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Inheritance of B subunits of glutenin and ω- and γ-gliadins in tetraploid wheats

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Abstract A double-1RS wheat-rye translocation line lacking all B subunits of glutenin was produced in durum wheat cv 'Langdon' for use in backcrosses and testcrosses in the study of the inheritance of low-molecular-weight (LMW) glutenin subunits in tetraploid wheats. The B subunits of glutenin and γ-and ω-gliadin bands present in parents derived from *Triticum durum* and *T. dicoccoides*, encoded by *Glu-3* and *Gli-1* loci, respectively, were found to be inherited mainly as units (blocks), as reported previously. Two rare recombination events between the *Glu-A3* and *Gli-A1* loci were detected in testcross progeny from 'Edmore' × *T. dicoccoides* landrace 19–27. Several rare recombinants were also detected within the 1BS-controlled B subunits of glutenin blocks, suggesting that there are two separate tightly linked loci (3.07±1.35 cM) within the *Glu-B3* 'locus'. Evidence was also obtained for the presence of an additional locus coding for a B subunit of glutenin in 'Edmore' that is loosely linked (20.9±3.18%) with the main *Glu-B3* 'locus'.

Key words Inheritance · Glutenins · Gliadins · *Glu-3* loci · *Gli-1* loci · *Triticum turgidum* var 'durum' and *T. dicoccoides*

Introduction

It has been shown that the B subunits of glutenin in durum wheats are coded by genes at the *Glu-3* loci and that these genes are tightly linked with the *Gli-1* loci coding for ω- and γ-gliadins on the short arms of group 1 chromosomes (Payne et al. 1984; Singh and Shepherd 1988; Pogna et al. 1990). However, these early studies only considered the inheritance of the low-molecular-weight (LMW)-1 and LMW-2 types, which are linked to γ-gliadin bands 42 and 45, respectively (Payne et al. 1984; Pogna et al. 1988, 1990). Subsequently, in an extensive survey of the LMW glutenin composition of tetraploid wheats carried out by Liu and Shepherd (1991), a large amount of variation was observed in these B and C glutenin subunit patterns, providing an opportunity to extend these inheritance studies.

In order to use testcross analyses to study the inheritance of LMW glutenin subunit patterns, a parent lacking all or most of the LMW glutenin subunit bands was required. A parent of this type would allow the segregation of contrasting banding patterns in the F_1 to be studied with minimum ambiguity from band overlap in the testcross progeny. Although such a phenotype was not identified in the cultivar survey, an attempt was made to utilise the 'triple translocation stock' used successfully earlier to study the inheritance of LMW glutenin subunits in hexaploid wheat (Gupta and Shepherd 1993). However, it was found that it could not be used directly in tetraploid × hexaploid crosses because the progeny seeds were shrivelled and had a low germination. Consequently, a breeding programme was undertaken to derive a double-1AL.1BL.1RS translocation stock in a tetraploid background from the double-1AL.1BL.1RS hexaploid translocation stock. This tetraploid double translocation stock was then used as the third testcross parent in the new inheritance studies. The development of this tetraploid double-1AL.1BL.1RS translocation stock and the results of using it in testcross inheritance studies are described in this paper.

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Materials and methods

Development of the double-1RS translocation stock in durum wheat

The durum wheat parent 'Langdon' was crossed with hexaploid wheat line 'Chinese Spring-Gabo' 1AL.1BL.1RS (Gupta and Shep-

herd 1993), and the F_2 seeds were screened by protein markers to detect the two separate single-translocation and one double-translocation stock. To distinguish these 1RS translocation lines from those produced earlier in hexaploid wheat (Gupta and Shepherd 1993), they are referred to as the single-1RS translocation (tetraploid) and double-1RS translocation (tetraploid) stocks, abbreviated to STr (4x) and DTr (4x), respectively.

Backcrosses

In order to identify unambiguously all the B subunits of glutenin present in the reference parents, 'Langdon' and 'Edmore' were each crossed with the tetraploid double-1RS translocation stock, and then each F_1 was backcrossed to the same stock to allow all the segregation of the B subunits from these parents to be scored clearly.

Parents and testcrosses

Durum wheat cvs 'Coulter', 'Beladi Bouhi' and 'Kharkof-5' and a wild tetraploid wheat line 19–27 (*Triticum turgidum* var 'dicoccoides') from Israel (Nevo and Beiles 1989) were found to have LMW glutenin subunit banding patterns that contrasted with those of cvs 'Langdon' and 'Edmore' (Liu and Shepherd 1991). Each of these four parents were crossed with either 'Langdon' or 'Edmore' as reference parents, since the genetic control of the B subunits had already been determined in these latter two cultivars (Gupta and Shepherd 1988). The F_1 hybrids were testcrossed as female parent to the DTr (4x) stock to generate progeny suitable for determining the inheritance of the parental differences in LMW glutenin subunits and gliadin patterns. Testcross combinations were produced as follows:

Testcross 1 ('Coulter' × 'Langdon') × DTr (4x)
Testcross 2 ('Kharkof-5' × 'Langdon') × DTr (4x)
Testcross 3 ('Beladi Bouhi' × 'Edmore') × DTr (4x)
Testcross 4 ('Edmore' × 19–27) × DTr (4x)

Protein extraction and electrophoresis

Single seeds were cut in half, and the brush ends were crushed with a hammer. The prolamins were extracted sequentially by the simple extraction procedure developed by Singh et al. (1991). For gliadin fractionation, a modified 7% acrylamide gel with a 3.0- or 1.5-mm gel thickness was utilised in the Acid-PAGE system as described by Bushuk and Zillman (1978). Glutenin subunits were separated by the SDS-PAGE using a 7.5–13% gradient gel with 1.0% cross-linking. Gliadin separation by SDS-PAGE (Singh and Shepherd 1988) was also used when required.

Genetic and statistical analysis

Recombination fractions (p) were calculated directly by dividing the observed number of recombinants (R) by the total number of progeny (n) analysed, excluding any aneuploid progeny. The standard error (Sp) of the recombination fraction was calculated using the formula for binomial distribution (Mather 1951). Map distances (cM) and their standard errors were calculated from recombination frequencies using the Kosambi function (Kosambi 1944). Where no recombinants were detected between two protein markers in the testcross progeny, the upper limit (95% confidence limit) was calculated using the method of Hanson (1959).

$p=[1-(0.05)^{1/n}]$ where n=number of euploid progeny analysed.

Contingency tables (2×2) were employed to test for the independence of the segregation of pairs of individual bands or group of bands.

Results

Production and characteristics of double-1RS translocation lines in tetraploid wheat

Durum wheat cv 'Langdon' was crossed with the hexaploid double-1RS translocation stock 'Chinese Spring-Gabo' 1AL.1BL.1RS, and the F_2 progeny seeds were screened for the presence and absence of protein markers located on the short arms of group 1 chromosomes. Progeny seeds lacking both *Tri-A1* and *Gli-B1* protein bands were selected as the putative double-translocation stock (1AL.1BL.1RS). The LMW glutenin subunit patterns of the DTr (4x) line lacked all of the prominent B subunits, but 8 prominent C subunits remained (see Fig. 2, lane o). The gliadin pattern lacked ω-gliadin bands (bands 20, 22, 38) and γ-gliadin band 42, as expected, and a strongly stained block of 6 secalin bands was present in the wheat ω- and γ-gliadin region (Figure not shown).

It is likely that many of the progeny seeds from the pentaploid F_1 still contained some D-genome chromosomes even though chromosome 1D protein markers were absent. Even after three generations of selfing following the initial cross, the selected single- and double-1RS translocation stocks still gave low seed set and some were self sterile. Despite possible cytological instability arising from the presence of extra D-genome chromosomes in this line, it could be readily crossed with tetraploid wheat to give viable seeds, and hence it was used as the third parent in the testcross.

Inheritance of LMW glutenin subunits and gliadins in backcross progeny

The analysis of the backcross progeny involving the reference parents 'Langdon' and 'Edmore' and the double 1RS translocation line allowed each 1AS- and 1BS-controlled block in these cultivars to be examined without the interference of bands segregating from another parent. As expected, the BC_1 progeny clearly revealed two distinct blocks of B subunits segregating in both 'Edmore' (Fig. 1A) and 'Langdon' (Fig. 1B).

One block in 'Edmore' consisted of all four B subunits (Fig. 1A, lane k, denoted ▷ and ■), and the other block consisted of only the slowest and the fastest bands (lane h, denoted ●), which overlapped bands of the former block. These two blocks segregated independently of each other, and the former block always co-segregated with γ-gliadin 45, a band known to be 1BS-controlled. Hence, the other block was presumed to be controlled by genes on 1AS. Obviously, it is difficult to identify the 1AS-bands when they are co-segregating with 1BS-bands, as reported earlier (Gupta and Shepherd 1988). It was noted that the fastest 1AS-band sometimes seemed to be much fainter than the slower band (eg. lanes i, j and l), but the reason for this is not known.

In 'Langdon', the three faster B subunits formed one block (Fig. 1B, lane x, denoted ▷) and the slowest dark band combined with a faint band (lane p, denoted ●) having the same mobility as the fastest dark band of 'Langdon' formed the other block. These are known to be controlled

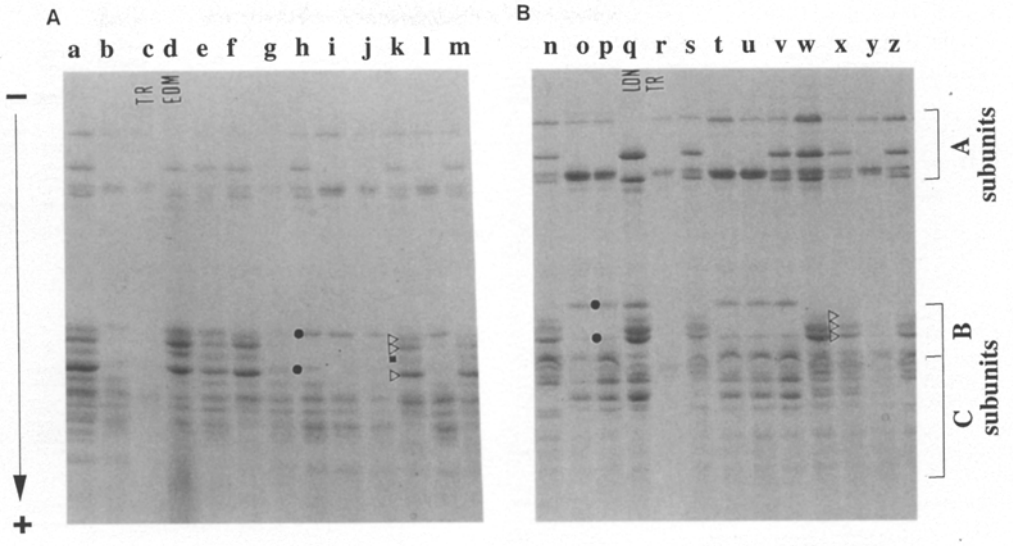

Fig. 1A,B SDS-PAGE patterns of glutenin subunits of backcross progenies 'Edmore'×[DTr (4x)]^2 (**A,** *lanes a, b,* and *e–m*), 'Langdon'×[DTr (4x)]^2 (**B,** *lanes n–p* and *s–z*) and their respective parents: *d* 'Edmore', *c, r* DTr (4x), *q* 'Langdon'. *EDM*, *LDN* and *TR* are abbreviations for parents 'Edmore', 'Langdon' and DTr (4x), respectively. ● and ▷ plus ■ refer to bands controlled by 1AS and 1BS, respectively

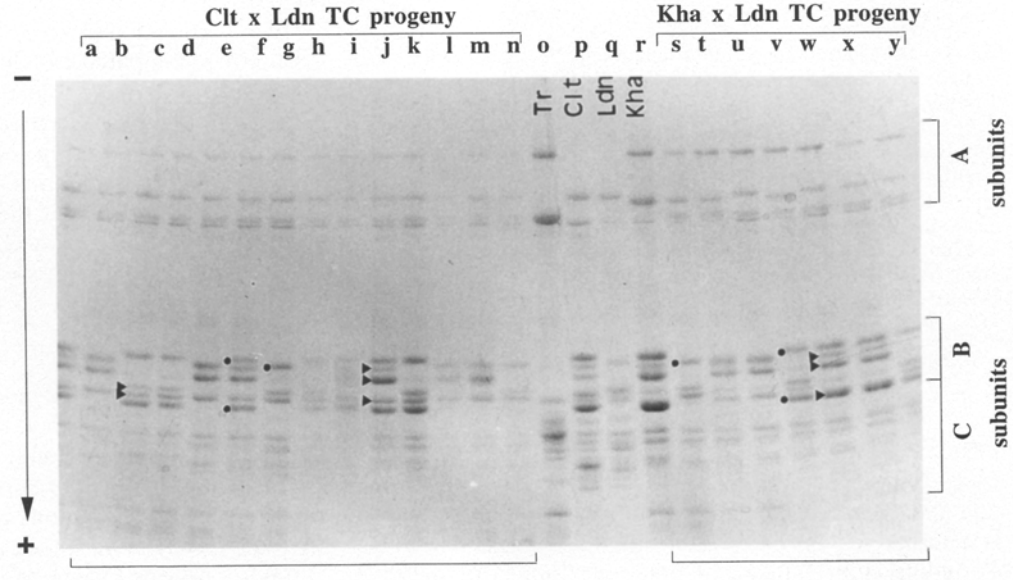

Fig. 2 SDS-PAGE patterns of glutenin subunits of two testcross progenies between 'Langdon' (*Ldn*) with 'Coulter' (*Clt*) and 'Kharkof-5' (*Kha*): 'Coulter'×'Langdon' testcross progeny (*a–n*); 'Kharkof-5'×'Langdon' testcross progeny (*s–y*). *o* DTr (4x) [Tr], *p* 'Coulter', *q* 'Langdon', *r* 'Kharkof-5'. ● and ▶ refer to bands controlled by 1AS and 1BS, respectively

by genes on chromosomes 1BS and 1AS, respectively (Gupta and Shepherd 1988), although the fainter faster band had not been identified in the earlier study.

When the gliadin bands were separated by Acid-PAGE from these two backcross progenies, two blocks of bands were also identified (Figure not shown). The 1AS-bands were the two slowest ω-gliadin bands in both parents, and the typical γ-45 in 'Edmore' and ω-38-γ-42 gliadin bands in 'Langdon', known to be controlled by genes on 1BS, segregated as alternatives with the 1AS-controlled bands (data not shown).

Inheritance of LMW glutenin subunits and gliadins in testcross progeny

The DTr (4x) line possesses HMW glutenin subunits 1 and 17 + 18 (Fig. 2, lane o), coded by *Glu-A1* and *Glu-B1*, respectively, and the presence of these bands in reduced SDS-PAGE was used to verify the correct parentage of all testcross progeny. Confirmation was also obtained by the presence of strongly stained secalin bands in unreduced SDS-PAGE and Acid-PAGE gels (Figure not shown).

A 'Langdon' as reference parent

Testcross 1 – 'Coulter' × 'Langdon'. There are least seven major differences with respect to the B subunits of glutenin between these two parents (Fig. 2, lanes p and q). In 98 testcross progeny analysed by SDS-PAGE and Acid-PAGE, the top and bottom darkly stained bands of 'Coulter' (Fig. 2, lane f, denoted ●) segregated together as a block and as alternatives to the slowest band of 'Langdon' (Fig. 2, lane g, denoted ●), known to be 1AS-controlled (Gupta and Shepherd 1988 and the backcross section). Hence,

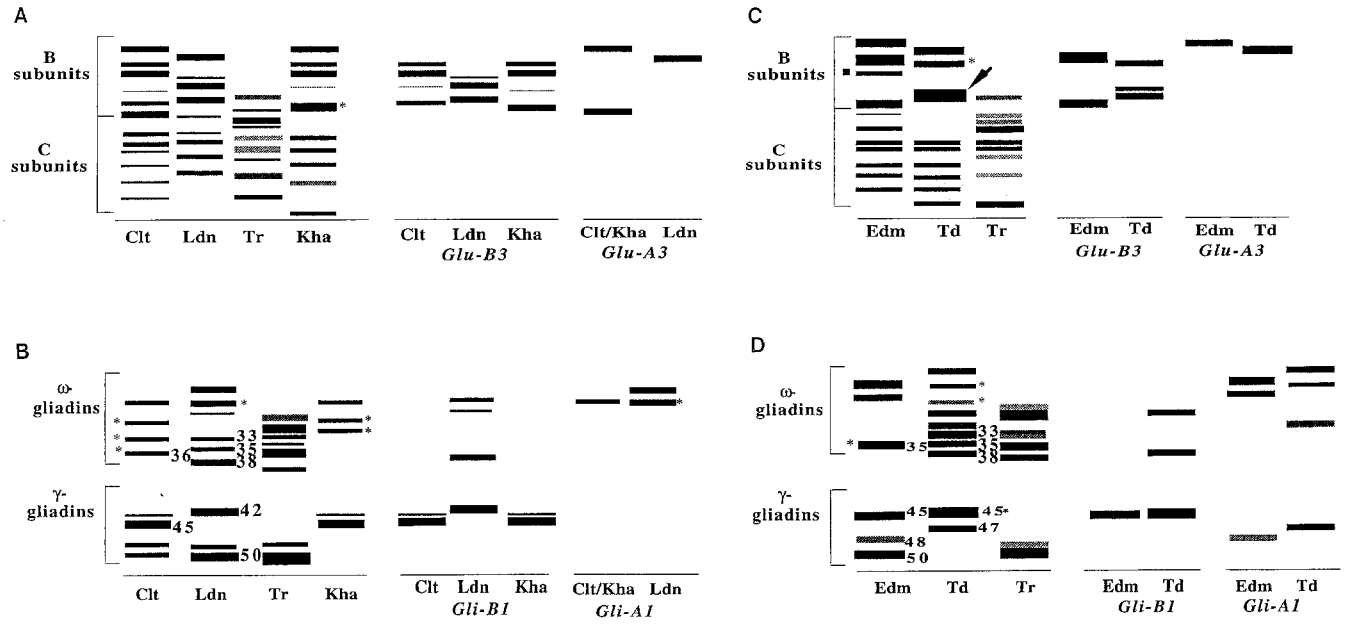

Fig. 3A–D Diagrammatic representation of the inheritance of B subunits (**A**, **C**) and ω- and γ-gliadins (**B**, **D**) of testcross parents 'Coulter' (Clt), 'Kharkof-5' (*Kha*), 'Langdon' (*Ldn*), 'Edmore' (*Edm*), *T. dicoccoides* line 19–27 (Td) and tetraploid double-1RS translocation stock DTr (4x) [Tr]. * represents bands common among parents that could not be scored in the testcross progeny, ■ refers to a band controlled by a gene loosely linked to the major *Glu-B3* locus-coding bands of 'Edmore'. The band *arrowed* in (**C**) was separated into 2 bands in a subsequent progeny test. *Numbers* assigned to bands in Acid-PAGE gels follow the nomenclature of Bushuk and Zillman (1978)

these 2 bands in 'Coulter' were also 1AS-controlled (Fig. 3A and Table 1). The middle bands of 'Coulter' (Fig. 2, lane j, denoted ▶) were also inherited as a block and as alternatives to the 3 fast-moving *Glu-B3* bands of 'Langdon' (Fig. 2, lane c, denoted ▶), indicating they are controlled by different alleles at the *Glu-B3* locus (Fig. 3A and Table 1).

The parents 'Coulter' and 'Langdon' possessed gliadin components γ-45 and γ-42 (Fig. 3B), respectively, known to be controlled by chromosome 1BS (Pogna et al. 1990). The slowest ω-gliadin band of 'Langdon' and 'Coulter' segregated independently from the *Gli-B1* bands of both parents, indicating that they are *Gli-A1* bands (Fig. 3B). In general, these respective gliadin blocks remained intact among the testcross progeny. No recombination within the gliadin components and between the gliadin and the LMW glutenin subunits was observed, indicating that the genes involved are allelic or closely linked.

Testcross 2 – 'Kharkof-5' × 'Langdon'. The B subunit pattern of 'Kharkof-5' appeared to be very similar to that of 'Coulter' and an analysis of segregation population in 121 testcross progeny showed that they possessed the same *Glu-A3* bands (Fig. 2, lanes v versus f, Fig. 3A). But the *Glu-B3* block in 'Kharkof-5' possessed a faster band in the group of 3 compared to 'Coulter' (Fig. 2, lanes w versus j, Fig. 3A). 'Kharkof-5' also had different ω-γ-gliadin patterns from those of 'Coulter' (having the γ-45-ω-32 gliadin bands as a block) (Fig. 3B). Therefore, the segregation behaviour of the B subunits and the ω-γ-gliadin bands was similar to that observed in the 'Langdon' × 'Coulter' cross. Again, no recombination within the gliadin components and between the gliadin and the LMW glutenin subunits was observed (Table 1).

B 'Edmore' as reference parent

Testcross 3 – 'Beladi Bouhi' × 'Edmore'. Figure 4 shows the B subunit patterns of some of the 108 testcross progeny between 'Edmore' and 'Beladi Bouhi' (lanes e and d). In the present experiment, however, the 1BS block of 'Edmore' revealed in the backcross was found to be segregated into two different groups. One group included 2 dark bands (Fig. 4, lane j, denoted ▶), another group the second fastest band (lane r, denoted ■); which had a relatively high recombination frequency (17.1±3.7%) (Table 2). The slowest band (lane l, denoted ●) segregated independently of the other bands and is assumed to be 1AS-controlled. All these blocks of 'Edmore' were not detected by Gupta (1989) in his study because of band overlap.

The two dark bands of 'Beladi Bouhi' (Fig. 4, lane c, denoted ▶) were considered to be 1BS-controlled since they segregated as alternatives to bands (▶ in lane J) known to be controlled by 1BS in 'Edmore' (Table 1). Furthermore, the slowest band of 'Beladi Bouhi' (Table 1 and Fig. 4, lanes k and l, denoted ●) was inherited mutually exclusively to the 1AS-controlled reference band in 'Edmore' and therefore is assumed to be controlled by genes on 1AS.

Three rare recombinants were detected involving the blocks of LMW glutenin bands controlled by 1BS in these two parents (Table 1). Type-1 recombinants (2 plants) pos-

Table 1 Segregation ratios of B subunit linkage blocks in testcrosses 1–4

LMW glutenin blocks (B subunits)	Observed frequency		χ^2 ($P_1:P_2=1:1$)	Probalility ($df=1$)
	Parental types ($P_1:P_2$)	Non-parental types		
Testcross 1				
1AS block (Coulter:Langdon)	49 : 48	0	≈ 0.00	≈ 1.00
1BS block (Coulter: Langdon)	56 : 41	0	2.00	> 0.10
Testcross 2				
1AS block (Kharkof-5:Langdon)	71 : 60	0	1.04	> 0.25
1BS block (Kharkof-5:Langdon)	64 : 57	0	0.38	> 0.50
Testcross 3				
1AS block (Edmore:Beladi Bouhi)	53 : 55	0	0.01	> 0.90
1BS block (Edmore:Beladi Bouhi)	53 : 52	3	≈ 0.00	≈ 1.00
Testcross 4				
1AS block (Edmore:19–27)	24 : 31	0	0.66	> 0.25
1BS block (Edmore:19–27)	26 : 27	2	≈ 0.00	≈ 1.00

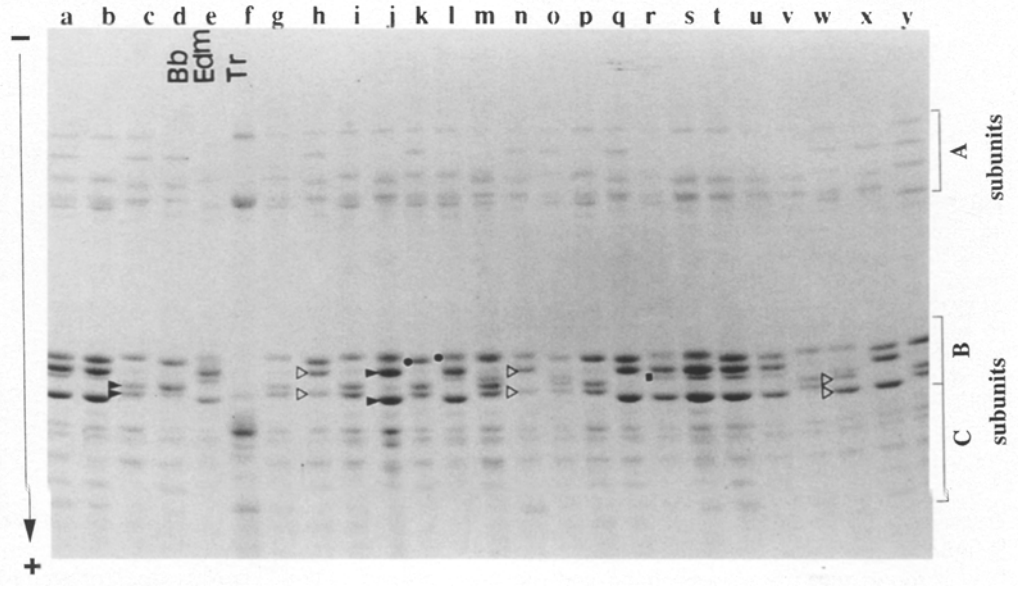

Fig. 4 SDS-PAGE patterns of glutenin subunits of testcross progeny [('Beladi Bouhi'×'Edmore')×DTr (4x)], and controls: *lanes a–c* and *g–y* testcross seeds, *m, y* non-testcross seeds, *d* 'Beladi Bouhi', *e* 'Edmore', *f* DTr (4x). *Bb*, *Edm* and *Tr* are abbreviations for parents 'Beladi Bouhi', 'Edmore' and DTr (4x). ● and ▶ refer to bands controlled by 1AS and 1BS, respectively, ▷ indicates suspected recombinants, ■ indicates band controlled by a gene on 1BS loosely linked with that controlling the major B subunits of 'Edmore'

sessed the fastest B subunit of 'Beladi Bouhi' and the second slowest band of 'Edmore' (Fig. 4, lanes h and n, denoted ▷), comprising bands from two different blocks. Type-2 recombinant (1 plant) had the fastest band of 'Edmore' and the second fastest band of 'Beladi Bouhi' (Fig. 4, lane w, denoted ▷), representing a different combination of bands from two different *Glu-B3* blocks. Two of these putative recombinants (seeds from lanes h and w in Fig. 4) survived to maturity, and their recombinant status was confirmed by testing the phenotype of their progeny. The progeny tests of recombinant type-1 showed that it also possessed the slowest band of 'Edmore' in the block, which again overlapped with the slowest-1AS-controlled B subunit.

Gliadin analysis: The 1BS-controlled ω- and γ-gliadin blocks in 'Beladi Bouhi' and 'Edmore' from Acid-PAGE gels were similar to those of the previous two testcross parents, i.e. γ-42 versus γ-45 gliadin bands, and segregated as alternatives in all testcross progeny. Similarly, 1AS-controlled bands were also identified from both parents, and they segregated mutually exclusively as expected (Figure not shown).

The rare type-1 recombinant had the 1BS-controlled gliadin pattern of 'Edmore' (i.e. γ-45), whereas recombinant type-2 had the 1BS-controlled gliadin bands of 'Beladi Bouhi' (i.e. γ-42) (Figure not shown). This indicates that the genes controlling the slowest and second slowest B subunit bands of 'Edmore' (in type-1) and the second fastest band of 'Beladi Bouhi' (in type-2) are situated nearer to the genes controlling their respective ω-γ-gliadin linkage blocks than that controlling the fastest bands of both parents.

Table 2 Joint segregation of the second fastest B subunit (■ in Figs 3C and 4) of 'Edmore' (Edm) with the major *Glu-3* blocks in testcrosses 3 and 4 involving cv 'Beladi Bouhi' (BB) and line 19–27

LMW glutenin blocks	■ band[a]	Testcross 3		Testcross 4		Combined data	
		BB block	Edm block	19–27 block	Edm block	19–27/BB block	Edm block
Glu-B3	+	12	45	4	20	16	65
	–	42	6	22	7	64	13
Recombination value		17.1 ± 3.7%		20.8 ± 5.6%		20.9 ± 3.2%	
Glu-A3	+	30	28	14	12	44	40
	–	21	29	10	19	31	48
Recombination value		No linkage		No linkage		No linkage	

[a] + = band present; – = band absent.

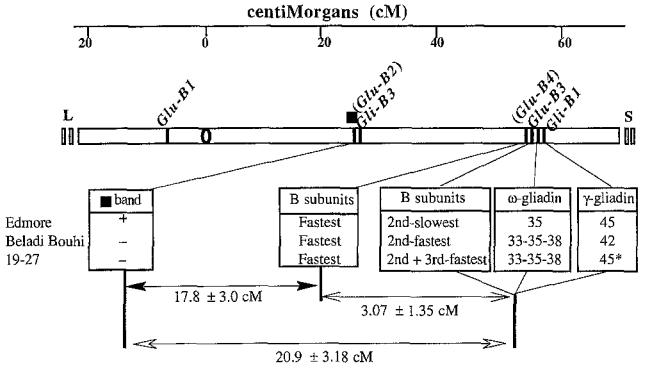

Fig. 5 Suggested location of the genes controlling storage protein on chromosome 1B of wheat (Lawrence and Shepherd 1981; Payne et al. 1982; Singh and Shepherd 1988; Pogna et al. 1990). The values given should not be regarded as absolute, since they are affected by the genetic background of the heterozygous parents as well as by environmental factors. However, the relative distances between loci are considered to be reliable, as the values between *Gli-B1* and *Gli-B3* shown were consistent with the previous results. *Glu*, *Gli*, *S* and *L* glutenin, gliadin, short arm and long arm, respectively

Testcross 4 – 'Edmore' × line 19–27. Line 19–27 had 3 dark B bands (Fig. 3C) in an analysis of 55 testcross progeny. It was found that the slowest band and the other pair of bands of 19–27 segregated as alternatives to the 1AS- and 1BS-controlled bands of 'Edmore', respectively (Table 1), and therefore they are likely to be controlled by alleles of *Glu-A3* and *Glu-B3*. Once again, one of the B subunits of 'Edmore' (Fig. 3C, ■ band) showed loose linkage (20.8±5.6%) with the bands controlled by the *Glu-B3* genes (Table 2).

Also, 2 additional rare recombinant plants were detected involving bands assigned to the *Glu-B3* locus (Table 1). One plant was similar to type-1 recombinants of testcross 3, and its phenotype was confirmed in progeny test. The second recombinant type appeared to possess all of the *Glu-B3* bands present in both parents. However, a progeny test showed that type-2 was composed of the 2 fastest bands of 'Edmore' and the second and third fastest bands of 19–27 (note: the fastest band of 19–27 was separated into 2 bands in the later analysis) and always segregated together in the selfing progeny (Figure not shown).

Gliadin analysis: The gliadin pattern of 19–27 exhibited a major γ-gliadin band, designated γ-45*, which was slightly slower than band 45 of 'Edmore' (Fig. 3D). Moreover, this band of 19–27 segregated together with the triplet (33–35–38) of ω-gliadin bands.

The rare type-1 recombinant was found to possess γ-gliadin 45 from 'Edmore', same as the type-1 recombinant in testcross 3, whereas the second recombinant type contained γ-45* from line 19–27 (Figure not shown). This suggests that the genes controlling the second and third fastest B subunits of 19–27 are situated nearer to the genes controlling 1BS-ω–γ-gliadin than the gene controlling the fastest B subunit of 19–27.

Two rare recombinants were also observed between *Glu-A3* and *Gli-A1* loci when the banding patterns of ω-gliadin and B subunits of glutenin in this testcross were compared.

Discussion

Value of the backcross procedure

The DTr (4x) stock proved to be very useful as a recurrent parent in backcrosses to study the inheritance of band blocks within a cultivar. Because of the absence of major B subunit bands in the DTr (4x) stock, the structure of the actual band blocks could be determined more clearly in the progeny, since segregation will produce BC_1 progeny carrying either a 1AS- (25%) or 1BS-block (25%) alone with the other 50% having both blocks or neither. In this way, many bands having the same or similar mobility to the major bands of other blocks can be detected. For example, in the present study the fainter faster bands of 'Langdon' and 'Edmore' coded by 1AS genes were detected for the first time. Earlier work had suggested that all of the B subunits of 'Edmore' were controlled by 1BS and that the *Glu-A3* allele was a null type not coding for any B subunit, since the overlapping of the slowest and the fastest bands with both 1BS and 1AS components could not be detected by just comparing the patterns of the two pairs of disomic substitution lines analysed (Gupta 1989).

The limitation of this BC_1 procedure for inheritance studies is that there is no recombination between any genes located on 1AS or 1BS because these chromosomes cannot pair with the 1RS chromosome arms introduced into the F_1 hybrid from the double rye translocation. Therefore, all genes located on the short arm will segregate as a unit, which explains the different inheritance pattern of the ■ band of 'Edmore' in the testcross and backcross procedures (see Figs 1, 3 and 4).

It should be noted that the selfing of testcross seeds obtained using the DTr (4x) stock achieves the same result as the backcrossing procedure, since the 1RS chromosome is present in a heterozygous state both in these progeny and in the F_1 hybrid used in backcrosses. One-fourth of the F_2 progeny are expected to have the 1AS- or 1BS-block alone, allowing linkage blocks to be visualised without overlap.

Value of the testcross approach for the inheritance of B subunits of glutenins

Segregation of the Glu-3 loci

The B subunit patterns encoded by *Glu-3*, with a few exceptions (see below), were inherited as blocks or units in all four testcross experiments. This observation is similar to the results observed in hexaploid wheat (Gupta 1989) and consistent with the conclusion that the B subunit blocks are encoded by a series of clustered structural genes mostly tightly linked (Singh and Shepherd 1988). The *Glu-B3* genes appear to code for at least two polypeptides since no singly occurring *Glu-B3* band has been observed so far. The exact band blocks in a parent could only be determined when there was no overlap with 1AS- and other 1BS-coded bands, as found with the backcross procedure used.

A total of five rare recombinants were detected within B subunit blocks from the crosses involving 'Edmore' as one parent, and four of these were confirmed in progeny tests. Pooled data from testcrosses 3 and 4 gave a combined estimate of 3.07±1.35% for the recombination value between the genes controlling the fastest B subunit band and the other major 1BS-controlled bands (Fig. 5), suggesting that the fastest B subunit bands of 'Edmore', 'Beladi Bouhi' and line 19–27 are controlled by a separate locus closely linked with the *Glu-B3* locus. On the other hand, the major *Glu-B3* locus [coding for the slower band(s)] did not recombine with the ω- and γ-gliadin blocks. Hence, we conclude that the fastest B subunits of the three parents studied are controlled by genes at a new locus, tentatively designated the *Glu-B4* locus (Fig. 5). This is the first report of such recombinants occurring within a locus controlling the LMW glutenin subunits of tetraploid wheats.

Previously it was thought that there were just two groups of homoeoloci, namely the *Gli-1* and *Glu-3* genes, controlling ω- and γ-gliadins and the major LMW glutenin subunits (B and C), on the distal part of the short arms of chromosomes 1A, 1B and 1D, and the *Gli-2* genes, controlling α- and β-gliadins, on the distal part of the short arms of group 6 chromosomes (Payne et al. 1984). The distance between *Glu-1* and *Gli-1* was calculated to be 66 cM (Payne 1987) (Fig. 5). However, it now appears that the number and structure of these loci are more complex. A new locus, designated *Gli-3* (Payne et al. 1988), has been located approximately midway between the *Glu-1* and *Gli-1* loci on chromosomes 1A and 1B of hexaploid wheats (Payne et al. 1988). The *Gli-B3* locus (Payne et al. 1988) [synonymous with the earlier *Gld-B6* (Galili and Feldman 1984) and *Glu-B2* (Jackson et al. 1985) loci on chromosome 1B, and equivalent to a *Gld-2-1A* locus on chromosome 1A (Sobko 1984)] was mapped with a genetic distance of 22–28 cM from the *Gli-B1* locus (see Pogna et al. 1993 for a review).

Recently, several other loci controlling minor gliadin components have also been detected on chromosome arm 1BS, for example *Gli-4* and *Gli-5* (Redaelli et al. 1992; Pogna et al. 1993). The *Gli-B5* locus was mapped 1.8±0.4 cM distally to the *Gli-B1* locus in hexaploid wheat (Pogna et al. 1993), whereas the *Gli-B4* locus was located 10 cM from the *Gli-A1* locus between the *Gli-A1* and *Gli-A3* loci (Redaelli et al. 1992). These loci probably correspond to the three loci previously reported to occur on chromosome 1AS and showing 13±3%, 5±1% and 1% recombination among the ω-gliadin bands of hexaploid wheat (Metakovsky et al. 1986).

A unique feature of the current inheritance studies was that the B subunits of 'Edmore' segregated into three groups rather than the expected two. In fact, the genes controlling the second fastest (■) band of 'Edmore' is apparently located in the same region as the *Gli-B3* locus, since it recombined with the major 1BS-controlled bands (i. e. the slower B subunits and ω- and γ-gliadin blocks) with about a 21% recombination rate (see Fig. 5 and testcrosses 3 and 4). In crosses between 'Edmore' and 'Beladi Bouhi' and 19–27, estimates of the distance between the genes coding for the ■ band and the 1BS-coded faster-moving B subunits (*Glu-B4*) was 17.8±3.0 cM, and for the blocks of 1BS-coded slower-moving B subunits and ω-γ-gliadins was 20.9±3.2 cM (Fig. 5). It has been proposed that the *Gli-B3* locus codes for ω-gliadins (e.g. Galili and Feldman 1984), or D subunits of glutenin (e.g. Jackson et al. 1985), whereas in the present study, this locus seemed to code for a B subunit of glutenin. Clearly further investigations are required to resolve this question. Because the ■ band appears to have aggregating properties and is biochemically similar to glutenin (Liu 1995), its locus has been redesignated *Glu-B2*, as first used by Jackson et al. (1985).

Segregation of the Gli-1 loci

The purpose of studying segregation of the gliadin bands was to monitor the co-inheritance of these bands with the B subunits of glutenin and to look for rare recombination between the *Glu-3* and *Gli-1* loci. It was found that all ω- and γ-gliadin patterns were inherited as blocks or clusters, as reported in earlier studies in both hexaploid and

durum wheats (Sozinov and Poperelya 1982; Pogna et al. 1990). For example, gliadin band γ-42 is known to form a block with ω-33-35-38 bands, whereas γ-45 is associated with ω-35 (Pogna et al. 1990); these are controlled by *Gli-B1* genes on chromosome 1BS and the combinations occur widely among the durum wheats. Some new blocks were also identified, such as the 1BS-coded bands of *T. dicoccoides* line 19–27 and 'Kharkof-5'. A rare recombination event in the Italian durum wheat cv 'Berillo' was reported by Pogna et al. (1988) who observed that gliadin band γ-42 had recombined with ω-35 giving rise to the γ-42-ω-35-LMW-2 phenotype. They constructed a genetic map showing the locus sequence (LMW-ω-gliadin)-γ-gliadins with a map distance of 2.0±0.8 cM separating the genes controlling the ω-35-B subunit block and the genes controlling the γ-gliadin bands (Pogna et al. 1990). However, all the *Gli-B1* blocks studied remained intact in the present study. That is, the rare recombination observed between different B subunits did not change these gliadin blocks, further suggesting that their structural genes are more tightly linked than those for the B subunit blocks, which now appear to involve at least three separate loci, i.e. *Glu-B3*, *Glu-B2* and *Glu-B4*. It was difficult to detect recombination with the 1AS block because of the large number of secalin bands in the testcross parent that overlapped the segregating bands. Variation at the *Gli-2* loci on homoeologous group 6 chromosomes was not investigated.

Recombination between the Glu-3 and Gli-1 loci

The joint segregation patterns for the ω-γ-gliadin bands and the B subunits of glutenin from the first two testcross progenies showed that they were co-inherited, hence no recombination was detected either within each individual locus or between the *Glu-3* and *Gli-1* loci (Table 1), the upper limit of recombination between the *Glu-3* and *Gli-1* loci and within both loci being 2.4–3.0% (95% confidence limit). Although there were three 1BS-controlled B subunit 'intra-block' recombinants involved in the third testcross progeny, no recombination was observed between the *Glu-3* and *Gli-1* loci, which provided an upper limit for the recombination value between them of 2.7% (with a 95% confidence limit). The close linkage of the ω- and γ-gliadin patterns with the major B subunits was consistent with results obtained in earlier work (Payne 1987; Gupta 1989). Unexpectedly, two rare recombinants were observed in the present experiment between *Glu-A3* and *Gli-A1* loci in the last testcross progeny, giving a recombination rate of 3.78±2.62%, even though the testcross progeny population were small (55 seeds). This value was close to an earlier value reported between *Gli-B1* and *Glu-B3* loci on chromosome 1BS in hexaploid wheat (Singh and Shepherd 1988).

The tight linkage between gliadins and LMW glutenin subunit genes on group 1 chromosomes supports the hypothesis that they have evolved from the same ancestral gene through duplication and divergence (Colot et al. 1989). However, the ancestral genes have shown much divergence at the genomic level, although they are known to share very high homology at the amino acid and nucleotide sequence levels. For example, the genes on chromosome 1BS always synthesise a larger number of polypeptide components (either gliadin or LMW glutenin subunits) than the genes on chromosome 1AS. This also applies to other prolamins in hexaploid as well as tetraploid wheats (Bushuk and Zillman 1978; Sozinov and Poperelya 1982; Payne 1987; Singh and Shepherd 1988; Gupta 1989), suggesting that genes in the A and B genomes have been subjected to quite different evolutionary forces after their incorporation into the polyploid wheats.

Our results have confirmed the results reported very recently by Ruiz and Carrillo (1993), who studied the segregational behaviour of the gliadin and glutenin banding patterns of F_2 progeny from four durum crosses. In addition to the general conclusion that the majority of the B subunits of glutenin were inherited as two independent groups controlled at the *Glu-A3* and *Glu-B3* loci, they also observed a B subunit (LMW 9) and two ω-gliadin bands recombined with a genetic distance of 19.5 cM from *Gli-B1* and 39.8 cM from *Glu-B1* loci (Ruiz and Carillo 1993). They suggested that the designated locus, *Gli-B3*, might be a complex controlling both ω-gliadin and B subunits. We assumed that their B subunit (LMW 9) corresponds to the band designated ■ in our work , which appears to be a glutenin subunit controlled by the *Glu-B2* locus (Liu 1995).

In summary, the current results are consistent with those from previous reports on recombination between genes controlling γ- and ω-gliadins and LMW glutenin subunits in durum (Payne et al. 1984; Pogna et al. 1988, 1990) as well as in bread wheats (Sozinov and Poperelya 1982; Singh and Shepherd 1988; Gupta 1989). This study was greatly facilitated by the availability of the DTr (4x) stock, which enabled a clear demonstration that all B subunits in tetraploid wheats are controlled by genes on chromosome arms 1AS and 1BS. The tight linkage between *Gli-1* and *Glu-3* was also confirmed. New information on the complex nature of the loci on chromosome arm 1BS controlling storage proteins was obtained.

Studies on the LMW glutenin subunits of durum wheats aim to document the allelic variation occurring for both LMW glutenin subunits and gliadins and to determine their genetic control and to associate particular bands with flour quality. It is now widely accepted that the LMW subunits are mainly responsible for flour quality, and the parallel relationship with gliadins is due to the tight linkage between the *Gli-1* and *Glu-3* loci (Metakovsky et al. 1990; Pogna et al. 1990). Thus, *Gli-1* alleles can still be used as genetic markers to select for required *Glu-3* alleles in breeding programmes, as the gliadins are easier to screen than the LMW alleles. Thus, further efforts to relate gliadin patterns (either Acid-PAGE or SDS-PAGE) with the LMW glutenin subunit patterns will be useful for selecting important types in breeding programmes (Metakovsky et al. 1990).

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