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Production of multiple wheat-rye 1RS translocation stocks and genetic analysis of LMW subunits of glutenin and gliadins in wheats using these stocks

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Summary. A triple (1AL.1RS/1BL.1RS/1DL.1RS) and three double (1AL.1RS/1BL.1RS, 1AL.1RS/1DL.1RS, 1BL.1RS/1DL.1RS) wheat-rye 1RS translocation stocks were isolated from a segregating population using the Gli-1, Tri-1 and Sec-1 seed proteins as genetic markers. These stocks carried 42 chromosomes and formed the expected multivalents (frequency of 14–25%) at metaphase 1. They gave floret fertility ranging from 40–60%. These stocks were subsequently used to determine the genetic control of low-molecular-weight (LMW) glutenin subunits in 'Chinese Spring' and 'Gabo' by means of two-step one-dimensional SDS-PAGE. All of the B subunits and most of the C subunits of glutenin were shown to be controlled by genes on the short arms of group-1 chromosomes in these wheats. The other C subunits were not controlled by group-1 chromosomes. The triple translocation line served as a suitable third parent in producing test-cross seeds for studying the inheritance of the LMW glutenin subunits and gliadins in wheat cultivars, e.g. 'Chinese Spring' and 'Orca'. The segregation patterns of the LMW glutenin subunits in these cultivars revealed that the subunits were inherited in clusters and that their controlling genes (*Glu-3*) were tightly linked with those controlling gliadins (*Gli-1*). The LMW glutenin patterns **d**, **d** and **e** in 'Orca' segregated as alternatives to the patterns **a**, **a** and **a** in 'Chinese Spring' controlled by *Glu-A3*, *Glu-B3* and *Glu-D3* loci on chromosome arms 1AS, 1BS and 1DS, respectively, thus indicating that these patterns were controlled by allelic genes at these loci.

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Introduction

The genetic control of low-molecular-weight (LMW) subunits of glutenin (B and C subunits) has been determined using nullisomic-tetrasomic and ditelocentric stocks of 'Chinese Spring' wheat, and the indications are that the majority of these subunits in 'Chinese Spring' are controlled by genes on the short arms of group-1 chromosomes in 'Chinese Spring' (Shepherd 1988). However, these stocks, which lack just one pair of chromosome or chromosome arms at a time, do not reveal the location of genes controlling protein bands that might overlap because of their similar electrophoretic mobility. This problem was encountered by Singh and Shepherd (1985) in an analysis of these stocks using a two-step separation procedure, and as a result they could not determine the chromosomal control of several of the LMW subunits of glutenin in 'Chinese Spring'. Some of these overlapping bands (particularly the C subunits), however, could be separated by two-dimensional electrophoretic methods (IEF/NEPHGE × SDS), and Jackson et al. (1983) were able to assign the genes controlling most of the B and C subunits to group-1 chromosomes. However, the genetic control of at least two major B subunits and three C subunits could not be determined by Jackson et al. (1983) because of their overlap with each other and/or with certain gliadins in the separation of total proteins using this method. Thus, it remains to be demonstrated whether or not all of the B or C subunits are controlled by genes on the short arms of

group-1 chromosomes. We have now addressed this question by producing stocks lacking two pairs or even three pairs of the short arms of group-1 chromosomes.

Very few studies have been carried out on the inheritance of the LMW glutenin subunits in common wheats and, in addition, these studies have been limited to only some of these subunits in the given cultivars. This has been mainly due to the lack of a suitable technique for screening these subunits reliably from a number of progeny. For example, one-dimensional separation of total reduced proteins allows only some of the B subunits to be scored due to an overlap in the mobilities of most of these bands with gliadins (Payne et al. 1984; Pogna et al. 1990). Singh and Shepherd (1988) were able to analyse these subunits free from gliadins by means of a two-step procedure, but they could study the segregation of only certain subunits at one time due to the introduction of an additional overlap from the tester parent used in the test-crosses. Furthermore, some of the aggregating albumin and globulin subunits interfered with the resolution of some of these subunits. Nevertheless, these groups of researchers were successful in mapping the genes controlling some of the B and C subunits to the *Glu-3* loci on the short arms of group-1 chromosomes. Scoring subunits can be further complicated by dosage effects if F_2 progeny are used. This latter problem is removed, however, if test-cross progeny are used, although this requires a tester parent that does not interfere with the segreation of these subunits from the F_1 hybrids, and an ideal parent for this purpose would be the one that lacks all of the LMW subunits of glutenin.

Thus, the aims of the study presented here were to establish the genetic control of the LMW subunits not accounted for by the removal of a pair of short arms of group-1 chromosomes in 'Chinese Spring' and 'Gabo' as well as to develop a suitable test-cross parent for inheritance studies of these subunits in common wheat. A genetic stock (called triple translocation stock) has now been produced that lacks all of the short arms of group-1 chromosomes. This was produced by replacing wheat arms 1AS, 1BS and 1DS by rye arm 1RS of 'Imperial' as this rye did not appear to have any LMW subunits of glutenin. Three double translocation stocks carrying just one of the wheat arms at a time were also isolated. Some of the general features (plant morphology, cytology) of these novel translocation stocks are described here. We also demonstrate that these stocks provide a novel and efficient approach for allocating LMW glutenin subunits to wheat chromosome arms 1AS, 1BS and 1DS, because the triple translocation line lacks all of the chromosome arms while the double translocations carry only one of these arms at any one time, thus permitting the identification of all the bands controlled by that arm, free of the problem of overlapping mobility with bands controlled by any of the other arms. The triple translocation stock has been used as an appropriate test-cross parent for studying the segregational behaviour of LMW glutenin bands in several wheat cultivars (Gupta and Shepherd 1988; Gupta 1989). Further data on the inheritance of certain LMW glutenin patterns and their allelic relationships with each other are presented here for cvs 'Orca' and 'Chinese Spring' using test-cross seeds. The inheritance patterns have been studied using a modified two-step procedure that does not involve the interference of aggregating albumins or globulins (Gupta and Shepherd 1990a). Since the completion of this study, however, more rapid methods than the two-step method have been developed (Gupta and MacRitchie 1991; Singh et al. 1991).

Materials and methods

Plant materials

The seeds of following genotypes were used in this study: 'Chinese Spring' (CS); CS nullisomic-tetrasomic lines 6A-6D, 6B-6D, 6D-6A, 6D-6B; CS ditelocentric lines 6AL, 6BL 6DL (Sears 1954); CS-'Imperial' rye translocation lines 1AL.1RS (1AL from cv 'Hope', see Singh and Shepherd 1988), 1DL.1RS and substitution line 1R (1B) (Shepherd 1973); 'Gabo' and 'Gabo'-'Imperial' rye translocations 1BL.1RS, 1DL.1RS and 1BL.1RS/1DL.1RS (K. W. Shepherd unpublished); Dutch wheat cv 'Orca', and rye cv 'Imperial'.

Isolation of multiple 1RS translocation stocks

F_1 seeds were produced by crossing the single translocation 1AL.1RS and double translocation 1BL.1RS/1DL.1RS. These seeds were then planted to provide F_2 seeds for isolating the three double and one triple translocation stocks.

Inheritance sutdies

Test-cross analysis was used to examine the inheritance of LMW glutenin subunits and gliadins in 'Chinese Spring' and 'Orca'. These cultivars, having contrasting LMW glutenin subunit and gliadin patterns, were intercrossed to give F_1 hybrids, which were then crossed as the female parent to the triple translocation stock, i.e. [('Chinese Spring' × '*Orca*') × triple translocation stock], to produce test-cross seeds.

Protein extraction and electrophoresis

The unreduced total proteins from individual seeds were extracted in TRIS-HCl buffer (pH 6.8) containing 4% SDS, while unreduced prolamins were extracted using 70% aqueous ethonal as described previously (Gupta and Shepherd 1990a). Protein extracts were separated by one-dimensional (1-D) SDS-PAGE (Lawrence and Shepherd 1980) and two-step 1-D SDS-PAGE (Singh and Shepherd 1988). The two-step SDS-PAGE was carried out on prolamins soluble in 70% ethanol at 50–60 °C (Gupta and Shepherd 1990a); the monomeric prolamins were electrophoretically removed from the polymeric prolamins (glutenin aggregates) in the first step, and then the gel strip containing glutenin aggregates was removed, reduced and electrophoresed in the second step for glutenin subunit composition. The gels were stained and destained according to Lawrence and Shepherd (1980).

Cytology

Standard Feulgen squashes of anthers at meiotic metaphase I were used to analyse chromosome configurations in the pollen mother cells (pmc) of the double and triple translocation stocks.

Locus and allele symbols

Locus (*Glu-3*) and allele (small letter viz. *a*, *b*, ...) symbols for LMW subunits of glutenin (B and C subunits) are given following the terminology of Singh and Shepherd (1988) and Gupta and Shepherd (1990a), respectively. *Gli-1* denotes genes on group-1 chromosomes controlling gliadins (Payne et al. 1984), and alleles are indicated by the small letters like the LMW alleles in this study.

Genetic analysis

Recombination fractions (p) were calculated directly by dividing the observed number of recombinants (R) by the total number of progeny analysed (n) excluding the aneuploid progeny. Where no recombinants were detected between two protein markers in the test-cross progeny, the upper limit (at the 95% confidence level) for the recombination fraction (p) was calculated using the method of Hanson (1959): $p = [1 - (0.05)^{-n}]$.

Results

Isolation of double and triple 1RS translocations

Production. A triple translocation heterozygote F_1 (1AL.1RS/1A, 1BL.1RS/1B, 1DL.1RS/1D) was obtained by crossing 'Chinese Spring' 1AL.1RS to 'Gabo' 1BL.1RS/1DL.1RS. This heterozygote carried chromosome arm 1RS from 'Imperial' rye, chromosome arm 1AS from 'Gabo' wheat and 1BS and 1DS from 'Chinese Spring' wheat. The F_2 seeds obtained from this plant were screened for their unreduced protein banding patterns by SDS-PAGE. The Gli-1 and Tri-1 bands controlled by these short arms in wheat (Singh and Shepherd 1988) were used to select for progeny lacking all three arms (1AS, 1BS and 1DS), viz. a triple 1RS translocation (TTr). Concurrently, seeds lacking only two of them at a time (double 1RS translocations, DTr) were also selected. Progeny seeds lacking Tri-A1 and Gli-B1 bands were expected to be DTr 1AL.1RS/1BL.1RS (Fig. 1, lane d). Similarly, progeny lacking Tri-A1, Tri-D1 and Gli-D1 bands were expected to be DTr 1AL.1RS/1DL.1RS (lane e). The seeds lacking all the triticin bands (Tri-A1, Tri-D1) and omega gliadins (Gli-B1, Gli-D1) were selected as a probable TTr 1AL.1RS/1BL.1RS/1DL.1RS (lane f). The F_3 seeds from these putative translocations were tested for the same markers to verify their homozygosity for the respective translocations. The seed homozygous for the triple translocations was not obtained in the F_2 generation, but it was isolated from the F_3 seeds of an F_2 selection heterozygous for 1AL.1RS (i.e. 1AL.1RS/1A) and homozygous for 1BL.1RS. and 1DL.1RS.

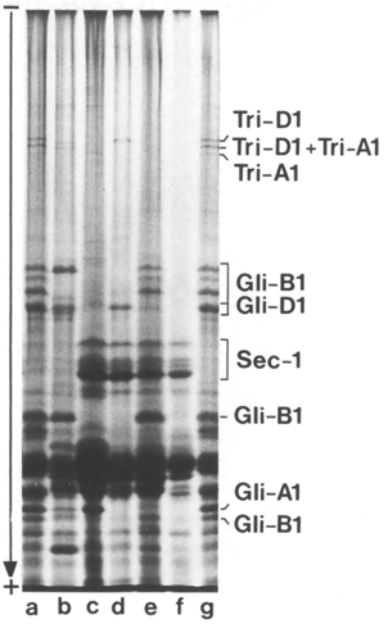

Fig. 1. One-dimensional SDS-PAGE patterns of total unreduced seed-protein extracts from background wheat parents 'Chinese Spring' *a*, *g* and 'Gabo' *b*, three double translocation lines disomic for 1BL.1RS/1DL.1RS *c*, 1AL.1RS/1BL.1RS *d* and 1AL.1RS/1DL.1RS *e* and a triple translocation line disomic for 1AL.1RS/1BL.1RS/1DL.1RS *f*

Cytology. From 30 to 120 pollen mother cells (pmc) from F_3 plants of these electrophoretically verified homozygous double and triple translocations were analysed for their chromosomal configurations at metaphase I. All the pmc analysed contained 42 chromosomes, and some formed multivalents as expected (Fig. 2). The DTr stocks formed quadrivalents at a frequency ranging from 14–17%, whereas the TTr line showed almost equal proportions (25%) of quadrivalents and hexavalents. These stocks also showed a few univalents. Two pmc from the TTr stock contained single trivalents. Thus, cytological data confirmed that these F_3 plants are genuine double and triple translocations.

Agronomic features. Observations on the plant morphology and fertility of these translocation lines, as well as on 'Chinese Spring' and 'Gabo' as controls, were made on single plants grown in 15-cm-wide pots during the spring of 1987 in the glasshouse. These stocks resembled 'Chinese Spring' more than the 'Gabo' parent in growth behaviour (not shown), spike morphology (Fig. 3) and maturity (time to spike emergence = 70–85 days). Spikes of the double translocation line 1AL.1RS/1BL.1RS, however, were awned and compacted at the apex. Similarly, the triple translocation line had sterile florets near the apex, and the apex was pointed. These features of a TTr have

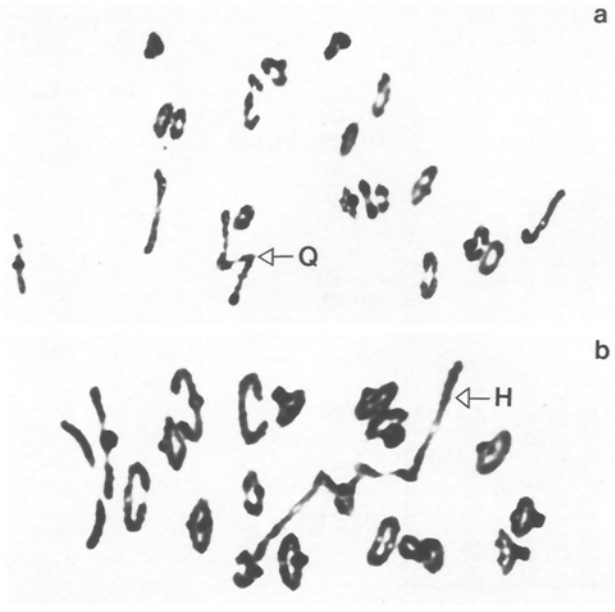

Fig. 2a, b. Chromosome configurations at metaphase I in pollen mother cells (pmc) of the triple translocation line. PMCs showing **a** $19^{II} + 1^{IV}$ and **b** $18^{II} + 1^{VI}$. *Q* Quadrivalent, *H* hexavalent

Fig. 3. Spikes (from *left to right*) from the parental cvs 'Gabo' and 'Chinese Spring', the F_2-derived double translocation lines 1BL.1RS/1DL.1RS, 1AL.1RS/1DL.1RS and 1AL.1RS/1BL.1RS and a triple translocation line 1AL.1RS/1BL.1RS/1DL.1RS

also been found to be common when it was transferred into the 'Gabo' background by backcrossing (figure not shown). In general, these stocks were fertile (Table 1) with vigorous vegetative growth.

Utilization of multiple 1RS translocation stocks

Genetic control of LMW glutenin subunits. Since these translocation stocks were produced in mixed backgrounds of 'Chinese Spring' and 'Gabo', with chromosome arm 1AS from 'Gabo' and arms 1BS and 1DS from 'Chinese Spring', the single translocation stocks lacking 1BS (i.e. 1BL.1RS) or 1DS (i.e. 1DL.1RS) in 'Gabo' or 1AS (i.e. 1AL.1RS) in 'Chinese Spring' were also required to allocate the LMW subunits of glutenin to group-1 chromosomes in these cultivars. The results are described for 'Gabo' and 'Chinese Spring' separately.

'Gabo'. 'Gabo' carries 12 LMW subunits of glutenin (numbered 1–12, Fig. 4, lane l). Bands 2 and 6–8 were absent in the 'Gabo' 1BL.1RS stock lacking chromosome arm 1BS (lane a), while 'Gabo' 1DL.1RS deficient in 1DS (lane b) lacked bands 5 and 10. These 6 bands were also absent in 'Gabo' 1BL.1RS/1DL.1RS lacking both 1BS and 1DS at the same time (lane c), thus confirming their control by genes on 1BS and 1DS. However, 2 other bands (numbered 3 and 4) were also missing in this stock, indicating that these bands consisted of an overlap of 2 bands, one controlled by 1BS and the other by 1DS. On the other hand, bands 9 and 12, which showed reduced staining intensity in the stock lacking 1DS (lane b), were not further affected by the simultaneous loss of 1BS (lane c), indicating the involvement of 1DS plus some chromosome arm(s) other than the 1BS in their control. Thus, 1BS and 1DS each controls 6 LMW subunits in 'Gabo'.

In addition to bands 9 and 12, the 1BL.1RS/1DL.1RS line (lane c) carried bands 1 and 11 of 'Gabo', and initially it was thought that they all might be controlled by 1AS. However, since bands 9 and 12 were still present in the 1AL.1RS/1BL.1RS/1DL.1RS stock lacking all three arms 1AS, 1BS and 1DS (lane i), it was concluded that only bands 1 and 11 are controlled by

Table 1. Some agronomic features of the parents and the novel translocation stocks

Genotype	Number of plants analysed	Spike/plant	Spikelets/ spike	Seeds/spikelet
Chinese Spring	1	42	14.8	1.6
Gabo	2	15	14.4	2.1
1AL.1RS/1BL.1RS	2	38	15.5	1.8
1AL.1RS/1DL.1RS	2	25	15.3	0.8
1BL.1RS/1DL.1RS	1	35	16.2	1.0
1AL.1RS/1BL.1RS/1DL.1RS	2	25	17.7	1.1

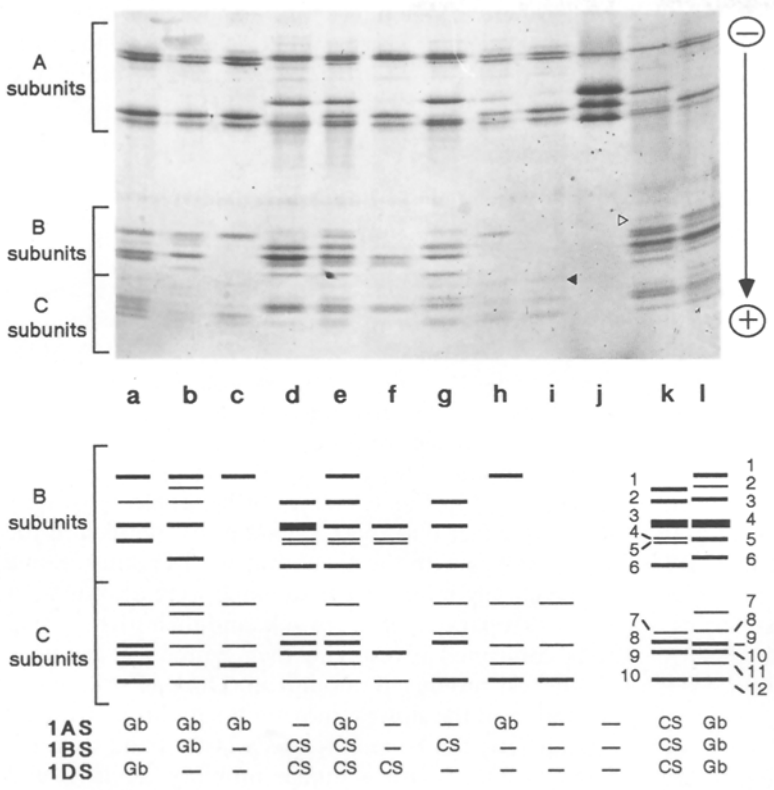

Fig. 4. Two-step 1-D SDS-PAGE patterns of endosperm proteins extracted in 70% ethanol from *a* 'Gabo' 1BL.1RS, *b* 'Gabo' 1DL.1RS, *c* 'Gabo' 1BL.1RS/1DL.1RS, *d* 'Chinese Spring' 1AL.1RS, *e* F_1 between 'Gabo' 1BL.1RS/1DL.1RS and 'Chinese Spring' 1AL.1RS; the F_2-derived stocks carrying double translocations *f* 1AL.1RS/1BL.1RS, *g* 1AL.1RS/1DL.1RS, *h* 1BL.1RS/1DL.1RS and a triple translocation *i* 1AL.1RS/1BL.1RS/1DL.1RS; rye cv 'Imperial' *j* and wheat cvs 'Chinese Spring' *k* and 'Gabo' *l*. *Gb* and *CS* refer to the presence of specified chromosome arms from 'Chinese Spring' and 'Gabo', respectively. The absence of these arms is indicated by —. ▷ only seen sporadically, ▶ probably controlled by 1RS

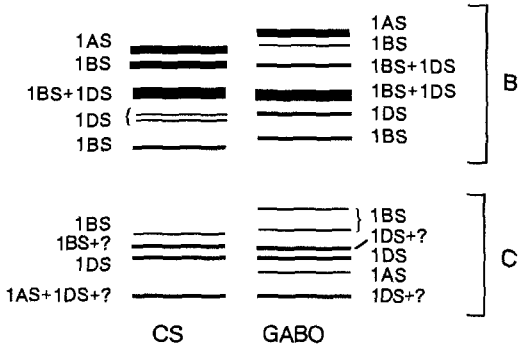

Fig. 5. Diagrammatic presentation of the B and C subunits of 'Chinese Spring' (*CS*) and 'Gabo' and their chromosomal controls

1AS and that bands 9 and 12 must be governed by 1DS plus some chromosome arm(s) other than 1AS and 1BS. There was another band (marked by ▶, lane i) seen in these 1RS lines that was in addition to those found in 'Gabo' (see below for further details). In summary, 'Gabo' carries at least 16 B and C subunits, 2 of which are controlled by 1AS, whereas 6 bands each are controlled by the 1BS and 1DS arms, respectively (Fig. 5).

'Chinese Spring'. 'Chinese Spring' (Fig. 4, lane k) has 10 LMW glutenin bands (numbered 1–10, bands 4 and 5 are seen as two distinct bands in lanes d–f). The band marked by ▷ was controlled by 1BS due to its presence in line carrying 1BS from 'Chinese Spring' (lane g), but since it has been seen only sporadically as D subunits (Payne et al. 1988), it was not included as one of the B and C subunits. Bands 1 and 10 were controlled by chromosome arm 1AS as band 1 was missing, whereas band 10 showed a much reduced staining intensity in the CS 1AL.1RS stock lacking 1AS (lane d). To determine whether the remaining 9 B and C subunits (including the remnant of band 10) are controlled by chromosome arms 1BS and 1DS, the banding patterns of double and triple translocation stocks involving these arms from 'Chinese Spring' were compared (see presence of these bands in the F_1 hybrid between CS 1AL.1RS and 'Gabo' 1BL.1RS/1DL.1RS, lane e).

In the derived 1BL.1RS/1DL.1RS line (lane h), bands 2–7 and 9 'Chinese Spring' were found to be absent, demonstrating that these bands must be controlled by genes on chromosome arms 1BS and 1DS. A similar response was seen in the triple translocation line 1AL.1RS/1BL.1RS/1DL.1RS (lane i), as expected. Bands 8 and 10 were still present in these lines, although weakly stained, suggesting that chromosome arms other than 1AS, 1BS and 1DS are also involved. Among the bands controlled by 1BS and 1DS, bands 2, 3, 6 and 7 appeared in the 1AL.1RS/1DL.1RS line

carrying only 1BS (lane g), while bands 3–5 and 9 showed their presence in the 1AL.1RS/1BL.1RS stock having only 1DS (lane f), thus confirming their control by these chromosome arms, respectively. Since band 3 was present in both of these stocks and was absent only when both 1BS and 1DS were absent simultaneously (lanes h, i), it represents 2 bands (one controlled by 1BS and the other by 1DS) of similar mobility. It can also be concluded that 1BS is involved in the control of band 8 of 'Chinese Spring' because the background band 8 in the TTr line (lane i) became darker when 1BS of CS was added to it (lane g). In addition to 1AS, chromosome arm 1DS is believed to be involved in the control of band 10 by the response of the latter to change in the dosage of chromosome arm 1DS (see Figs. 2 and 3A in Gupta and Shepherd 1990b), although it stained too faintly to be seen in the line carrying only 1DS here (lane f); it seemed that this line may also be lacking the band 10 of triple 1RS line (lane i), thus giving it a very faint appearance. Thus, out of 14 B and C subunits in 'Chinese Spring' (Fig. 5), 12 are controlled by chromosome arms 1AS (2), 1BS (5) and 1DS (5).

Triple translocation stock. Of the 3 LMW glutenin bands remaining over in the triple translocation stock, the band marked by ► (Fig. 4, lane i) was not present in 'Chinese Spring' or 'Gabo' but was only seen in those stocks that carried rye arm 1RS. It was generally more intense when 1RS was present in four or six doses, viz. double and triple translocation stocks (lanes g–i), indicating that it may be controlled by genes on this arm. Arm 1RS in these stocks was derived from 'Imperial' rye, but this band was not visible in 'Imperial' rye, probably because it was produced in even smaller amounts in rye with just two doses of 1RS. Bands with a similar mobility to the remaining 2 bands, however, were present in both 'Chinese Spring' (bands 8 and 10) and 'Gabo' (bands 9 and 12).

To investigate the genetic control of these bands in 'Chinese Spring', nullisomic-tetrasomic and ditelocentric lines of group 6 chromosomes were analysed. These particular stocks were chosen for analysis because all of the prolamins in wheat are apparently controlled by group-1 and group-6 chromosomes (see Shepherd 1988 for a review). The two-step banding patterns of these stocks indicated that the removal of the group-6 chromosomes did not have any marked effect on any of the LMW glutenin bands in 'Chinese Spring' (figure not shown). However, since these C subunit bands were faintly stained and overlapping in this separation system with some relatively darkly stained C subunits controlled by group-1 chromosomes, the possibility of their control by the group-6 chromosomes cannot be ruled out. It is also possible that these bands in 'Chinese Spring' and 'Gabo' may be controlled by two different chromosomes, and thus they may be present or absent simultaneously; e.g. the band corresponding to band 10 of CS and 12 of 'Gabo' did not appear to be present in lane f (Fig. 4).

Inheritance of LMW subunits of glutenin and gliadin bands

LMW glutenin subunits. One hundred and seven seeds from [('Chinese Spring' × 'Orca') × triple translocation stock] test-crosses were screened by two-step SDS-PAGE. All of the B subunits segregating from the F_1 could be scored separately in the test-cross seeds because the triple translocation stock does not carry any B subunits. However, some of the C subunits, as marked by * in Fig. 6, could not be scored separately due to the fact that their mobilities overlapped in the F_1 as well as with the C subunits of TTr stock. Some of the closely migrating B subunits were also difficult to score separately in some gels, and their identity had to be confirmed by resolving them better. For example, the fastest moving B subunit in 'Orca' (P_1) contains 3 bands and the slowest moving B subunit of 'Chinese Spring' (P_2) can be resolved as a faster band than the band in 'Orca' with a similar mobility. Thus, B and C subunit patterns of these cultivars are also shown diagrammatically (Fig. 6). In the gel photograph given here, however, only those differences which can be easily seen are labelled.

Segregation data from the test-cross seeds showed that the LMW subunits from 'Chinese Spring' were inherited in three groups. These groups could be assigned to chromosome arms 1AS, 1BS and 1DS on the basis of their absence in the stocks lacking these arms (see Fig. 5 for a summary). The band patterns of these arms in 'Chinese Spring' have been designated **a**, **a** and **a**, respectively (Fig. 6). The 1BS pattern **a** consisted of at least 3 bands inheriting as a unit, indicating that the genes controlling them were tightly linked. Similarly, LMW subunits from 'Orca' segregated as three clusters, **d**, **d** and **e**, and these could be allocated to 1AS, 1BS and 1DS, respectively, on the basis of their mutually exclusive segregation (in a ratio of 1:1) to those controlled by these arms in 'Chinese Spring' (Table 2). In other words, all of the test-cross seeds possessed either pattern **a** or **d** (bands shown by ► lane T_4 and T_3, respectively) from 1AS, pattern **a** or **d** (bands marked by ▷ in lane T_3 and T_2, respectively) from 1BS or either **a** or **e** (bands marked by →, lanes T_1 and T_4, respectively) from 1DS. Only 1 seed was found to lack both patterns **a** and **d** from 1BS simultaneously, but since it also lacked the HMW subunits of glutenin and gliadins controlled by the long and short arms of chromosome 1B in the F_1 parents (figure not shown), it was classified as an

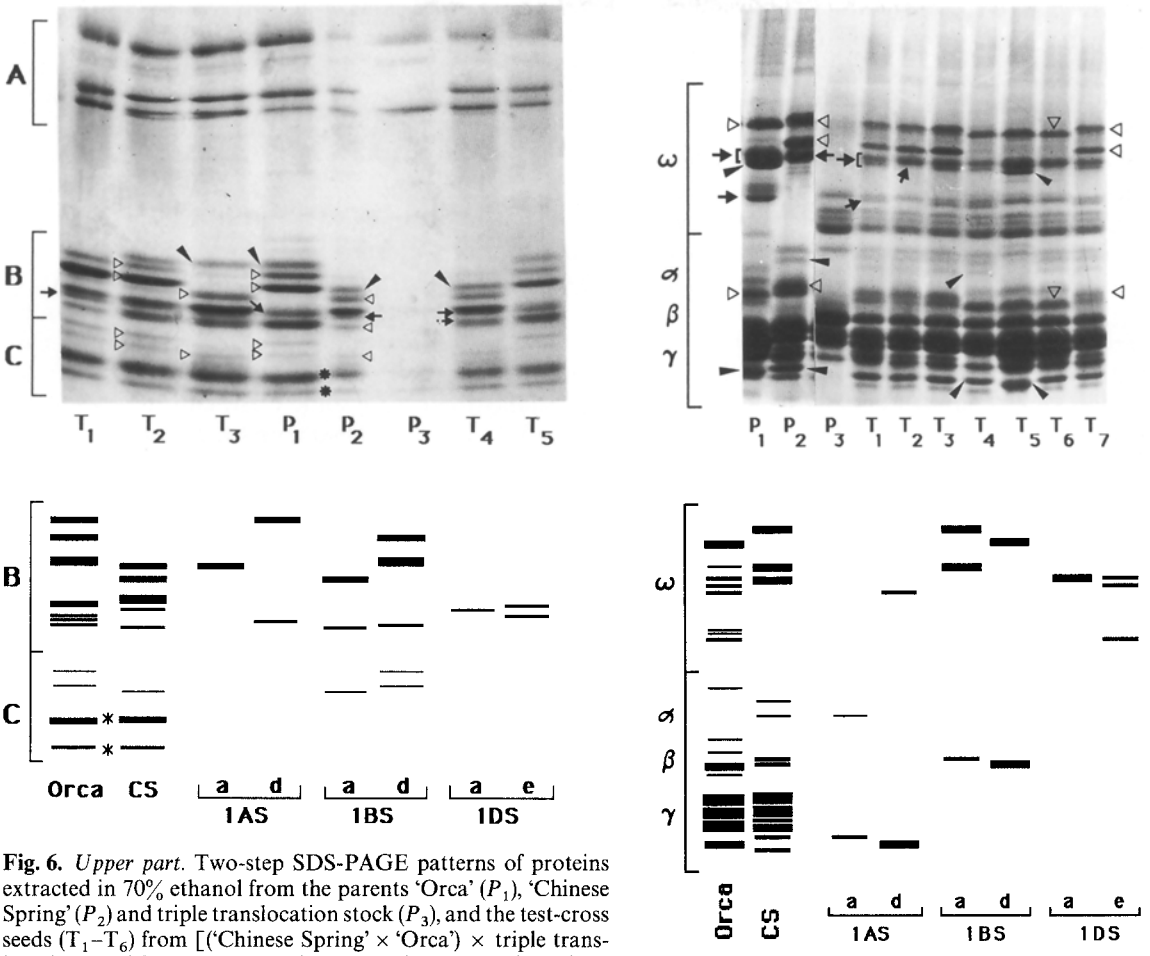

Fig. 6. *Upper part.* Two-step SDS-PAGE patterns of proteins extracted in 70% ethanol from the parents 'Orca' (P_1), 'Chinese Spring' (P_2) and triple translocation stock (P_3), and the test-cross seeds (T_1–T_6) from [('Chinese Spring' × 'Orca') × triple translocation stock]. *Lower part.* Diagrammatic presentation of the *B* and *C* subunits (*Glu-3*) of 'Chinese Spring' and 'Orca' divided into three inheritance groups. ▸ Bands controlled by 1AS, ▷ bands controlled by 1BS, →bands controlled by 1DS, * the bands with common mobilities in P_1 and P_2, which could not be scored separately in the test-cross seeds

Fig. 7. *Upper part.* One-dimensional SDS-PAGE patterns of unreduced proteins extracted in 70% ethanol from the parents 'Orca' (P_1), 'Chinese Spring' (P_2) and triple translocation stock (P_3) and the test-cross seeds (T_1 –T_7) from [('Chinese Spring' × 'Orca') × triple translocation stock]. *Lower part.* Diagrammatic presentation of the gliadins (*Gli-1*) of 'Chinese Spring' and 'Orca, divided into three inheritance groups. ▸ Bands controlled by 1AS, ▷ bands controlled by 1BS, →bands controlled by 1DS

Table 2. Segregation of LMW subunits of glutenin and gliadins among 107 test-cross seeds from [('Chinese Spring' × 'Orca') × triple translocation stock]

LMW glutenin/gliadin pattern	Observed frequency of progeny		χ^2 value (1:1)($df = 1$)	Probability
	Parental	Nonparental[a]		
a:d (1AS)	52:55	0	0.08	0.7–0.8
a:d (1BS)	46:60	1	1.84	0.1–0.2
a:e (1DS)	45:62	0	2.70	0.1–0.2

[a] The nonparental type was excluded from the analysis

aneuploid rather than a recombinant. Thus, **d**, **d** and **e** patterns on 'Orca' were concluded to be controlled by genes allelic to those controlling **a**, **a** and **a** in 'Chinese Spring' at the *Glu-A3* (1AS), *Glu-B3* (1BS) and *Glu-D3* (1DS) loci, respectively. As expected, the patterns from one chromosome arm segregated independently of the patterns from the other chromosome arms, which was consistent with a segregation ratio of 1:1:1:1 ($\chi^2 df = 3 < 6.6$; $P > 0.05$) for two independent loci in test-cross seeds.

Gliadins. The unreduced protein phenotypes of 'Orca' and 'Chinese Spring' showed that they also differed for some gliadins (Fig. 7, lanes P_1 and P_2). Since the triple translocation stock (lane P_3) did not have any gliadin bands overlapping the parental differences, the latter could be scored in test-cross seeds without difficulty. The segregation patterns of the gliadins were scored in the same 107 test-cross seeds in order to examine their inheritance and the linkage relationships with the Glu-3 bands in these cultivars. The chromosome arm location of all the ω- and certain γ-gliadins segregating from 'Chinese Spring' could easily be established using the 1RS translocation lines (see Fig. 1 for examples), and the bands controlled by 1AS, 1BS and 1DS have been labelled by ▶, ▷ and →, respectively (lane P_2). The gliadins in 'Orca' controlled by these arms have also been marked by the same symbols (lane P_1); the chromosome arm location of these gliadins is based on their allelism to those of 'Chinese Spring' (Table 2).

Like the Glu-3 subunits, the gliadin bands controlled by each of these arms were also inherited as a unit in the test-cross seeds, indicating their synthesis by closely linked genes at the *Gli-1* loci. Moreover, they did not show recombination with the Glu-3 subunits controlled by the same chromosome arms, suggesting that they are controlled by tightly linked genes. Because of this tight linkage, the Gli-1 patterns have been designated with the same allelic letters as the Glu-3 patterns, e.g. **a**, **a**, **a** in 'Chinese Spring' and **d**, **d** and **e** in 'Orca'. The absence of any recombinants amongst the 107 test-cross seeds sets an upper limit of 2.7% for the recombination fraction between the *Glu-3* and *Gli-1* loci (at the 95% confidence level).

Discussion

Although the double and triple translocation stocks form multivalents due to multiple doses of the rye 1RS arm, they have been found to be cytologically stable ($2n = 42$) over a number of generations in our meiotic analyses and in a Giemsa C-banding analysis of root tip cells at the F_6 generation (M. Baum, personal communication). Furthermore, these stocks are fertile with vigorous vegetative growth, an indication that the wheat chromosome arms 1AS, 1BS and 1DS are genetically compensated by the rye chromosome arm 1RS. This provides further support to the homoeologous relationship between group-1 chromosomes of wheat and rye (Shepherd 1973).

It is also shown that these translocation lines provide a novel approach for allocating B and C subunits to chromosome arms 1AS, 1BS and 1DS in wheat. The triple translocation stock, which lacks all of these arms simultaneously, has revealed unambiguously for the first time that all of the B subunits are controlled by these arms in both cultivars. On the other hand, there were some C subunits that were not controlled by these arms. There were 12 and 16 LMW subunits of glutenin in 'Chinese Spring' and 'Gabo', respectively, that were allocated to these short arms. The number of B and C subunits that could be assigned to these arms in 'Chinese Spring' is much greater than could be done previously using a two-step 1-D method (Singh and Shepherd 1985) or two-dimensional methods (Jackson et al. 1983), and this is mainly because even those bands which had overlapping mobility and were controlled by more than one of these chromosome arms could be allocated to these arms using the double and triple translocation stocks. For example, bands 3 and 10 in 'Chinese Spring' and bands 3 and 4 in 'Gabo' were each assigned to chromosome arms 1BS and 1DS on the basis that they were present in stocks carrying any of these arms and were absent only when both of these arms were absent. Because of this overlap, the chromosomal control of these compound bands could not be determined by previous investigators using nullisomic-tetrasomic or ditelocentric lines, which lack only one pair of chromosome or chromosome arms at a time.

The particular chromosome arms involved in the synthesis of the two C subunits present in the triple translocation line could not be determined due to their overlap with some other C subunits (Glu-3 bands) in the two-step gels. However, it is interesting to note that some prolamins of similar sizes (40–50 kDa) with polymeric ability have been assigned to chromosome 6B in bread wheat (see Kasarda et al. 1987 and references therein). It remains to be seen, however, whether the two C subunits detected here in 'Chinese Spring' and 'Gabo' wheats are controlled by group-6 chromosomes. Since these subunits were the part of the alcohol soluble-native glutenin analysed by the two-step procedure and since bands with similar mobilities to these C subunits of the triple translocation line were present in both 'Chinese Spring' and 'Gabo', they are presumed to be the wheat glutenin proteins. However, the confirmatory answer to this will come by their presence in a genetic stock lacking all three of the wheat chromosome arms 1AS, 1BS and 1DS but without rye arm 1RS or by the assignment of these subunits to wheat chromosomes by using an equivalent separation method but with a greater resolution power for the C subunits than the two-step. Unfortunately, none of these options is presently available.

Since all of the B and most of the C subunits were absent in the triple translocation stock, it served as a very useful third parent in the test-crosses produced for studying the inheritance of these subunits and their allelic relationships. Segregation data obtained from such test-crosses as shown here and elsewhere (Gupta and

Shepherd 1988; Gupta 1989) have provided evidence for the allelic relationships for nearly all the patterns detected at *Glu-3* loci on chromosome arms 1AS, 1BS and 1DS in hexaploid wheats (Gupta and Shepherd 1990a). The allelic patterns consisting of two or more B and C subunits were inherited as a single unit with no recombination, suggesting that they are controlled by a cluster of structural genes at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci. Our SDS-PAGE results showing that the genes at the *Gli-A1*, *Gli-B1* and *Gli-D1* loci coding for gliadins are also present in clusters confirmed earlier reports using Acid-PAGE (Sozinov and Poperelya 1980). Moreover, the co-inheritance of the Gli-1 and Glu-3 bands is in agreement with a tight linkage between the *Gli-1* and *Glu-3* loci (Payne et al. 1984; Singh and Shepherd 1988). A knowledge of linkage relationships between these two groups of different proteins is of evolutionary and breeding significance. The inheritance of these polypeptides in tightly linked clusters along with their homology at amino acid and nucleotide levels suggests that the genes controlling them may have arisen from a single ancestral gene through duplication and mutation (Kreis et al. 1985; Singh and Shepherd 1988; Colot et al. 1989). However, the divergence of the ancestral gene is likely to have occurred in the common progenitor of the Triticeae because both of the LMW subunits of glutenin and gliadins are present in the diploid *Triticum* species related to the A, B and D genome of wheat and also in other diploid wheat relatives (Gupta and Shepherd 1990b; Gupta 1989). Regarding the value of this knowledge in wheat quality improvement, allelic variation in both gliadins and LMW glutenin subunits has been shown to be related to differences in flour quality (Sozinov and Poperelya 1980; Gupta and Shepherd 1988; Metakovsky et al. 1990; Pogna et al. 1990), although only the LMW subunits of glutenin have cause-effect relationships with flour quality (Pogna et al. 1990). This parallel relationship can be ascribed to the tight linkages between the *Gli-1* and *Glu-3* loci encoding these proteins, indicating that *Gli-1* alleles can be used as markers for selecting for appropriate *Glu-3* alleles in breeding programmes as these are easier to screen than the LMW alleles. Thus, further efforts to relate gliadin patterns (SDS-PAGE or Acid-PAGE based) with the LMW glutenin subunit patterns (SDS-PAGE) will be worth continuing (Metakovsky et al. 1990).

In conclusion, the novel translocation lines have demonstrated that all of the B and most of the C subunits in common wheats are controlled by genes on the 1AS, 1BS and 1DS chromosome arms. The triple translocation line serves as a useful tester parent, thus facilitating the segregational studies of LMW glutenin and gliadin bands. We were able to confirm that genes controlling the B and C subunits (*Glu-3*) on these arms are tightly linked to those controlling gliadins (*Gli-1*). Apart from this, the multiple 1RS lines have been useful in the genetic characterization of certain monoclonal antibodies raised against total glutenin (Brett et al. 1991), in the molecular analysis of the rye genome (Rogowsky et al. 1991) and in determining functional relationships between glutenin subunits and dough visco-elasticity, including dough stickiness (Gupta et al. 1990). These stocks may also be useful for isolating DNA clones or purifying LMW subunits of glutenin specific to the wheat chromosome arms 1AS, 1BS or 1DS. Finally, genetic lines with multiple deletions or nulls for gliadin and glutenin-coding loci/arms (but without involving rye 1RS) are currently being developed at the CSIRO centre for use in future genetic and functional characterization of storage proteins in wheat.

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