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Seed-storage-protein loci in RFLP maps of diploid, tetraploid, and hexaploid wheat

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Abstract Linkages between high- and low-molecular-weight (M_r) glutenin, gliadin and triticin loci in diploid, tetraploid and hexaploid wheats were studied by hybridization of restriction fragments with DNA clones and by SDS-PAGE. In tetraploid and hexaploid wheat, DNA fragments hybridizing with a low-M_r glutenin clone were mapped at the *XGlu-3* locus in the distal region of the maps of chromosome arms 1AS, 1BS, and 1DS. A second locus, designated *XGlu-B2*, was detected in the middle of the map of chromosome arm 1BS completely linked to the *XGli-B3* gliadin locus. The restriction fragments mapped at this locus were shown to co-segregate with B subunits of low-M_r glutenins in SDS-PAGE in tetraploid wheat, indicating that *XGlu-B2* is an active low-M_r glutenin locus. A new locus hybridizing with the low-M_r clone was mapped on the long arm of chromosome $7A^m$ in diploid wheat. No glutenin protein was found to co-segregate with this new locus. Triticin loci were mapped on chromosome arms 1AS, 1BS, and 1DS. A failure to detect triticin proteins co-segregating with DNA fragments mapped at *XTri-B1* locus suggests that this locus is not active. No evidence was found for the existence of *Gli-A4*, and it is concluded that this locus is probably synonymous with *Gli-A3*. Recombination was observed within the multigene gliadin family mapped at *XGli-A1* (1.2 cM). Although these closely linked loci may correspond to the previously named *Gli-A1* and *Gli-A5* loci, they were temporarily designated *XGli-A1.1* and *XGli-A1.2* until orthology with *Gli-A1* and *Gli-A5* is established.

Key words *Triticum* · Storage-protein loci · Glutenin · Gliadin · Triticin

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Introduction

The potential of wheat flour for breadmaking depends to a large extent on the quantity and quality of gluten (for a recent review see Weegels et al. 1996). Gluten is a protein complex composed of monomeric gliadins and polymeric glutenins. Glutenin polypeptides form high-molecular-weight polymers via intermolecular disulfide bonds. These polymers facilitate retention of gas by the dough; therefore, their characteristics are critical for the properties of dough and the quality of bread. Gliadins form only intramolecular disulfide bonds and do not aggregate. They are, therefore, less critical for the breadmaking potential of wheat flour.

The gliadins are separated into four groups, denoted as α-, β-, γ-, and ω-gliadins, when fractionated by polyacrylamide-gel electrophoresis at low pH. The α- and β-gliadins are similar to each other structurally and are referred to as α-type gliadins. They exhibit some differences from the γ-gliadins that are referred to as γ-type gliadins (Tatham et al. 1990). The α- and γ-type gliadins [and also the low-molecular-weight (M_r) glutenins] contain appreciable levels of sulphur amino acids. The ω-gliadins, in contrast, are deficient, and in some cases completely lacking, in sulphur amino acids (Tatham et al. 1990). They have the highest M_r and are the least charged.

The subunits of glutenin are categorized according to their M_r into high- and low-M_r classes (Holt et al. 1981; Jackson et al. 1983; Payne et al. 1984). The low-M_r glutenin subunits have been subdivided into three groups, designated B, C, and D; A was reserved for the high-M_r glutenin subunits (Payne and Corfield 1979). The B subunits have an M_r ranging from 42 to 51 kDa and constitute a major group. The C subunits have an M_r ranging from 30 to 40 kDa and constitute a minor group (Payne and Corfield 1979; Pogna et al. 1994). The B and C low-M_r glutenin subunits are distantly related to γ-gliadins (Colot et al. 1989; Cassidy and Dvorak 1991). Another minor group are the D subunits which are highly acidic and are related to ω-gliadins (Jackson et al. 1983; Masci et al. 1993).

The high-M_r glutenin subunits are encoded at the *Glu-1* loci on the long arms of wheat chromosomes 1A, 1B, and 1D. The low-M_r B and C glutenin subunits are encoded at the *Glu-3* multigene loci tightly linked to the *Gli-1* multigene loci. The *Gli-1* multigene loci encode γ- and ω-gliadins and some β-gliadins at the distal ends of the short arms of chromosomes 1A, 1B and 1D (Jackson et al. 1983; Singh and Shepherd 1988; Pogna et al. 1990; Ruiz and Carrillo 1993; Dubcovsky and Dvorak 1995; Dubcovsky et al. 1995; Gale et al. 1995). Additional genes encoding ω-gliadins have been located at the *Gli-3* loci. These loci were originally designated *Gld-B6* (Galili and Feldman 1984) and *Gld-2* (Sobko 1984) and have been mapped in the middle of the maps of the short arms of chromosomes 1A and 1B (Payne et al. 1988; Ruiz and Carrillo 1993; Dubcovsky and Dvorak 1995; Dubcovsky et al. 1995; Van Deynze et al. 1995). Most β- and all α-gliadins are encoded at multigene loci on the short arms of chromosomes 6A, 6B, and 6D (Wrigley and Shepherd 1973).

The D subunits of low-M_r glutenin appear in the glutenin (aggregated) fraction in gel-filtration chromatography but are non-covalently bound to glutenin (Payne et al. 1988). Genes encoding D subunits are completely linked to the *Gli-D1* locus on chromosome 1D (Payne et al. 1986; Pogna et al. 1995). However, on chromosome 1B they are at a separate locus, which was originally designated as *Glu-B2*. This locus was mapped between *Glu-B1* and *Gli-B1* (Jackson et al. 1985). Genes at this locus are completely linked to the ω-gliadin genes mapped at the locus and originally designated as *Gld-B6* (Galili and Feldman 1984). Because of the close functional and linkage relationships between ω-gliadins and D subunits encoded at the *Gld-B6* and *Glu-B2* loci, Payne at al. (1988) concluded that *Gld-B6* and *Glu-B2* are synonymous and re-designated the locus as *Gli-B3*. The *Gld-2* locus on chromosome 1A (Sobko 1984) was assumed to be orthologous to this locus and was re-designated as *Gli-A3* (Payne et al. 1988).

A gene encoding a low-M_r glutenin of the B class that appears to have aggregating properties was recently found to be closely linked to the gliadin genes at the *Gli-B3* locus (Ruiz and Carrillo 1993; Liu 1995; Liu and Shepherd 1995). The locus was designated *Glu-B2* (Liu 1995), a name used before for the locus encoding the D glutenin subunits (Jackson et al. 1985) but abandoned for reasons explained above. Since genes encoding low-M_r glutenin subunits are related to gliadins, a gliadin may mimic a low-M_r glutenin subunit if it acquires an additional cystein enabling it to aggregate (Tao and Kasarda 1989; Lew et al. 1992). Since gliadin subunits make up to 20% of the low-M_r glutenin (Lew et al. 1992), it cannot be excluded on the basis of existing evidence that the low-M_r glutenin gene mapped at the *Glu-B2* locus by Liu (1995) was a mutant gliadin.

Two additional low-molecular-weight subunits of glutenin with relative molecular masses of 30 kDA and 32 kDA were reported on chromosomes 1D (*Glu-D4*) and 7D (*Glu-D5*) respectively (Sreeramulu and Singh 1997). Although both subunits appear in the aggregated fraction in alkylated glutenin preparations, the N-terminal amino-acid sequence of the subunit from chromosome 7D is related to α-type gliadins (Lew et al. 1992; Sreeramulu and Singh 1997).

Low-M_r glutenin genes that have been cloned are sufficiently different at the nucleotide sequence level from the gliadin genes so that they do not hybridize with them (Cassidy and Dvorak 1991; Dubcovsky and Dvorak 1995; Dubcovsky et al. 1995). Hence, mapping of DNA restriction fragments hybridizing with cloned gliadin or low-M_r glutenin genes provides a convenient strategy to ascertain whether a mapped storage protein was coded by a glutenin or gliadin gene. We report here the linkage relationships among genes encoding low-M_r glutenin and gliadin proteins in diploid, tetraploid and hexaploid wheats, using DNA hybridization. DNA markers for other storage proteins, such as high-M_r glutenins (Anderson et al. 1989) and triticin (Singh et al. 1993) were also included in this study.

Material and methods

Mapping populations

Diploid wheat

The genetic maps of diploid wheat, *T. monococcum* L., were based on two populations. One population comprised 74 F_2/F_3 families from the cross cultivated *T. monococcum* ssp. *monococcum* DV92 × *T. monococcum* ssp. *aegilopoides* G3116 from Lebanon.The other population comprised 76 F_2/F_3 families from the cross *T. monococcum* ssp. *aegilopoides* G2528 × *T. monococcum* ssp. *aegilopoides* G1777. Linkage maps of chromosome $1A^m$ from these crosses have already been reported (Dubcovsky et al. 1995, 1996).

Tetraploid wheat

Chromosome 1A of *T. turgidum* L. was mapped using 85 recombinant substitution lines (RSLs) derived from a cross between

T. turgidum ssp. *durum* (Desf.) Husnot cultivar (cv) Langdon and a Langdon disomic substitution (DS) line in which chromosome 1A was replaced by a *T. turgidum* L. ssp. *dicoccoides* (Korn.) Thell. homologue (Joppa and Cantrell 1990). The F_1 was crossed with a disomic substitution line in which Langdon chromosome 1A was replaced by chromosome 1D of *T. aestivum* (Joppa and Williams 1988). The resulting double 1A-1D monosomics were self-pollinated and disomic RSLs homozygous for chromosome 1A were selected. Chromosome 1B was mapped using 92 RSLs derived from analogous crosses involving a DS line, in which *T. turgidum* ssp. *dicoccoides* chromosome 1B replaced its Langdon homologue. The parental disomic substitution lines are designated DSTdic1A and DSTdic1B.

Hexaploid wheat

A chromosome 1B map was constructed using 74 disomic RSLs obtained from a cross between *T. aestivum* cv Chinese Spring double ditelosomic 1B and a Chinese Spring disomic substitution line in which chromosome 1B was replaced by a homologous chromosome pair of cv Cheyenne (Morris et al. 1966). This line will henceforth be designated as DSCnn1B. The map of chromosome 1D was based on 87 RSLs from a cross between Chinese Spring double ditelosomic 1D and a disomic substitution line in which Chinese Spring chromosome 1D was replaced by *T. tauschii* chromosome 1D from the synthetic amphihexaploid RL5405 (Jones et al. 1991). The disomic substitution line was designated DSTt1D.

Other genetic stocks

The presence of low-M_r glutenin and gliadin genes on chromosomes of homoeologous group 7 was investigated in the following stocks: (1) Chinese Spring ditelosomics 7AS, 7BS and 7DS (Sears and Sears 1979); (2) disomic substitution lines with *Lophopyrum elongatum* (Host) Löve chromosome 7E individually substituted for Chinese Spring chromosomes 7A, 7B, and 7D (Dvorak 1980); and (3) a disomic addition line of chromosome 7H from *H. vulgare* L. in Chinese Spring (further DA7H) (Islam et al. 1981).

Clones and mapping

Nuclear DNAs were isolated from leaves of single plants following the procedure of Dvorak (1988). Southern hybridization was performed as described earlier (Dubcovsky et al. 1994). After hybridization, the membranes were washed in 2 × sodium citrate buffer (SSC) at 65°C for 30 min and 1 × SSC at 65°C for 30 min. The final wash was 0.2 × SSC at 65°C for 30 min for wheat clones and 0.5 × SSC at 65°C for 30 min for heterologous clones. The following clones were used in the mapping of seed-storage-protein loci: hordein (γ-gliadin) clone pcP387 (Forde et al. 1985 a), α-gliadin clone pTU1 (D'Ovidio et al. 1992), high-M_r glutenin clone pDY10A/KS- (Anderson et al. 1989), low-M_r glutenin clone pTdUCD1 (Cassidy and Dvorak 1991), and triticin clone Tri25-11 (Singh et al. 1993). Probes used for the mapping of the remaining loci were as described by Dubcovsky et al. (1996).

Maps were constructed with the computer program Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992) using the Kosambi mapping function (Kosambi 1943). Multipoint analysis was used on individual linkage groups employing a LOD threshold of two. Preferred orders were checked by the "RIPPLE" command with a window size of 5 and a LOD threshold of 2. Markers with LOD < 2 were placed in the preferred locations and are indicated by non-italicized parentheses in Fig. 1. The goodness-of-fit of the segregation of each pair of alleles was tested with χ^2 tests. Segregation of the double ditelosomic and bibrachial chromosome states was used to map the centromeres in *T. aestivum*. In *T. turgidum*, the positions of the centromeres were inferred from the position of markers flanking the centromere in *T. aestivum* (Dubcovsky et al. 1995). The significance of the difference in the lengths of a specific interval between maps was tested by the z-test. To perform these tests, interval lengths in cM were converted back into percentage of recombination using the Kosambi function, and variances of the estimates of percentages of recombination were calculated according to Allard (1956).

Most chromosomal loci of seed-storage-protein genes mapped here are designated by symbols proposed by other workers. For ease of reading, the designations of orthologous loci on wheat homoeologous chromosomes (e.g., *Glu-A1*, *Glu-B1*, *Glu-D1*) are used only when the context does not indicate which of the orthologous loci is being considered.

Protein analyses

Glutenins were extracted from single seeds using a sequential extraction procedure (Singh et al. 1991b) . Briefly, gliadins were extracted twice (30 min) with a 50% (v/v) propan-1-ol solution and discarded. Glutenins were then extracted from the residue, alkylated, and fractionated by one-dimensional SDS-PAGE using 10–12% (w/v) polyacrylamide gels. The protein extraction, the alkylation of SH groups, and the treatment of propanol extracts with SDS sample buffer were all carried out at 65°C (Singh et al. 1991b). Triticins were extracted according to Singh et al. (1991a) and electrophoretically fractionated in 10% (w/v) polyacrylamide gels.

Results

Chromosome 1A

A map of chromosome 1A of *T. turgidum* employing 21 molecular markers and one morphological marker, black glume (*Bg*), was 140 cM long (Fig. 1). The centromere was inferred to be within the 3.6-cM *Xbcd446-XEm* interval. The interval centromere-*XGlu1* on the long arm was 36.2–39.8 cM long, which is significantly longer (z-test, $P < 0.05$) than the same interval on the maps of chromosomes 1B (19.9 and 21.7 cM), 1D (17.9 cM) (Fig. 1), and of previously published maps of chromosome 1A of *T. aestivum* (18.2–22.2 cM) (Dubcovsky et al. 1995) and $1A^m$ of *T. monococcum* (5.4 cM and 18.5 cM) (Dubcovsky et al. 1995, 1996). Intervals distal to *XGlu-1*, *XGlu1-XAdh3* and *XAdh3-Xcmwg710* on the map of chromosome 1A of *T. turgidum* were greatly compressed ($P < 0.01$) relative to the corresponding intervals on the *T. turgidum* 1B map (Fig. 1), a *T. aestivum* 1A map (Dubcovsky et al. 1995), and two *T. monococcum* $1A^m$ maps (Dubcovsky et al. 1995, 1996).

Four seed-storage-protein loci were mapped on the short arm. The most proximal was triticin locus *XTri*, which was 6.0–9.6 cM from the centromere, and the most distal was the gliadin locus *XGli-1*, which was 40.4–45.2 cM from the centromere (Fig. 1). A crossover was detected within the *XGli-1* locus and the two resulting loci were temporarily designated *XGli-1.1* and *XGli-1.2* (Fig. 1). Another gliadin locus was detected in the middle of the map of the short arm

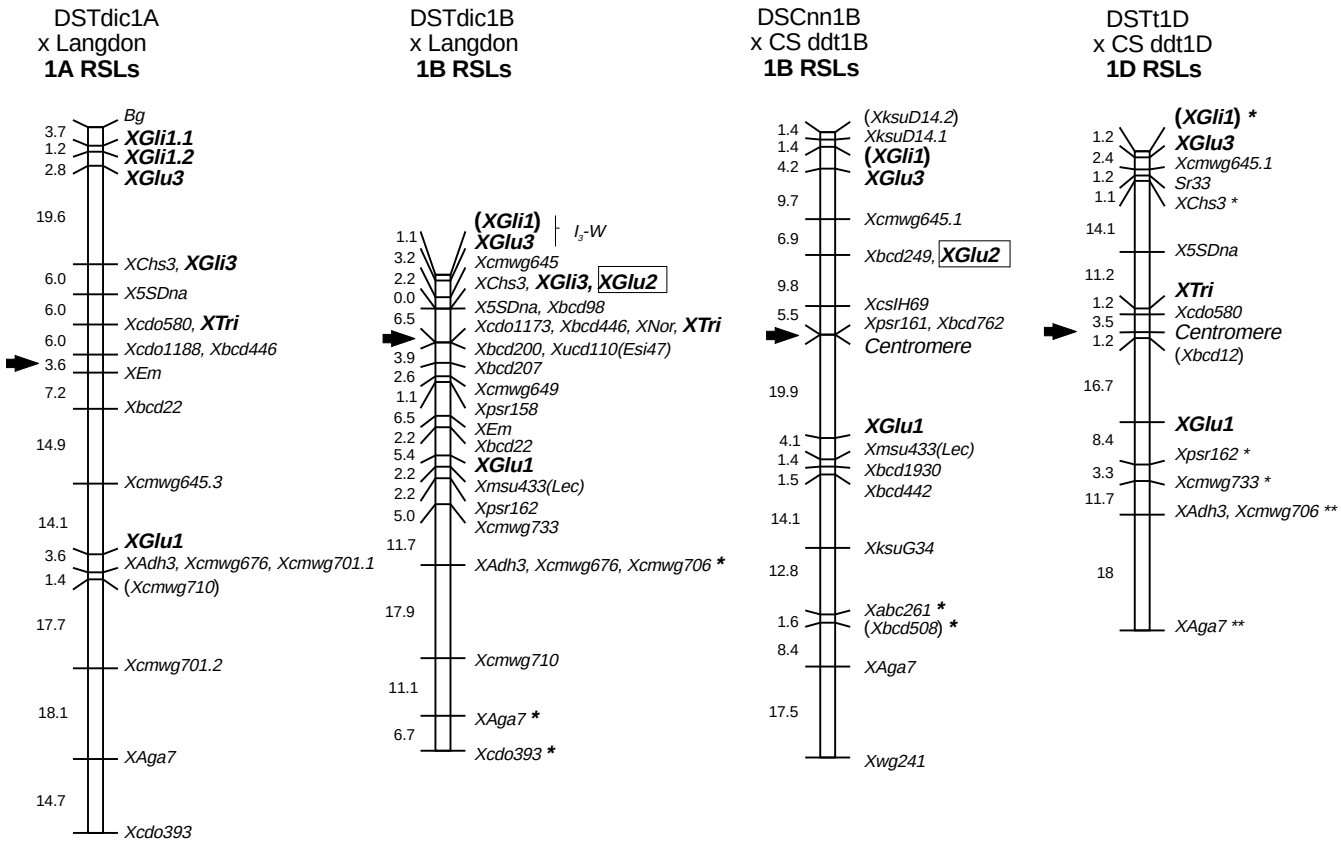

Fig. 1 Linkage maps of storage protein loci (*in bold*) detected with DNA markers on chromosomes 1A, 1B, and 1D. Distances are in cM and *arrows* indicate centromeres. Loci mapped at LOD scores <2 are indicated by *non-italicized parentheses. One and two asterisks* indicate segregation distortions at $P < 0.05$ and $P < 0.01$ levels respectively (χ^2 test)

completely linked to the *XChs3* locus. We assume that this is *XGli-3*. DNA fragments hybridizing with the low-M_r glutenin clone pTdUCD1 were mapped 2.8 cM proximally to *XGli-1*. We assume that this is the *XGlu-3* locus.

Distances observed among storage-protein loci on the maps of the short arm of chromosome 1A of *T. turgidum* were similar to those found on the same arm of chromosome $1A^m$ of *T. monococcum* (mapping populations DV92 × G3116 and G2528 × G1777). In the two mapping populations, the *XTri* locus was 6.3 and 3.4 cM from the centromere, respectively. The gliadin probe detected *XGli-1* and *XGli-3* loci in each diploid population. On the G2528 × G1777 map, *XGli-3* was completely linked to *XChs3*, while on the DV92 × G3116 map it was 7.7 cM distal to *XChs3*. On a *T. aestivum* chromosome-1A map, the *XGli-3* locus was 3.4 cM distal from to the *XChs3* locus (Dubcovsky et al. 1995). The consistent position of the *XGli-3* locus relative to the *XChs3* locus on all maps leaves little doubt that loci mapped as *Gli-3* on 1A, $1A^m$ and 1B (Fig. 1) are orthologous. The low-M_r glutenin locus *Glu-3* was completely linked to *Gli-1* on the DV92 × G3116 map and 0.7 cM proximal to it on the G2528 × G1777 map.

Chromosome 1B

Two maps of chromosome 1B were constructed (Fig. 1). The *T. aestivum* map comprised 19 molecular markers and the centromere, and was 120-cM long. The *T. turgidum* map comprised 29 molecular markers and one morphological marker, waxless spike, and was 91-cM long. The waxless phenotype is dominant over waxy. The dominant allele was contributed by the *T. turgidum* ssp. *dicoccoides* parent, which has spikes devoid of waxy bloom. Loci controlling waxy bloom that have been mapped in wheat and related species are dominant foliage wax inhibitors on chromosomes 2B ($I_1 - W$) and 2D ($I_2 - W$) (Tsunewaki 1966). We designate the locus on chromosome 1B as $I_3 - W$.

On the *T. aestivum* map, the position of the centromere was determined from the segregation of a centromere polymorphism (double telosomic state vs bibrachial state) whereas on the *T. turgidum* map the centromere was mapped by mapping markers flanking the centromere (Fig. 1).

The length of the long-arm map of the *T. turgidum* chromosome (78.5 cM) did not differ significantly from

that of the long arm in *T. aestivum* (81.3 cM). However, the maps of the short arm were of greatly different lengths. The *T. turgidum* short-arm map was 13.0 cM long but that of *T. aestivum* was 38.9 cM long. It appears that it was the *T. turgidum* map that was anomalous since this map was also significantly shorter than the maps of chromosome arms 1AS and 1DS. The positions of common markers on the two 1BS maps, *XGli-1*, *XGlu-3*, *Xmwg645.1*, and *XGlu-2*, suggest that the 1BS map was reduced in length due to a reduced crossover frequency in the entire *T. turgidum* 1BS linkage group, rather than in a specific region of it, as was the case in the long arm of chromosome 1A.

The high-M_r glutenin clone pDY10A/KS- hybridized with restriction fragments that mapped 21.7 cM and 19.9 cM from the centromere on the long arm of chromosome 1B in *T. turgidum* and *T. aestivum*, respectively (Fig. 1). The triticin locus *XTri* was completely linked to the centromere and the *XNor* locus in *T. turgidum*. Triticins from seeds of RSLs of chromosome 1B with different *XTri* RFLP alleles and from Langdon DS1D(1A) and DS1D(1B) were analyzed by SDS-PAGE. Only the triticin band from chromosome 1A, identified using DS1D(1A), was observed in the RSLs of chromosome 1B. This suggests that the *XTri-B1* locus is inactive. The *XTri* locus was not mapped in *T. aestivum* due to the lack of RFLPs.

DNA fragments hybridizing with gliadin clone pcP387 were mapped at two loci on the short arm of the *T. turgidum* chromosome (Fig. 1). The distal locus was *XGli-1*. The proximal locus was completely linked to *XChs3,* like the *XGli-3* locus on chromosome 1A. This indicates that the proximal gliadin loci on 1A and 1B are orthologous. The *Gli-3* locus was not mapped on the *T. aestivum* chromosome since no DNA fragment polymorphic between Chinese Spring and Cheyenne DNAs was found in this region of the map.

Low-M_r glutenin clone pTdUCD1 hybridized with DNA fragments mapping at two loci (Fig. 2, *Bgl*II). One locus, *Glu-3,* was 1.1 cM proximal to *XGli-1* in *T. turgidum* and 4.5 cM in *T. aestivum* (Fig. 1). The other locus, *Glu-2,* was located in the middle of the maps of the short arm in both species. The *XGlu-2* locus was completely linked to *XGli-3* in *T. turgidum* and to *Xbcd249* in *T. aestivum* (Fig. 1). Unfortunately, the linkage relationship between *XGlu-2* and *XGli-3* could not be investigated in *T. aestivum* because no restriction fragment for the *XGli-3* locus was polymorphic in the *T. aestivum* mapping population. A Southern blot of *T. turgidum* RSL DNAs digested with *Xba* I was sequentially hybridized with gliadin clone pcP387 and LMW clone pTdUCD1. There was no fragment common to the two profiles (Fig. 2, *Xba*I). The same result was obtained with *Bgl*II-digested DNAs (Fig. 2).

Five *T. turgidum* RSLs recombined between *XGlu-2* and *XGlu-3* and five RSLs with the parental genotypes were analyzed by SDS-PAGE (Fig. 3). Six low-M_r glutenin subunits of the B type (indicated by arrowheads in Fig. 3) co-segregated with DNA fragments from the Langdon or *T. turgidum* ssp. *dicoccoides XGlu-3* alleles, and two B-type subunits (indicated by arrows in Fig. 3) co-segregated with DNA fragments from the Langdon or *T. turgidum* ssp. *dicoccoides XGlu-2* alleles (compare Figs. 2 and 3).

Chromosome 1D

The map of chromosome 1D involved 14 molecular markers, the centromere, and the stem rust-resistance locus *Sr33* (Jones et al. 1991). The total length of the map was 95 cM. Strong segregation distortion favoring Chinese Spring alleles was observed in the distal portion of the long arm. Only 10.6% (expected 50%) of *T. tauschii* alleles were detected at *XAga*7. The most distal loci *Xcdo393* and *Xwg241* could not be mapped because only a few of the RSLs carried *T. tauschii* alleles at these loci.

The high-M_r glutenin locus was mapped 17.9 cM from the centromere on the long arm. This 1D interval was significantly shorter (z-test, $P < 0.05$) than the same interval on the map of chromosome 1A (Fig. 1) but did not significantly differ from the *XGlu-1*–centromere interval on either map of chromosome 1B.

The *XTri* locus was mapped 4.7 cM from the centromere on the short arm. The *XGli-1* locus was 36 cM from the centromere. Only one RSL showed recombination between *XGli-1* and *XGlu-3*, yielding a distance of 1.2 cM between the loci. No *XGli-3* or *XGlu-2* locus was detected on chromosome 1D.

Chromosome $7A^m$

In DNAs of the DV92 × G3116 *T. monococcum* population digested with *Dra*I, *Eco*RI, *Sst*I, and *Xba*I and hybridized with pTdUCD1, a single restriction fragment was observed to segregate independently of DNA fragments mapping at the *XGlu-3* locus. The G3116 parent contributed this restriction fragment. The fragment mapped on the long arm of chromosome $7A^m$ between markers *Xwg380* and *Xabg461* (Fig. 4). Cultivated *T. monococcum* DV92 had a null allele at this locus. In DNAs of the G2528 × G1777 *T. monococcum* population digested with *Bgl* II, pTdUCD1 hybridized with one G2528 and two G1777 restriction fragments that segregated independently of the DNA fragments mapping at the *XGlu-3* locus (Fig. 5). The three fragments were mapped to chromosome $7A^m$ between RFLP markers *Xwg380* and *Xwg420* (Fig. 4). We

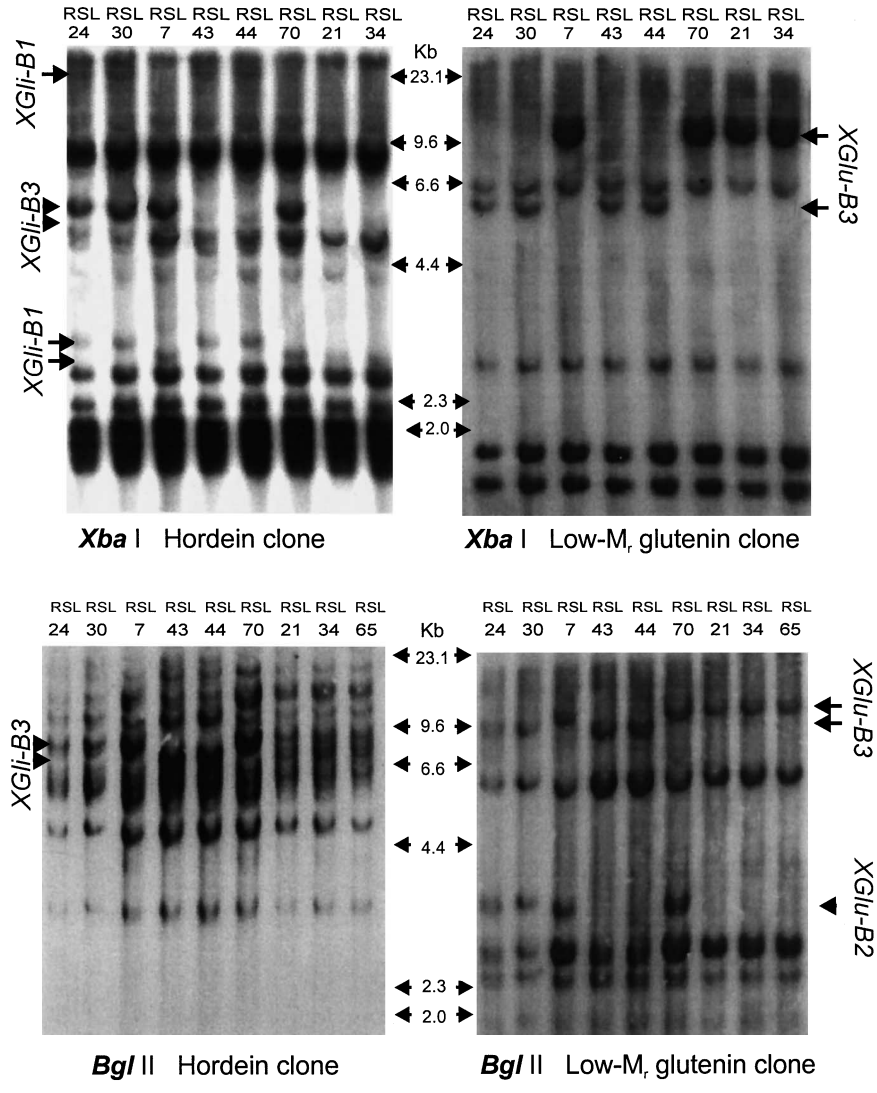

Fig. 2 Sequential hybridization of *Xba*I (*upper two panels*) and *Bgl*II (*lower two panels*)-digested DNAs with hordein clone pcP387 and low-M_r glutenin clone pTdUCD1. Note that these clones hybridize with different restriction fragments in the same Southern-blots (*Bgl*II Southern-blot hybridized with hordein clones is included only for comparison). RSL24 and RSL30 have Langdon alleles at *XGlu-B3* and *XGlu-B2*; RSL21, RSL34, and RSL65 have *T. Turgidum* ssp. *dicoccoides* alleles at *XGlu-B3* and *XGlu-B2*; and RSL7, RSL43, RSL44, and RSL70 show recombination between *XGlu-B3* and *XGlu-B2*

designate this new low-M_r glutenin locus *Xucd1* (*Low M_r-Glu*).

To investigate the expression of this locus, SDS-PAGE profiles of the low-M_r glutenin of the parental lines were compared with profiles of different F_2 individuals recombinant for *XGlu3* and *Xucd1* (*Low M_r-Glu*). No protein co-segregating with *Xucd1* (*Low M_r-Glu*) was found, suggesting that the *Xucd1* (*Low M_r-Glu*) locus is not expressed.

Chromosome 7D

The presence of low-M_r glutenin and gliadin genes on chromosomes of homoeologous group 7 was investigated in Chinese Spring ditelosomics, disomic substitutions and disomic addition lines (see Material and methods). Southern blots of DNAs of these genetic stocks digested with restriction enzymes *Bgl*II, *Dra*I, *Eco*RI, and *Xba*I were hybridized with clones pTdUCD1, pcP387 and pTU1, and the hybridization profiles were compared with those of Chinese Spring (Fig. 5). No restriction fragment was absent from, or gained by, any of these stocks relative to Chinese Spring, suggesting that there is no low-M_r glutenin or gliadin gene on chromosome 7 in the A, B, and D genomes of *T. aestivum*, the E genome of *L. elongatum*, or the H genome of *H. vulgare* (Fig. 5). However, since Southern-blot hybridization with the α-gliadin clone pTU1 produced a dark background, we cannot conclusively exclude the possibility of the presence of an α-type gliadin fragment on homoeologous group-7 chromosomes.

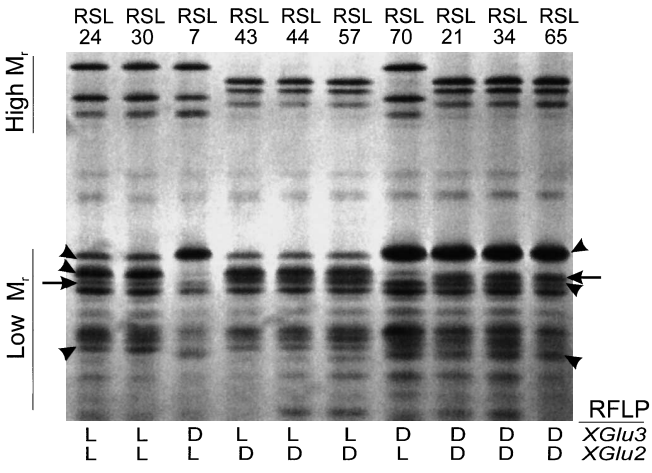

Fig. 3 One-dimensional SDS-PAGE (10% acrylamide). *Glu-B3* low-M_r glutenin subunits are indicated by *arrowheads* and *Glu-B2* low-M_r glutenin subunits by *arrows*. Alleles detected at *XGlu-B3* and *XGlu-B2* by DNA hybridization are indicated by *L* (Langdon) and *D* (*T. turgidum* ssp. *dicoccoides*)

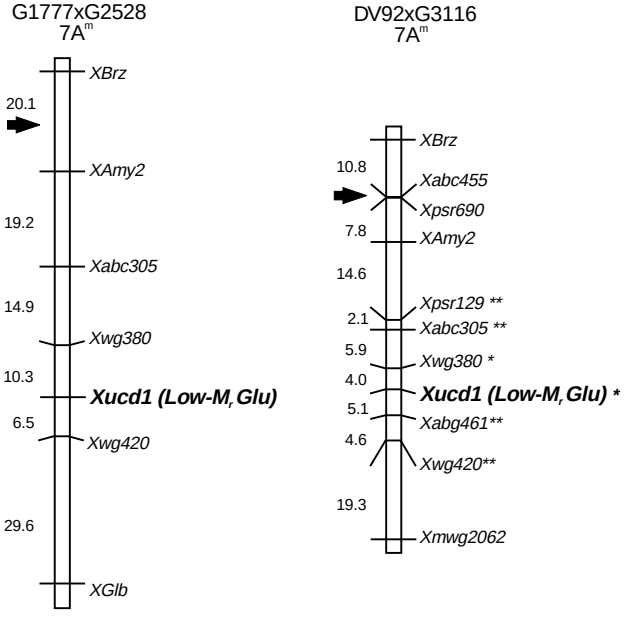

Fig. 4 Linkage maps of *T. monococcum* chromosome 7A^m. New low-M_r glutenin locus is indicated in *bold*. Distances are in cM and *arrows* indicate centromeres. *One and two asterisks* indicate segregation distortions at $P < 0.05$ and $P < 0.01$ levels respectively (χ^2 test)

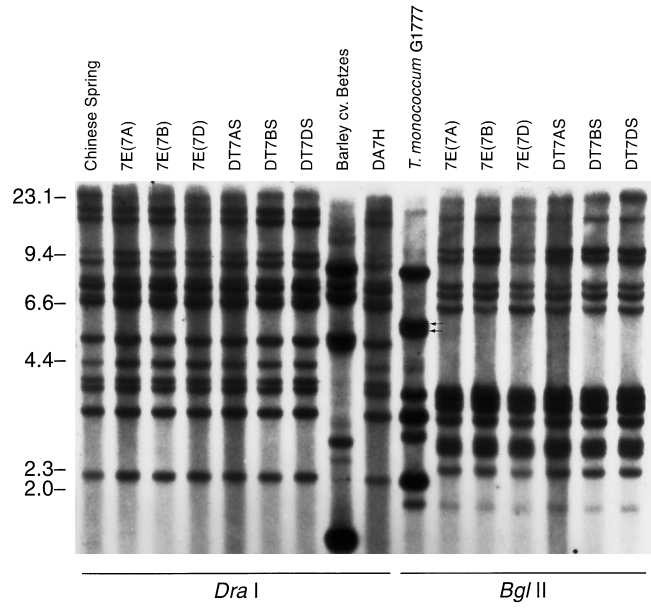

Fig. 5 Hybridization of *Bgl*II- and *Dra*I digested DNAs of *L. elongatum* disomic substitution lines 7E(7A), 7E(7B), and 7E(7D); Chinese Spring ditelosomics 7AS, 7BS, and 7DS; and barley disomic addition line DA7H; with low-Mr glutenin clone pTdUCD1. Note the strong hybridization signal for the *Triticum monococcum* DNA fragments mapped at the *Xucd1*(*Low-M_r Glu*) locus on chromosome 7A^m (indicated by *arrows*)

Discussion

Low-M_r glutenins

Ruiz and Carrillo (1993) mapped a low-M_r glutenin subunit, LMW9, at the *Gli-3* locus in the B genome (*Gli-B3*) of *T. turgidum* ssp. *durum* cv Mexicali. This subunit had an electrophoretic mobility characteristic of the B subunits. Liu (1995) found a similar subunit in durum cv Edmore. He designated the locus at which the subunit was encoded as *Glu-2*. Although Liu (1995) demonstrated that the subunit was linked to glutenin by disulphide bonds, these observations did not rule out the possibility that the subunit is a mutant gliadin that acquired an odd cysteine that facilitates its covalent bonding to glutenin by means of an intermolecular disulphide bond (Lew et al. 1992). This possibility was however, ruled out in the present study. Low-M_r glutenin clone pTdUCD1 has less than 70% homology with the gliadin genes at the nucleotide-sequence level and does not hybridize with them (Cassidy and Dvorak 1991). Restriction fragments that hybridized with the clone and mapped at the *XGlu-2* locus did not hybridize with the gliadin clone while those that mapped at the *XGli-B3* locus and hybridized with the gliadin clone did not hybridize with the low-M_r glutenin clone. This showed that the aggregating polypeptide mapped at the *Glu-2* locus is indeed a low-M_r glutenin subunit and not a mutated gliadin. In view of the greatly reduced level of recombination shown by the short arm of the *T. turgidum* chromosome, the lack of recombination between the *XGlu-2* and *XGli-3* loci on 1B of *T. turgidum* has little weight for deciding whether one or two loci are involved. In this study, it was not possible by SDS-PAGE to find a protein

subunit that co-segregates with the restriction fragments mapped at *XGlu-B2* in the hexaploid mapping population.

The low-M_r glutenin subunit mapped here and by Ruiz and Carrillo (1993) and Liu (1995) is a B-subunit and not the D-subunit originally mapped at *Glu-2* in the B genome by Jackson et al. (1985). In contrast to the B-subunits of low-M_r glutenin, the D-subunits are related to ω-gliadins. When the D-subunits were found to be at the *Gli-3* and *Gli-1* loci in the B and D genomes, respectively, the *Glu-2* name was abandoned (Payne et al. 1988). Liu (1995) re-used the *Glu-2* designation for the locus encoding the B-subunit in the middle of the short arm of chromosome 1B.

Another new low-M_r glutenin locus, designated *Xucd1* (*Low-M_r Glu*), was detected with clone pTdUCD1 on the long arm of chromosome $7A^m$ in *T. monococcum* ssp. *aegilopoides*. The absence of this locus on *T. monococcum* ssp. *monococcum* chromosome $7A^m$, and on chromosomes from homoeologous group 7 in other *Triticeae* genomes, suggests that this duplication is recent. Co-linearity between chromosome $7A^m$ and barley chromosome 7H for markers flanking the *Xucd1* (*Low-M_r Glu*) locus, *Xabc455*, *XAmy2*, *Xpsr129*, *Xabc305*, *Xabg461*, *Xwg420* and *Xmwg2062* (Dubcovsky et al. 1996) indicates that the *Xucd1* (*Low-M_r Glu*) locus does not owe its origin to a translocation or a duplication of a large segment of chromosome $1A^m$ in chromosome $7A^m$. Synthesis of the prolamin polypeptides is endosperm-specific (Forde et al. 1985 b; Colot et al. 1987) and consequently it is unlikely that a low-M_r glutenin mRNA can be reverse transcribed and integrated as a processed pseudogene in the germ line. Positional changes of multigene loci without alteration of chromosome co-linearity (gene nomadism) were also reported for the 18*S*-5.8*S*-26*S* and 5*S*rRNA multigene families (Dubcovsky and Dvorak 1995; Dubcovsky et al. 1996). Although the exact nature of this process is unknown, intrachromosomal recombination between closely linked DNA direct repeats and excision of a circular DNA molecule containing the intervening sequences can provide a possible explanation (Peterhans et al. 1990; Gal et al. 1991). Intrachromosomal recombination between direct repeats occurs regularly in plants (Gal et al. 1991), and in one case the 'popped out' DNA sequence was found integrated elsewhere in the genome (Peterhans et al. 1990).

The low-M_r glutenin locus *Xucd1* (*Low-M_r Glu*) detected on chromosome $7A^m$ of *T. monococcum* is probably not orthologous to the *Glu-D5* locus reported on chromosome 7D by Sreeramulu and Singh (1997). No DNA fragment was detected on chromosome 7D using a low-M_r glutenin clone that strongly hybridized with the $7A^m$ L DNA fragments in the same Southern blot (Fig. 5). Moreover, the VXVPV N-terminal amino-acid sequence of the subunit mapped by Sreeramulu and Singh (1997) is characteristic of α-type gliadins and not of low-M_r glutenins. Payne et al. (1987) and Tercero et al. (1991) reported four α-type hordeins on homoeologous group 7 of *Hordeum chilense*. Clearly, determination of the position of the *Glu-D5* and *Hor-Hch3* in relation to molecular markers that have been mapped on chromosome $7A^m$ should receive high priority in order to ascertain the orthology between these two loci and *Xucd1* (*Low-M_rGlu*).

Gliadins

In homoeologous group 1, the major component of γ- and ω-gliadins is encoded at the *Gli-1* loci at the distal ends of the maps of the 1AS, 1BS, and 1DS chromosome arms. In the A and B genomes, another locus (*Gli-3*) encoding gliadins is halfway between *Gli-1* and the centromere. In addition to these well-documented loci, another locus proximal to *Gli-1* has been claimed to exist on arm 1AS. Metakovsky et al. (1986) mapped this putative locus 13% recombination units proximal to *Gli-1* while Redaelli et al. (1992) mapped it 10 cM proximal to *Gli-1* and named it *Gli-4*. However, neither study provided evidence that this locus is not *Gli-3*. The rationale for assigning a new symbol to the locus was its shorter distance from *Gli-1*. The *Gli-3* locus had been reported to be 25–36% proximal to *Gli-1* (Galili and Feldman 1984; Payne et al. 1988). It is nevertheless possible that *Gli-4* is actually the same locus as *Gli-3* and that the low recombination with *Gli-1* reflected heterozygosity for the distal 1AS/1DS translocation that differentiated the parents of the Redaelli et al. (1992) mapping population. Heterozygosity for an analogous terminal 1AS/1DS homoeologous translocation was observed to reduce recombination in the proximal, homologous region by 62% (Dubcovsky et al. 1995). Moreover, shorter distances between *Gli-3* and *Gli-1* ranging from 13 cM to 27 cM were reported recently (Dubcovsky et al. 1995, 1996) and those ranging from 7 to 22 cM were observed here. Since none of the five 1A or $1A^m$ chromosomes for which RFLP maps have been constructed showed simultaneously the presence of the *Gli-3* and *Gli-4* loci, it is prudent to consider *Gli-4* actually to be *Gli-3* until conclusive evidence for the existence of *Gli-4* is obtained.

The *Gli-1* loci are complex and include a large number of genes. Occasional recombination within these clusters has been reported for hexaploid wheat chromosomes 1A (Metakovsky et al. 1986; Felix et al. 1996), 1B (Pogna et al. 1993, 1995), and 1D (Metakovsky and Sozinov 1987; Metakovsky 1990). Recombination within the *XGli-1* locus on chromosome 1A of tetraploid *T. turgidum* was observed here. Recombination within the *Gli-1* loci raises concerns as to the validity of assigning additional locus designations to genes tightly linked to *Gli-1*. A ω-gliadin locus 1.5–1.8 cM distal to the genes in the *Gli-B1* locus was recently designated *Gli-B5* (Pogna et al. 1993). The same authors speculated that crossovers within *Gli-1* on chromosomes 1A

(Metakovsky et al. 1986) and 1D (Metakovsky and Sozinov 1987; Metakovsky 1990) actually reflect the existence of separate loci on those chromosomes as well. The distal loci were suggested to be orthologous to *Gli-5* on chromosome 1B. These authors further speculated that the wheat *Gli-5* locus is orthologous to the minor hordein loci (*HrdC*, *HrdD*, and *HrdE*) coding for the ω-gliadin-like hordein loci that map 2.5–3.0 cM distally from the main hordein locus (*Hor2*) on chromosome 1H (Jensen 1987) and the distal ω-type secalin locus closely linked to the 40 kDa γ-secalin genes on chromosome 1R (Carrillo et al. 1992). These inferences are valid only if crossovers within the cluster of gliadin genes always occur between the same groups of genes and not at various sites within the cluster. Since it is very difficult to establish if the former assumption is true, and since gliadins were not analyzed at the protein level in this cross, the two products of a crossover in the *Gli-A1* locus were temporarily designated *XGli-A1.1* and *XGli-A1.2* until orthology with *Gli-A1* and *Gli-A5* is established.

Triticin

Triticins are storage proteins synthesized during seed development and deposited in the protein bodies of the wheat endosperm (Singh and Shepherd 1985b). The amino-acid sequence of triticin shows strong homology with the legumin-like proteins of oats (12*S* globulin), and rice (glutelin), and the legumin proteins of legume seeds (Singh et al. 1993). Synteny mapping by hybridization of the triticin cDNA clone with ditelocentric and nullisomic-tetrasomic Chinese Spring group-1 aneuploids showed that triticin genes are located on chromosome arms 1AS, 1BS, and 1DS. The intensity of the bands in euploid wheats was similar for all three chromosomes, suggesting an equal number of genes on each chromosome (Singh et al. 1993). To-date, protein products have been found only for loci on chromosomes 1A and 1D; no 1B-encoded protein has been detected. It was therefore speculated that chromosome 1B may carry a null allele (Singh and Shepherd 1985a; Singh et al. 1991a).

Triticin genes were mapped in the present study on the short arms of chromosomes 1A, 1B and 1D by DNA hybridization. Distances between *XTri* and the centromere in chromosomes 1AS (6–9.6 cM) and 1DS (4.7 cM) are similar to those based on protein analysis (11 cM and 10 cM, respectively) (Singh and Shepherd 1984). The *XTri* locus shows similar linkage relationships on chromosomes 1A, 1B, and 1D, indicating the orthology of the three loci.

Variation in recombination

Two of the maps of homoeologous group 1 reported here are based on recombination between complete chromosomes, and two others on recombination between a complete chromosome and a double ditelosome. Distances between the centromere and the *XGlu-1* locus based on recombination between complete chromosomes and double ditelosomes (Fig. 1, 18–20 cM) were not significantly different from distances between the same markers based on recombination between complete chromosomes (18–22 cM, present data and see Dubcovsky et al. 1995). However, a valid comparison of the effect of telosomes on recombination requires the use of similar mapping populations with complete chromosomes and telosomes and the adjustment of the genetic distances for pairing frequencies of the telosomes (J. Dubcovsky and J. Dvorak, in preparation).

The *XGlu-1* locus was further from the centromere on the map of chromosome 1A of *T. turgidum* than on any other map of a *Triticum* chromosome 1 (present data and Payne et al. 1982, 1984; Snape et al. 1985; Curtis and Lukaszewski 1991; Gill et al. 1991; Lagudah et al. 1991; Wang et al. 1991; Dubcovsky et al. 1995; Van Deynze et al. 1995; Dubcovsky et al. 1996). This is probably compensation for a five-fold reduction in recombination in the juxtaposed distal *XGlu-1–XAdh3* interval, which is caused by polymorphism for a paracentric inversion that differentiates *T. turgidum* ssp. *dicoccoides* chromosome 1A from Langdon chromosome 1A (J. Dvorak, unpublished).

Another anomaly on the *T. turgidum* maps reported here was a three-fold reduction in recombination in the short arm of chromosome 1B relative to that in the *T. aestivum* chromosome 1B reported earlier (Dubcovsky and Dvorak 1995). Heterozygosity between homologous chromosomes in wheat reduces chiasma frequency (Dvorak and McGuire 1981; Dvorak 1988). The chromosomes of the B genome, particularly the short arms, such as 1BS, show the greatest reduction of chiasmatic pairing in intervarietal hybrids. Presumably, a high level of polymorphism between the *durum* and *T. turgidum* ssp. *dicoccoides* 1B chromosomes was the cause of this anomaly. Recombination was low in all intervals on the short arm, which agrees with the observation that heterozygosity reduces chiasma frequencies throughout the entire length of chromosome arms (Crossway and Dvorak 1984; Dvorak and Chen 1984). However, some other factor must be involved in this anomaly since the long arm of the same chromosome pair did not show any reduction in recombination relative to that in *T. aestivum* chromosome 1B or *T. turgidum* chromosome 1A. A detailed study of recombination between wheat homoeologous chromosomes 4B and 4D in the absence of the *Ph1* locus also revealed more reduced recombination in the short arm than in the long arm compared to homologous chromosomes (Dvorak et al. 1995). Low recombination in chromosome arm 1BS compared to that in 1AS was also observed in a F_2/F_3 mapping population derived from the cross, synthetic wheat

(*T. turgidum* × *T. tauschii*) × *T. aestivum* cv Chinese Spring (Gale et al. 1995). However, in a single-seed-descent mapping population from a cross involving a different synthetic wheat (*T. turgidum* cv Altar 84 × *T. tauschii*) no reduction in recombination in 1BS was reported (Van Deynze et al. 1995). It is likely that this difference reflected the nature of the mapping populations (F_2/F_3 vs single-seed descent) since there is no reason to believe that the two pairs of parents differed in the level of polymorphism.

The drastic reduction in genetic length of the map of chromosome arm 1BS was not observed in the map of chromosome arm 1DS, although the latter was based on recombination between homologues which originated from different species (*T. aestivum* and *T. tauschii*). This is, however, in full agreement with the assumption that reduced crossover frequencies are related to the level of polymorphism between chromosomes. There was a very low level of RFLP between the *T. aestivum* chromosome 1D and the *T. tauschii* homologue (9% of polymorphic probe × enzyme combinations) compared with the level of polymorphism between *T. turgidum* ssp. *durum* chromosome 1B and the *T. turgidum* ssp. *dicoccoides* homologue (36% of polymorphic probe × enzyme combinations).

Although wide crosses are an appealing strategy to bypass the low level of RFLP among wheat cultivars, they can result in anomalies in recombination that may confound comparative mapping results. In addition to segregation distortion, an extreme form of which was observed here in the distal region of the 1DL arm of the *T. aestivum* and *T. tauschii* chromosome pair, one should expect large variation in genetic distances. It is therefore imperative that conclusions concerning the orthology of loci are based on their position relative to tightly linked flanking markers rather than simply on the strength of linkage, as has been pointed out for gliadin loci above.

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References

Allard, RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Anderson OD, Greene FC, Yip RE, Halford NG, Shewry PR, Malpica-Romero J-M (1989) Nucleotide sequences of the two high-molecular-weight glutenin genes from the D genome of hexaploid wheat, *Triticum aestivum* L. cv Cheyenne. Nucleic Acids Res 17:461–462

Carrillo JM, Vázquez JF, Orellana J (1992) Identification and mapping of the *Gli-R3* locus on chromosome 1R of rye (*Secale cereale* L.). Theor Appl Genet 84:237–241

Cassidy BG, Dvorak J (1991) Molecular characterization of a low-molecular-weight glutenin cDNA clone from *Triticum durum*. Theor Appl Genet 81:653–660

Colot V, Robert LS, Kavanagh TA, Bevan MW, Thompson RD (1987) Localization of sequences in wheat endosperm protein genes which confer tissue-specific expression in tobacco. EMBO J 6:3559–3564

Colot V, Bartels D, Thompson R , Flavell R (1989) Molecular characterization of an active wheat LMW glutenin gene and the relation to other wheat and barley prolamin genes. Mol Gen Genet 216:81–90

Crossway A, Dvorak J (1984) Distribution of nonstructural variation along three chromosome arms between wheat cultivars Chinese Spring and Cheyenne. Genetics 106:309–324

Curtis CA, Lukaszewski AJ (1991) Genetic linkage between C-bands and storage protein genes in chromosome 1B of tetraploid wheat. Theor Appl Genet 81:245–252

D'Ovidio R, Tanzarella OA , Porceddu E (1992) Isolation of an alpha-type gliadin gene from *Triticum durum* Desf. and genetic polymorphism at the *Gli-2* loci. J Genet Breed 46:41–48

Dubcovsky J, Dvorak J (1995) Ribosomal RNA loci: nomads in the Triticeae genomes. Genetics 140:1367–1377

Dubcovsky J, Galvez AF, Dvorak J (1994) Comparison of the genetic organization of the early salt-stress-response gene system in salt-tolerant *Lophopyrum elongatum*and salt-sensitive wheat. Theor Appl Genet 87:957–964

Dubcovsky J, Luo M-C, Dvorak J (1995) Differentiation between homoeologous chromosomes 1A of wheat and $1A^m$ of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. Proc Natl Acad Sci USA 92:6645–6649

Dubcovsky J, Luo M-C, Zhong G-Y, Bransteiter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996) Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Dvorak J (1980) Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can J Genet Cytol 22:237–259

Dvorak J (1988) Cytogenetical and molecular inferences about the evolution of wheat. Proc 7th Int Wheat Genet Symp. Cambridge. Bath Press, Bath, England, pp 187–192

Dvorak J , Chen K-C (1984) Distribution of no structural variation between wheat cultivars along chromosome arm 6*Bp*: Evidence from the linkage map and physical map of the arm. Genetics 106:325–333

Dvorak J, McGuire PE (1981) Nonstructural chromosome differentiation among wheat cultivars with special reference to differentiation of chromosomes in related species. Genetics 97:391–414

Dvorak J, Dubcovsky J, Luo M-C, Devos KM, Gale MD (1995) Differentiation between wheat chromosomes 4B and 4D. Genome 38:1139–1147

Felix I, Martinant JP, Bernard M, Bernard S, Branlard G (1996) Genetic characterization of storage proteins in a set of F_1-derived haploid lines in bread wheat. Theor Appl Genet 92:340–346

Forde BG, Kreis M, Williamson MS, Fry RP, Pywell J, Shewry PR, Bunce N , Mifflin BJ (1985 a) Short tandem repeats shared by B- and C-hordein cDNAs suggest a common evolutionary origin for two groups of cereal storage-protein genes. EMBO J 4:9–15

Forde BG, Heyworth A, Pywell J, Kreis M (1985 b) Nucleotide sequence of a B1 hordein gene and the identification of possible upstream regulatory elements in endosperm storage-protein genes from barley, wheat and maize. Nucleic Acids Res 13:7327–7339

Gal S, Pisan B, Hohn T, Grimsley N, Hohn B (1991) Genomic homologous recombination *in planta*. EMBO J 10:1571–1578

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. Proc 8th Int Wheat Genet Symp, Beijing. China Agricultural Scientech Press, pp 29–40

Galili G, Feldman M (1984) Mapping of glutenin and gliadin genes located on chromosome 1B of common wheat. Mol Gen Genet 193 : 293–298

Gill KS, Lubbers EL, Gill BS, Raupp WJ , Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat. Genome 34 : 362–374

Holt LM, Astin R, Payne PI (1981) Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. 2. Relative isoelectric points determined by two-dimensional fractionation in polyacrylamide gels. Theor Appl Genet 60 : 237–243

Islam AKMR, Shepherd KW , Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46 : 161–174

Jackson EA, Holt LM , Payne PI (1983) Characterization of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. Theor Appl Genet 66 : 29–37

Jackson EA, Holt LM , Payne PI (1985) *Glu-B2*, a storage-protein locus controlling the D group of LMW glutenin subunits in bread wheat (*Triticum aestivum*). Genet Res 46 : 11–17

Jensen J (1987) Co-ordinators report. Chromosome 5. Barley Genet Newslett 17 : 111–113

Jones SS, Dvorak J, Knott DR, Qualset CO (1991) Use of double-ditelosomic and normal chromosome 1D recombinant substitution lines to map *Sr33* on chromosome arm 1DS in wheat. Genome 34 : 505–508

Joppa LR , Cantrell RG (1990) Chromosomal location of genes for grain-protein content of wild tetraploid wheat. Crop Sci. 30 : 1059–1064

Joppa LR , Williams ND (1988) Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. Genome 30 : 222–228

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12 : 172–175

Lagudah ES, Appels R , Brown ADH (1991) The molecular-genetic analysis of *Triticum tauschii*, the D genome donor to hexaploid wheat. Genome 36 : 913–918

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) MAPMAKER: an integrated computer package for construction of primary linkage maps of experimental and natural populations. Genomics 1 : 174–181

Lew EJ-L, Kuzmicky DD , Kasarda DD (1992) Characterization of low-molecular-weight glutenin subunits by reversed-phased high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino-acid sequencing. Cereal Chem 69 : 508–515

Lincoln S, Daly M , Lander E (1992) Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report 3rd ed., Cambridge, MA

Liu C-Y (1995) Identification of a new low-Mr glutenin subunit locus on chromosome 1B of durum wheat. J Cereal Sci 21 : 209–213

Liu C-Y, Shepherd KW (1995) Inheritance of B subunits of glutenins and omega- and gama-gliadins in tetraploid wheats. Theor Appl Genet 90 : 1149–1157

Masci SM, Lafiandra D, Porceddu E, Lew EJ-L, Tao MP, Kasarda DD (1993) D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal Chem 70 : 581–585

Metakovsky EV (1990) Organization of gliadin-encoding genes which are genetic markers in wheat. Molecular mechanisms of genetic processes. Nauka, Moscow, pp 157–168

Metakovsky EV, Akhmedov MG , Sozinov AA (1986) Genetic analysis of gliadin-coding genes reveals gene clusters as well as single remote genes. Theor Appl Genet 73 : 278–285

Metakovsky EV, Sozinov AA (1987) Organization, variability and stability of the family of the gliadin-coding genes in wheat: genetic data. Gluten proteins. Proc 3rd Int Workshop, Singapore New Jersey Hong Kong. World Sci, pp 30–45

Morris R, Schmidt JW, Mattern PJ, Johnson VA (1966) Chromosomal location of genes for flour quality in the wheat variety Cheyenne using substitution lines. Crop Sci 6 : 119–122

Payne PI, Corfield KG (1979) Subunit composition of wheat glutenin proteins isolated by gel filtration in dissociating medium. Planta 145 : 83–88

Payne, PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Theor. Appl Genet 63 : 129–138

Payne PI, Holt LM, Jackson EA, Law CN (1984) Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Phil Trans R Soc Lond 304 : 359–371

Payne PI, Roberts MS, Holt LM (1986) Location of genes controlling the D group of LMW glutenin subunits on chromosome 1D of bread wheat. Genet Res 47 : 175–179

Payne PI, Holt LM, Reader SM, Miller TE (1987) Chromosomal location of genes coding for endosperm proteins of *Hordeum chilense*, determined by two-dimensional electrophoresis of wheat – *H. chilense* chromosome-addition lines. Biochem Genet 25 : 53–65

Payne P, Holt LM, Krattiger AF, Carrillo J (1988) Relationships between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. J Cereal Sci 7 : 229–235

Payne PI, Holt LM, Lister PG (1988) *Gli-A3* and *Gli-B3*, two newly designated loci coding for omega-type gliadins and D subunits of glutenins. Proc 7th Int Wheat Genet Symp, Cambridge. Bath Press, Bath, England, pp 999–1002

Peterhans A, Schlupmann H, Basse C, Paszkowski J (1990) Intrachromosomal recombination in plants. EMBO J 9 : 3437–3445

Pogna NE, Autran J-C, Mellini G, Lafiandra D, Feillet P (1990) Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. J Cereal Sci 11 : 15–34

Pogna NE, Metakovsky EV, Redaelli R, Raineri F, Dachkevitch T (1993) Recombination mapping of *Gli-5*, a new gliadin-coding locus on chromosomes 1A and 1B in common wheat. Theor Appl Genet 87 : 113–121

Pogna NE, Redaelli R, Dachkevitch T, Curioni A , Dal Belin Peruffo A (1994) Genetics of wheat quality and its improvement by conventional and biotechnological breeding. Wheat, production, properties and quality. Blakie Academic and Professional, Chapman and Hall, Glasgow, UK, 128–142

Pogna NE, Redaelli R, Vaccino P, Biancardi AM, Peruffo ADB, Curioni A, Metakovsky EV, Pagliaracci S (1995) Production and genetic characterization of near-isogenic lines in the bread-wheat cultivar Alpe. Theor Appl Genet 90 : 650–658

Redaelli R, Pogna NE, Dachkevitch T, Cacciatori P, Biancardi AM, Metakovsky EV (1992) Inheritance studies of the 1AS/1DS chromosome translocation in the bread-wheat variety Perzivan-I. J Genet Breed 46 : 253–262

Ruiz M, Carrillo RM (1993) Linkage relationships between prolamin genes on chromosomes 1A and 1B of durum wheat. Theor Appl Genet 87 : 353–360

Sears ER, Sears LMS (1979) The telocentric chromosomes of common wheat. Proc 5th Int Wheat Genet Symp, New Delhi, India, pp 389–407

Singh NK, Shepherd KW (1984) A new approach to study the variation and genetic control of disulphide-linked endosperm proteins in wheat and rye. Proc 2nd Int Workshop Wheat Gluten Proteins, Wageningen, pp 129–136

Sing NK, Shepherd KW (1985 a) The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor Appl Genet 71 : 79–92

Singh NK, Shepherd KW (1985 b) Solubility behaviour, synthesis, degradation and subcellular location of a new class of disulphide-linked proteins in wheat endosperm. Aust J Pl Physiol 14 : 245–252

Singh NK, Shepherd KW (1988) Linkage mapping of the genes controlling endosperm proteins in wheat. 1. Genes on the short arms of group-1 chromosome. Theor Appl Genet 75 : 628–641

Singh NK, Shepherd KW, Langridge P, Gruen LC (1991 a) Purification and biochemical characterization of triticin, a legumin-like protein in wheat endosperm. J Cereal Sci 13: 207–219

Singh NK, Shepherd KW, Cornish GB (1991 b) A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J Cereal Sci 14: 203–208

Singh NK, Donovan GR, Carpenter HC, Skerritt JH, Langridge P (1993) Isolation and characterization of wheat triticin cDNA revealing a lysine-rich repetitive domain. Pl Mol Biol 22: 227–237

Snape JW, Flavell RB, O'Dell M, Hughes WG, Payne PI (1985) Intrachromosomal mapping of the nucleolar organizer region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*) Theor Appl Genet 69: 263–270

Sobko TA (1984) Identification of a locus controlling the synthesis of endosperm alcohol-soluble proteins in winter bread wheat. J Agric Sci 7: 78–80

Sreeramulu G, Singh NK (1997) Genetic and biochemical characterization of novel low-molecular-weight glutenin subunits in wheat (*Triticum aestivum* L.) Genome 40: 41–48

Tao HP, Kasarda DD (1989) Two-dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. J Exp Bot 40: 1015–1020

Tatham AS, Shewry PR, Belton PS (1990) Structural studies of cereal prolamins, including wheat gluten. Adv Cereal Sci Technol 10: 1–78

Tercero JA, Bernardo A, Jouve N (1991) Encoding genes for endosperm proteins in *Hordeum chilense*. Theor Appl Genet 81: 127–132

Tsunewaki K (1966) Comparative gene analysis of common wheat and its ancestral species. II. Waxiness, growth habit and awnedness. Jap J Bot 19: 175–254

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group-1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38: 45–59

Wang ML, Atkinson MD, Chinoy CN, Devos KM, Harcourt RL, Liu CJ, Rogers WJ, Gale MD (1991) RFLP-based genetic map of rye (*Secale cereale* L.) chromosome 1*R*. Theor Appl Genet 82: 174–178

Weegels PL, Hamer RJ, Schofield JD (1996) Functional properties of wheat glutenin. J Cereal Sci 23: 1–18

Wrigley CW, Shepherd KW (1973) Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209: 154–162