estimated by analysing the backcross-one progeny of two different crosses. To estimate recombination between the D subunit genes and *Gli-B1*, half grains were analysed by two-dimensional electrophoresis. The *Gli-B1* locus contains genes for the B group of LMW glutenin subunits, γ-gliadins and ω-gliadins although only the latter were made use of in this study to distinguish the parental alleles. Additionally, the complementary half grains were analysed by sodium dodecyl sulphate, polyacrylamide-gel electrophoresis to estimate recombination between *Gli-B1* and *GluB1*, coding for high-molecular-weight (HMW) glutenin subunits. The D subunit genes occur at a new locus, provisionally defined as *Glu-B2*, which lies in between *Glu-B1* and *Gli-B1*, 17 cM from the former and 22 cM from the latter. On the basis of previous mapping data involving *Gli-B1*, it was concluded that the D subunit genes occur close to the nucleolar organizing region and probably on the short-arm satellite, like *Gli-B1*.

1. INTRODUCTION

Glutenin, the viscoelastic component of wheat flour, is a heterogeneous mixture of large proteins which are built up from several different types of subunit linked together by disulphide bonds (Wall, 1979). Four groups of subunits are currently recognized, and they have been called A, B, C and D subunits (Payne & Corfield, 1979; Jackson, Holt & Payne, 1983). The A subunits are now more commonly described as high-molecular-weight (HMW) subunits (Payne, Law & Mudd, 1980; Lawrence & Shepherd, 1981) and are clearly distinguishable from other subunits and from other endosperm proteins by their slow mobility during SDS–PAGE. In line with this alternative terminology, the B, C and D subunits may be collectively described as low-molecular-weight (LMW) subunits.

The B subunits are the most common subunits of glutenin and by SDS–PAGE they have nominal molecular weights of 42000–51000 (Payne & Corfield, 1979).

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They have, on average, slower mobilities than the α, β- and γ-gliadins during SDS-PAGE although there is some overlap. By the two-dimensional procedure of O'Farrell, Goodman & O'Farrell (1977) they are clearly distinctive, for they are much more basic proteins than the gliadins (Jackson *et al.* 1983). The minor C subunits have similar mobilities in SDS-PAGE, both to each other and to α-, β- and γ-gliadins (Payne & Corfield, 1979), but they have a wide range of isoelectric points, from slightly acidic to highly basic. Unfortunately, many of them are difficult to study because they have identical electrophoretic mobilities to some gliadins which are produced in much larger amounts. The remaining group, the D subunits, were only discovered by the use of two-dimensional electrophoresis (Brown & Flavell, 1981; Jackson *et al.* 1983). They have slower mobilities than the B and C subunits and form one of the most acidic groups of proteins in the endosperm.

It has been known for some time that the genes controlling the A (HMW) subunits are located on the long arms of chromosomes 1A, 1B and 1D (Bietz, Shepherd & Wall, 1975; Lawrence & Shepherd, 1980). However, it has only recently been shown that the B subunits, some of the C subunits and all of the D subunits are controlled by genes on the short arms of the same set of chromosomes (Jackson *et al.* 1983). Subsequent recombination mapping showed that the B subunit genes examined were tightly linked to ω- and γ-gliadins on chromosomes 1A or 1B and so therefore must be located at loci *Gli-A1* and *Gli-B1* respectively and presumably also at *Gli-D1* (Payne *et al.* 1984*b*).

In this study the location on the short arm of chromosome 1B of genes controlling D subunits of glutenin was determined by recombination mapping with genes at *Gli-B1* on the short arm and *Glu-B1* on the long arm.

2. METHODS

The bread-wheat varieties used were taken from the collection held at the Plant Breeding Institute, Cambridge, UK.

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

The method used to fractionate the total proteins of wheat endosperm after reduction with 2-mercaptoethanol and to detect allelic variation at *Glu-B1* was described previously (Payne *et al.* 1980, 1981). The method fractionates proteins mainly according to their molecular weight.

(ii) *Two-dimensional electrophoresis*

This method, which uses isoelectric focusing (IEF) in the first dimension and SDS–PAGE in the second (abbreviated 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 in ω-gliadins coded by chromosome 1B and the D subunits of glutenin.

3. RESULTS

To map the D subunit genes on chromosome 1B the progeny from two crosses were analysed. The first was:

Chinese Spring ♀ × Sicco ♂ (Primary cross)
↓
F_1 ♀ × Sicco ♂ (Backcross)
↓
grain for analysis

The two varieties have contrasting allelic variants for HMW subunits of glutenin at *Glu-B1* and contrasting allelic variants for ω-gliadins at *Gli-B1* as shown in Table 1. Chinese Spring contains a D subunit of glutenin controlled by chromosome 1B, called DB1, but Sicco contains a more basic allelic variant, called DB2.

Table 1. *Allelic variants for the loci under study in Sicco, Chinese Spring and landrace V 538*

		Proteins translated		
Protein	Locus	Sicco	Chinese Spring	V 538
HMW glutenin subunits	*Glu-B1*	7+9*	7+8*	20*
ω-gliadins	*Gli-B1*	A	B+C+D†	F+G
D-glutenin subunits	?	DB2	DB1	DB1

* The HMW subunit numbering system of Payne *et al.* (1981) is used. The allele for 7+9 was designated *Glu-B1c*, that for 7+8 *Glu-B1b* and that for 20 *Glu-B1e* by Payne & Lawrence (1983).
† By SDS–PAGE, only C is revealed.

To study the segregation of *Gli-B1* alleles and D subunit alleles from the primary cross, half grains were first analysed by two-dimensional electrophoresis, and an example of four separations, two on each second-dimension gel slab, is shown in Plate 1. The samples fractionated in Plate 1, Figs. 1 and 2, contain four ω-gliadins coded by chromosome 1B. The ω-gliadins B, C and D must have been inherited from Chinese Spring, a primary-cross parent, and ω-gliadin A from the Sicco backcross parent. Both of the progeny are therefore Chinese Spring (C) allele types. In contrast the progeny of Plate 1, Figs. 3 and 4 lack ω-gliadins B, C and D but contain ω-gliadin A at a much higher dosage than in Figs. 1 and 2. The progeny must have inherited two doses of A from the Sicco primary parent and one from the Sicco backcross parent and so contain Sicco (S) allele types. The segregation of the 1B-encoded D subunits of glutenin is also shown in Plate 1. Subunit DB 1 from Chinese Spring is present in Plate 1, Figs. 1, 2 and 4 but is absent in Plate 1, Fig. 3 whereas subunit DB2 from Sicco is present at low dosage in Plate 1, Figs. 1, 2 and 4 but at relatively higher dosage in Fig. 3. Therefore, for chromosome 1B-encoded D subunits of glutenin, the progeny in Plate 1, Figs. 1, 2 and 4 have Chinese Spring (C) alleles and that in Fig. 3 has the Sicco (S) allele. In all, 22 grains were analysed and all could be classified unambiguously for C and S alleles of D subunit genes and *Gli-B1*.

To study the segregation of *Glu-B1* alleles from the primary cross in the same progeny, the half grains remaining from the above analysis were fractionated by SDS–PAGE. Chinese Spring contains two HMW glutenin subunits coded at *Glu-B1*, subunits 7 and 8, whereas Sicco contains 7 and 9 (Table 1). The subunit 7 associated with 8 has a slightly greater electrophoretic mobility than subunit 7 associated with

Table 2. *Genotypic classification of the progeny from the two crosses for* Glu-B1, *D subunit genes and* Gli-B2

	Allele types			Observed frequencies		
Genotype	*Glu-B1*	D subunit genes	*Gli-B1*	Cross 1	Cross 2	Combined
1	S	S	S	6	16	22
2	S	C	C	2	0	2
3	S	S	C	2	3	5
4	S	C	S	1	1	2
5	C	S	C	1	0	1
6	C	C	S	2	3	5
7	C	S	S	1	4	5
8	C	C	C	7	13	20

Recombination percentages assuming locus order *Glu-B1*–D subunit genes–*Gli-B1*:

Glu-B1 and D subunit genes $= \frac{2+4+5+7}{\text{total}} \times 100 = 16{\cdot}1 \pm 4{\cdot}7\,\%$*

D subunit genes and *Gli-B1* $= \frac{3+4+5+6}{\text{total}} \times 100 = 21{\cdot}0 \pm 5{\cdot}2\,\%$*

Glu-B1 and *Gli-B1* $= 16{\cdot}1 + 21{\cdot}0 = 37{\cdot}1 \pm 6{\cdot}1$*

C = Chinese Spring allele for cross 1 and landrace allele for cross 2.
S = Sicco allele for both crosses.
* = Standard deviation.
The two parental genotypes are 1 and 8.

9 but in the analysis, a selection of which are shown in Plate 2, the presence or absence of subunit 8 and the relative intensity of subunit 9 was sufficient to assign all progeny unambiguously to either C-type or S-type *Glu-B1* alleles. This gel procedure also distinguishes some of the 1B-encoded ω-gliadins from the two parents and so was used as an independent check of allele assignments for *Gli-B1* from the two-dimensional separations.

For the three loci under study, there are eight possible genotypes. These, and their frequencies of occurrence amongst the progeny, are listed in Table 2.

Using the two gel electrophoresis procedures a further 40 grains were analysed from a second crossing procedure in which a bread-wheat landrace from Morocco, V 538, replaced Chinese Spring as one of the primary-cross parents. The landrace has different alleles at *Glu-B1* and *Gli-B1* from both Sicco and Chinese Spring but has the same 1*B*-encoded D subunit of glutenin (DB1) as Chinese Spring (Table 1). Interpretation of the gels was exactly comparable with that of the first cross.

The frequencies of the eight possible genotypes from this cross are also shown in Table 2 and, as they are not significantly different from the frequencies obtained for the first cross, the results of the 62 progeny were combined. Since two of the

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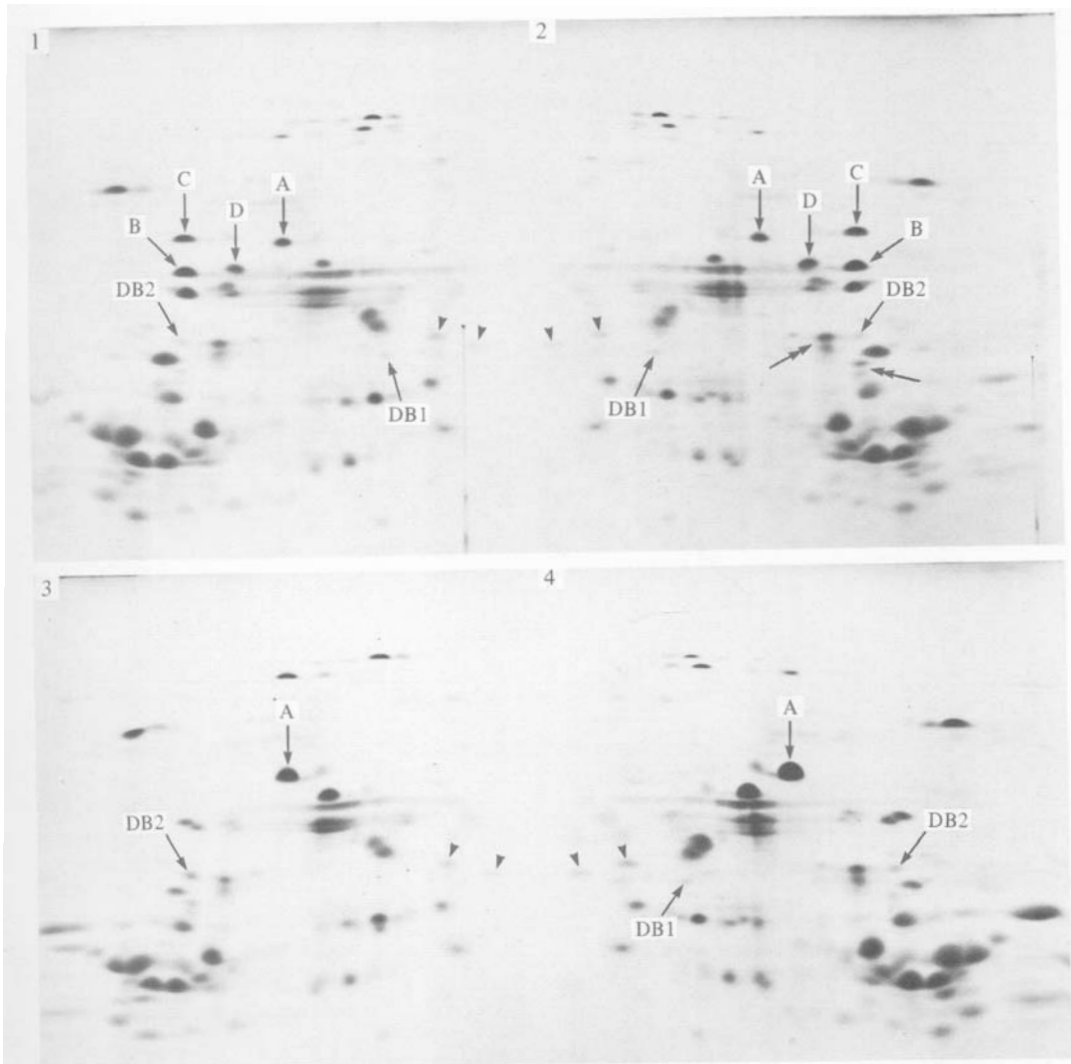
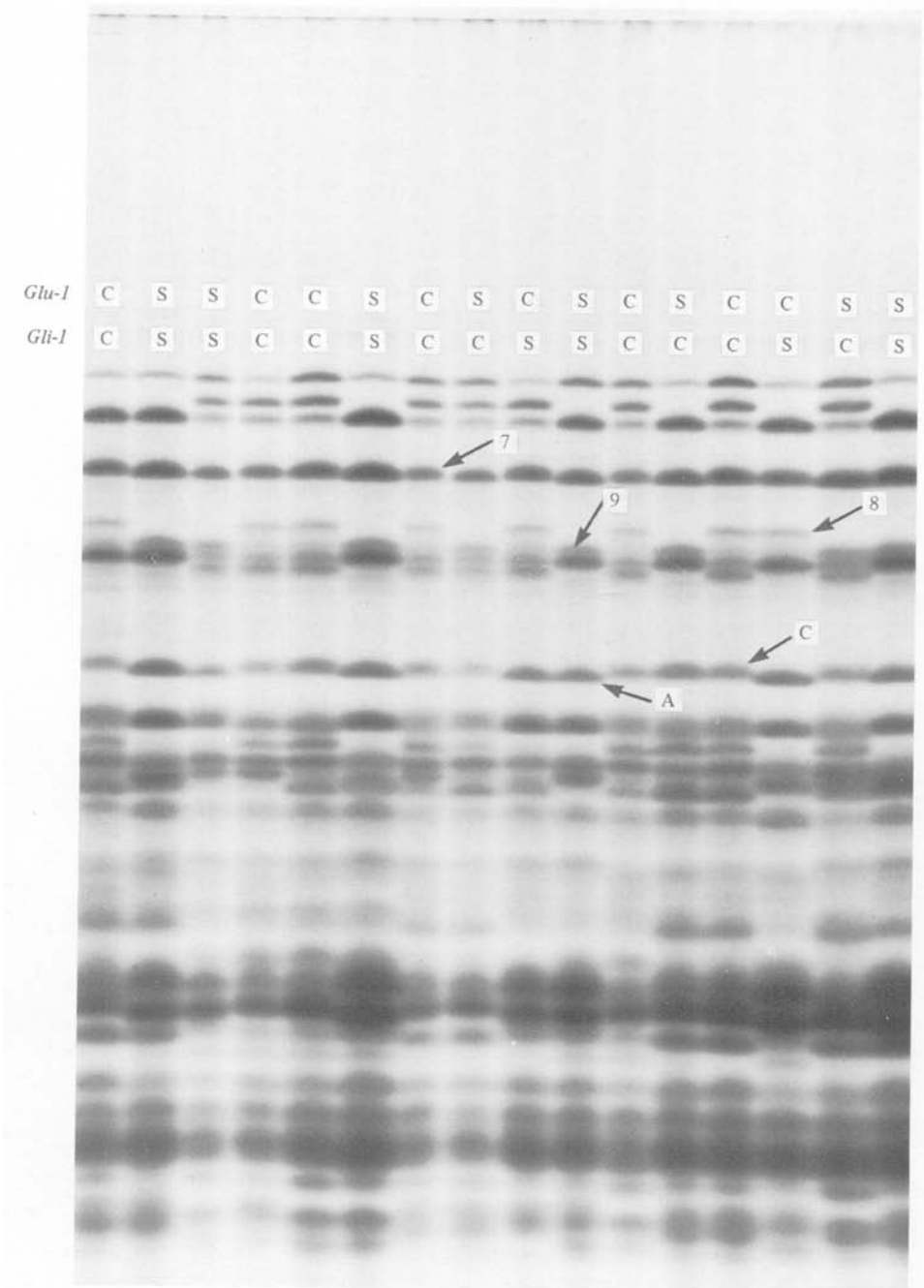


Two-dimensional fractionation of endosperm proteins from four different progeny (Figs. 1–4) of the cross (Sicco × Chinese Spring) × Sicco. Proteins in each experiment were extracted from an embryo-less halfgrain. The ω-gliadin coded by chromosome 1 B and derived from Sicco is arrowed A; those from Chinese Spring are arrowed B, C and D. DB 1 and DB 2 are 1 B-encoded D subunits of glutenin derived from Chinese Spring and Sicco respectively. The 1 D-encoded D subunits are indicated by tail-less arrows. The two sets of large subunits derived from the triplet bands are indicated by double-headed arrows, and each set consists of two components. The set with slower mobility during SDS–PAGE are controlled by chromosome 1 D and the other by chromosome 1 A. In the experimental procedure, two first-dimension tube gels were placed side by side with their origins (basic end) distant from each other, which is contrary to normal. This ensured that the acidic D subunits separated in the middle of the gel instead of at the extreme edge.



SDS–PAGE of 16 half grains. The HMW glutenin subunits coded by chromosome 1B and marked 9 and 8 are inherited from Sicco and Chinese Spring respectively. The major 1B-encoded subunit 7 occurs in both varieties. The ω-gliadin-labelled A is inherited from Sicco and that labelled C from Chinese Spring. The progeny were classified into allele types (C or S) for both *Glu-B1* and *Gli-B1*.

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three loci under study occur on the short arm of chromosome 1B and the other is on the long arm, only two locus orders are possible; either *Gli-B1* is proximal to the D subunit genes on the short arm or it is distal. The actual order can be predicted from Table 2 by determining the number of progeny which are double recombinants, i.e. allele types S–C–S and C–S–C, where recombinations occur on either side of the central locus and are thus relatively rare. If *Gli-B1* is proximal to the D subunit genes then genotypes 3 and 6 of Table 2 are the double recombinants and these occur in 10 of the 62 progeny. If *Gli-B1* is distal then the double recombinants are genotypes 4 and 5. As there are only three of these the locus order is probably *Glu-B1*–D subunit genes–*Gli-B1*. On this basis recombination between the three loci was calculated (Table 2) and the D subunit genes were shown to be approximately equidistant between *Glu-B1* and *Gli-B1*. Using the Kosambi function as previously described (Payne *et al.* 1982) chromosome map distances were calculated in centiMorgans (cM). They were *Glu-B1* to *Glu-B2*, $16{\cdot}7 \pm 5{\cdot}2$ cM, and *Glu-B2* to *Gli-B1*, $22{\cdot}4 \pm 6{\cdot}3$ cM.

4. DISCUSSION

The D subunits of glutenin, whilst contributing to the heterogeneous glutenin complex, have distinctive biochemical and genetical properties. Individual components are expressed in much smaller amounts than most storage protein components and allelic variation is very limited, as was shown in a survey of some 25 varieties (Jackson, Holt & Payne, in prep.). In this study the limited variation was exploited to map their controlling genes. The subunits are likely to be true storage proteins, for they are freely soluble in 70% aqueous ethanol at room temperature, which is a characteristic of the prolamins. The D subunits occur in the aggregated state, even in the presence of highly dissociating solvents, and it is presumed that they are disulphide-linked, although the composition of aggregates containing them is not known. Gel-filtration chromatography experiments have indicated that the D-subunits are preferentially associated with the smaller-sized aggregates of glutenin (E. A. Jackson, unpublished work).

The work presented here shows that the controlling genes of the D subunits do not occur at *Gli-B1*, as do the genes for the major LMW glutenin subunits, but at a separate locus. Since the D subunits are subunits of glutenin, it is reasonable to call this new locus *Glu-B2* provisionally. It is assumed that the locus is part of a homoeologous series, and two D subunits controlled by chromosome 1D have similar electrophoretic properties to the chromosome 1B-encoded D subunits studied here. However, no D subunit controlled by chromosome 1A has been detected so far. The recombination observed between *Gli-B1* and *Glu-B2*, 21%, is considerably less then that found between ribosomal RNA genes (*Nor1*) and *Gli-B1* (37%) by Snape *et al.* (1985), although it is only slightly less than an indirect estimate of the same distance (23%) determined by Payne *et al.* (1984*a*). The balance of evidence therefore suggests that *Glu-B2* occurs fairly close to *Nor1* but on the satellited segment of the short arm of chromosome 1B. Direct evidence for the physical location of *Glu-B2* on the short arm of chromosome 1B comes from the two-dimensional analysis of the proteins of a mutant line which lacks the 1B

satellite by a chromosome cleavage at the nucleolar organizing region (Payne *et al.* 1984*a*). The primary parents of the line were Cappelle-Desprez, which contains the 1B-encoded D subunit DB1, and Hope, which contains DB2. The mutant contains neither of these proteins.

Recently, publications from two different laboratories have also shown that some minor endosperm proteins are controlled by genes on the short arms of the group 1 chromosomes, but which map separately to *Gli-1*. Singh & Shepherd (1984) showed the presence of a triplet of bands (called Trp-1, Trp-2 and Trp-3) in non-reduced fractionations by SDS–PAGE. Upon reduction the protein in the bands dissociated into two large subunits controlled by chromosomes 1A and 1D and two small subunits, controlled by the same chromosomes. The triplet band larger subunits in our two-dimensional procedure are labelled by double-headed arrows in Plate 1, fig. 2. The results of Singh & Shepherd (1984) and our own unpublished mapping data show that the controlling genes for these proteins occur much closer to the centromere on chromosomes 1A and 1D than the locus under study in this paper, *Glu-B2*, which occurs on chromosome 1B. They are therefore very unlikely to form a homoeologous series.

Prior to the work of Singh & Shepherd (1984), Galili & Feldman (1984) demonstrated the presence of a distinctive protein in SDS–PAGE fractionations of the variety Thatcher and the intervarietal chromosome substitution line Chinese Spring (Thatcher 1B), which apparently is uncommon amongst varieties (Galili & Feldman, 1983). Its electrophoretic mobility was similar to that of the D subunits of glutenin and to the larger subunits of the triplet bands. The biochemical properties of the protein, called B 30, have not yet been described. Although it could be a rare 1B-encoded triplet band subunit, the common form being a null, this is unlikely from the gene mapping data of Galili & Feldman (1984). The B 30 genes are approximately equidistant between *Glu-B1* and *Gli-B1* like the genes at *Glu-B2*. Whether B 30 is an unusual allelic variant of 1B-encoded D subunits or whether it is quite a different protein coded at yet another endosperm protein locus remains to be seen.

The *Trp* and *Glu-2* loci add to several other gene loci mapped on the short arm of chromosome 1B (Payne *et al.* 1984*a*; Snape *et al.* 1985) and will serve as useful genetic markers. Any evolutionary relationships between these two minor loci and the major storage protein loci *Glu-1* and *Gli-1* have yet to be established.

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