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Biochemical and genetic characterization of a monomeric storage protein (T1) with an unusually high molecular weight in *Triticum tauschii*

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Abstract The protein named T1, present in *Triticum tauschii*, was previously characterized as a high-molecular-weight (HMW) glutenin subunit with a molecular size similar to that of the y-type glutenin subunit-10 of *Triticum aestivum*. This protein was present along with other HMW glutenin subunits named 2^t and T2, and was considered as part of the same allele at the *Glu-D^{t}1* locus of *T. tauschii*. This paper describes a re-evaluation of this protein, involving analyses of a collection of 173 accessions of *T. tauschii*, by SDS-PAGE of glutenin subunits after the extraction of monomeric protein. No accessions were found containing the three HMW glutenin subunits. On the other hand, 17 lines with HMW glutenin subunits having electrophoretic mobilities similar to subunits 2^t and T2 were identified. The absence of T1 protein in these gel patterns has shown that protein T1 is not a component of the polymeric protein. Rather, the T1 protein is an ω-gliadin with an unusually high-molecular-weight. This conclusion is based on acidic polyacrylamide gel electrophoresis (A-PAGE), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and two-dimensional gel electrophoresis (A-PAGE+ SDS-PAGE), together with analysis of its N-terminal amino-acids sequence. The inheritance of ω-gliadin T1 was studied through analyses of gliadins and HMW glutenins in 106 F_2 grains of a cross between synthetic wheat, L/18913, and the wheat cv Egret. HMW glutenin subunits and gliadins derived from *T. tauschii* (*Glu-D^{t}1* and *Gli-D^{t}1*) segregated as alleles of the *Glu-D1* and *Gli-D1* loci of bread wheat. A new locus encoding the ω-gliadin T1 was identified and named *Gli-DT1*. The genetic distance between this new locus and those of endosperm proteins encoded at the 1D chromosome were calculated. The *Gli-DT1* locus is located on the short arm of chromosome 1D and the map distance between this locus and the *Gli-D1* and *Glu-D1* loci was calculated as 13.18 cM and 40.20 cM, respectively.

Keywords *Triticum tauschii* · HMW glutenin subunits · ω-gliadins · D-genome

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Introduction

Triticum tauschii (syn. *Aegilops tauschii*, *Aegilops squarrosa*, 2n=2*x*=14 DD) is well recognized as one of the diploid progenitors of bread wheat, *Triticum aestivum* (2n=6*x*=42 AABBDD). The origin of hexaploid wheats has been related to an apparently small number of *T. tauschii* genotypes (Lagudah and Halloran 1989) producing a relatively small degree of genetic diversity for the D genome in *T. aestivum*. Consequently, a wider range of *T. tauschii* accessions is expected to provide useful genetic resources for increasing the genetic diversity of common wheat and for improving various agronomic characteristics and quality aspects of bread wheat. This wild ancestor of hexaploid wheats is also an important source of genetic diversity for those endosperm proteins (Lagudah and Halloran 1988a; Williams et al. 1993) that are associated with bread-making quality in wheat (Payne et al. 1987).

It is now well established that the HMW glutenin subunits are encoded at the *Glu-1* loci on the long arms of the group-1 chromosomes (1A, 1B and 1D) in hexaploid wheat (Payne et al. 1987; Shewry et al. 1989, 1992 for a

review). Each locus consists of two genes that are not always expressed in hexaploid wheats. One of these genes encodes a HMW glutenin subunit with slow electrophoretic mobility, named the x-type subunit, while the other encodes a HMW glutenin subunit with faster mobility, termed the y-type subunit (Shewry et al. 1992; Margiotta et al. 1993). However, in some accessions of the diploid species *T. tauschii*, Lagudah and Halloran (1988a) and Williams et al. (1993) have reported the presence of three HMW glutenin subunits (named 2^t, T1 and T2) encoded at the *Glu-D^{t}1* locus. The three subunits were inherited together and hence considered to belong to the same allele (Lagudah and Halloran 1988b). No other alleles at the *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) have been reported encoding three genes of HMW glutenin subunits in *Triticum* species. For this reason the genetic control of this protein is extremely interesting. According to Williams et al. (1993), when these three HMW glutenin subunits were present in *T. tauschii*, it was possible to observe one x-type subunit, i.e. 2.1^t, 1.5^t, 2^t or 3^t, along with two subunits, termed T1 and T2. Subunit T1, in SDS-PAGE, has a mobility similar to HMW glutenin subunit Dy-10 in hexaploid wheats and subunit T2 shows a faster mobility than HMW glutenin subunit Dy-12. They also assumed that T1 and T2 proteins always occur together. Nevertheless, Mackie et al. (1996) have reported recently that one of these presumptive HMW glutenin subunits, from *T. tauschii* T1, was not a true glutenin subunit, on the basis of its solubility in 70% ethanol. At a genetic level, only HMW glutenin genes have been located on the long arm of the group-1 chromosome. These contradictory results pose questions about the nature of this protein and its chromosomal location.

The aim of the present work was to better characterize the T1 protein present in *T. tauschii* accessions. The biochemical studies included electrophoretic analyses as well as analysis of its N-terminal amino-acid sequence. The genetic characterization was carried out by analysis of F_2 grains from a cross between the synthetic hexaploid wheat, L/18913, which contains the T1 protein, and the cv Egret of *T. aestivum*.

Materials and methods

Plant material

A set of *T. tauschii* accessions that had three proteins in the region of the HMW glutenin subunits (analyzed by SDS-PAGE of total proteins) was selected from a collection of 173 *T. tauschii* accessions (Gianibelli 1998; Gianibelli et al. 2001). A synthetic hexaploid L/18913 obtained from colchicine treatment of hybrids between *Triticum turgidum* var. *durum* Langdon and *T. tauschii* AUS18913 (with subunits 2^t+T1+T2) was used in inheritance studies of endosperm proteins. L/18913 was crossed with the bread wheat cv Egret, and 106 F_2 grains were analyzed to determine their gliadin and HMW glutenin subunit composition.

SDS-PAGE analyses

The HMW glutenin subunit composition was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the total proteins. One third of a grain (F_2 grains) was extracted with buffer Tris-HCl (pH 6.8) containing 2% SDS, 5% 2-mercaptoethanol and 30% glycerol. Proteins were separated using a discontinuous SDS-PAGE system with 10% acrylamide concentration for the separating gel (pH 8.8) and 3% acrylamide concentration for the stacking gel (pH 6.8). The electrode buffer of Tris-glycine at pH 8.3 was as described by Laemmli (1970). The gels were stained with Coomassie Brilliant Blue R-250 overnight. To overcome the difficulty of determining the HMW glutenin subunit composition in those F_2 grains, where the Dy-10 subunit appeared to co-migrate with the T1 protein, a modification of the SDS-PAGE procedure described above was carried out. The concentration of acrylamide and bis-acrylamide in the separating gels was increased to obtain a better separation of these two proteins. The optimized concentrations were 14% acrylamide and 0.6% bis-acrylamide.

A-PAGE analysis

Gliadins were extracted with 70% ethanol and were analyzed by A-PAGE (pH 3.1) according to Tkachuk and Mellish (1980).

Two-dimensional electrophoresis

The two-dimensional procedure involved two electrophoresis steps. The first dimension is a normal A-PAGE of gliadins (soluble in 70% ethanol) with SDS-PAGE in the second dimension. The first dimension was carried out according to Tkachuk and Mellish (1980) in a vertical slab (1.5-mm thick). The gels were equilibrated in a medium containing 2% SDS and loaded on top of a thick slab (2 mm) of 10% acrylamide and subjected to normal SDS-PAGE overnight (Payne et al. 1982). The gels were stained with Coomassie Brilliant Blue G-250 overnight following the method of Neuhoff et al. (1988).

N-terminal sequence

The spots with T1 protein from the SDS-PAGE slab gel were excised and the protein eluted at 37°C in 0.1% (w/v) SDS in 100 mM of sodium acetate, pH 8.8, by vortexing small pieces of gel with 400 µl of buffer overnight according to Kurth and Stofeel (1990). The protein was concentrated on a PVDF (polyvinylidene difluoride) membrane prior to N-terminal amino-acid sequence analysis, which was performed according to Walsh et al. (1995). Sequencing was carried out with an automated protein sequencer (ABI 470A, Applied Biosystems, Foster City, Calif.) equipped with an on-line RP-HPLC system for identification of the phenylthiohydantoin-amino acid derivatives. Sequence determination was carried out by both visual inspection of the chromatograms and by analysis of data using software version 1.61, Applied Biosystems.

Nomenclature

The gene symbols and the numbers for HMW glutenin subunits were designated following the system proposed by Lagudah and Halloran (1988a) and Williams et al. (1993). The superscript ‘t’ is used to differentiate the *T. tauschii* genome from that of *T. aestivum.*

Genetic Analysis

The location of genes controlling the proteins derived from the *T. tauschii* genome were made on the basis of their segregation relative to the known allelic variants in the bread wheat cv Egret. Gliadins and HMW glutenin subunits were extracted from one-third of each grain from a population of 106 F_2 grains of a cross between L/18913 and cv Egret. A map was constructed with the

computer program Mapmaker version 2.0 (Lander et al. 1987). Markers were placed with a LOD ($\log_{10}$ odds likelihood) threshold of 3.0 to develop the linkage maps. The percentages of recombination were calculated according to Allard (1956). Map distances (in cM) were estimated by Kosambi functions (Kosambi 1944). The goodness of fit of the segregation of each pair of alleles was tested with a χ^2 test.

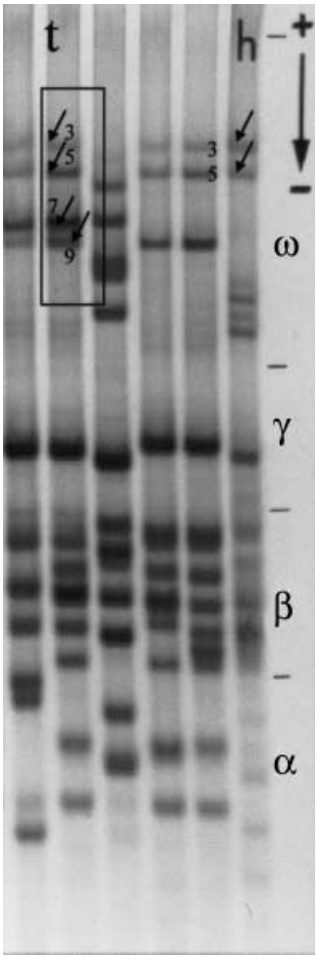

Fig. 1 A-PAGE separation of gliadins from wheat cv Chinese Spring (*h*) and accession AUS18913 (*t*) of *T. tauschii*. Small arrows indicate ω-gliadin components encoding at the *Gli-D1* locus of Chinese Spring (*h*) and ω-gliadin components of AUS18913 (t)

Results

Biochemical characterization of T1

The gliadin composition of the accessions with T1 protein was analyzed by A-PAGE, and a typical gel of gliadins in A-PAGE of several *T. tauschii* accessions is shown in Fig. 1. The gliadins of *T. tauschii* accession AUS18913 have been named compared with those corresponding to the hexaploid wheat cv Chinese Spring. The two slowest ω-gliadins from AUS18913 have the same electrophoretic mobility as those present in Chinese Spring. Additional ω-gliadins of fast mobility are also present in *T. tauschii* accession AUS18913. To identify the molecular weight of each ω-gliadin of the accession AUS18913, a two-dimensional analysis was carried out (Fig. 2). A-PAGE in the first dimension allowed the separation of gliadins by their different electrical charges, and SDS-PAGE in the second dimension separated the proteins by differences in their molecular weights. Analysis of the two-dimensional gel clearly identified a polypeptide with high-molecular-weight that corresponds to the third slowest in mobility of the ω-gliadins of A-PAGE, designated ω-7 (Fig. 1).

Analysis of the amino-acid sequences of the amino-terminal region of the endosperm proteins has been shown to be an excellent approach to further identify endosperm proteins on the basis of their structural features (Kasarda et al. 1983; Shewry et al. 1989; Lew et al. 1992). For that reason, N-terminal sequence analysis was also conducted on the T1 protein purified from spots separated by SDS-PAGE. The first 12 amino acids of its N-terminal sequence, obtained by the Edman degradation procedure, are shown in Table 1. The homology of the N-terminal sequence of T1 protein to other endosperm proteins has been studied using the database of the Australian National Genomic Information Service (ANGIS). The amino-acid sequence started with threonine and had homology with the first seven amino acids

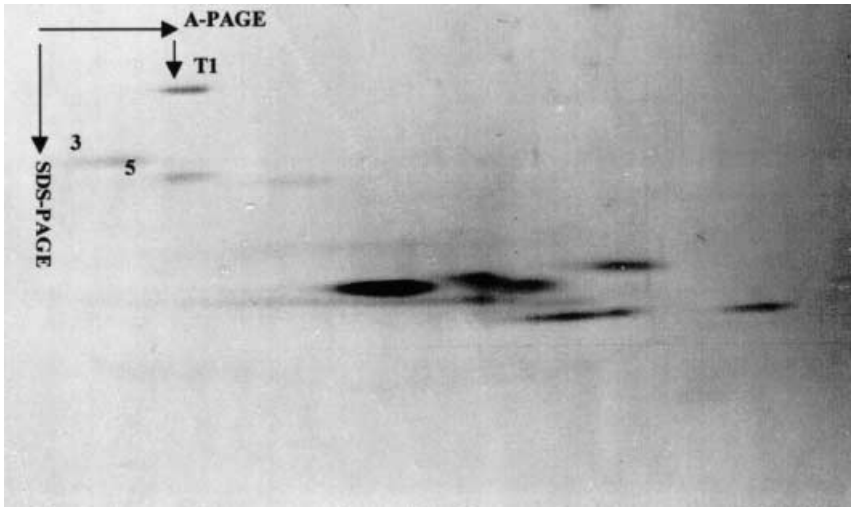

Fig. 2 Two-dimensional A-PAGE x SDS-PAGE separation of gliadins of accession AUS18913 of *T. tauschii*. The *small arrow* indicates ω-gliadin T1. *Numbers 3 and 5* indicate ω-gliadins encoded at the *Gli-D^t 1* locus

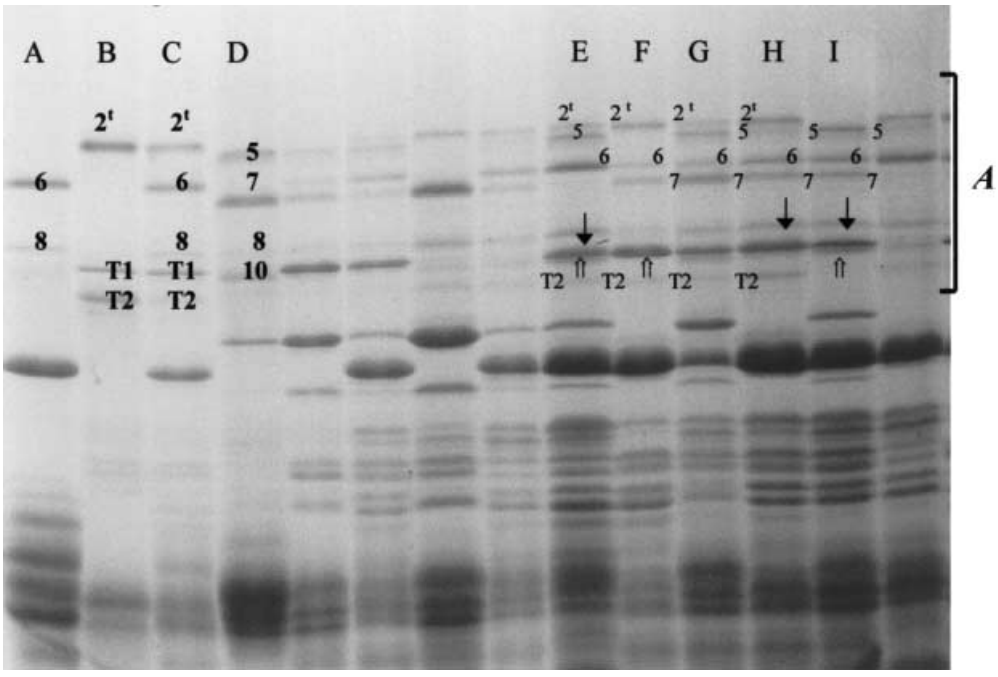

Fig. 3 SDS-PAGE separation of total proteins of F_2 progeny from the cross of the synthetic hexaploid wheat L/18913× cv Egret (*E–I*). *Arrows* indicate (→) HMW glutenin subunit 10 from cv Egret, and *double arrows* (⇑) indicate T1 protein from *T. tauschii*. Controls: tetraploid wheat cv Langdon (*A*), *T. tauschii* accession AUS18913 (*B*), hexaploid synthetic wheat L/18913 (*C*) and hexaploid wheat cv Egret (*D*). *A*=high-molecular-weight glutenin subunit region

Table 1 N-terminal amino-acid sequence of T1 protein and other related ω-gliadins (genome designation in brackets). Data from Tatham and Shewry (1995)

Species		N-terminal amino-acid sequence
T. speltoides (S)	*ω-2	ARQLNPSNKELQSPQQSFYQQQQQQ
T. monococcum (A)	*ω-2	ARQLNPSDQELQSPQQLYPQQPYQQPY
T. monococcum (A)	*ω-3	TRQLSPRGMELQTPQ
T. tauschii (D)	T1	TRQLSPRSKELG

Table 2 F_2 segregation data for progeny from cross L/18913× Egret deduced from electrophoretic analysis

Locus	Protein type		No. of progeny				χ^2 (1:2:1)	χ^2 (3:1)	Conclusion
	A	B	A–	–B	AB	–			
Glu-1D	5+10	2^t+T2	29	24	53	–	0.47 n.s.	–	Allelism
Gli-D1	ω–1.5	–	71	–	–	35	–	3.63 n.s.	Allelism
Gli-DT1	–	T1	75	–	–	31	–	1.01 n.s.	Allelism

n.s.=not significant

from the ω-gliadin sequence published by Tatham and Shewry (1995) for *Triticum monococcum* (TRQLSPR) (Table 1).

Genetics of storage proteins

The parents of the cross, the synthetic hexaploid wheat, L/18913, and the wheat cv Egret, differ from each other with respect to allelic composition at the *Glu-D1*, *Gli-D1* loci and in the presence/absence of the ω-gliadin T1. Typical total protein patterns obtained from F_2 grains are shown in Fig. 3. Four different samples were also included in each gel as controls: *T tauschii* AUS18913, *T. turgidum* var. *durum* Langdon, *T. aestivum* cv Egret and the synthetic hexaploid wheat L/18913. The segregation of the HMW glutenin subunits 5+10 and 2^t+T2 as well as ω-gliadin T1 can be followed unambiguously. The HMW glutenin subunits 5+10 from Egret and 2^t+T2 from *T. tauschii* along with their heterozygous composition were inherited according to the expected proportion of 1:2:1. This observation is consistent with these subunits segregating as allelic variants of the *Glu-D1/Glu-D^{t}1* locus (χ^2=0.47) (Table 2). The gliadins of both parents L/18913 and Egret are shown in Fig. 4. The alleles at the *Gli-D1* locus of both parents are very similar. Thus the *Gli-D^{t}1* allele from *T. tauschii* present in L/18913 is similar to the allele *a* identified in the hexaploid wheat Chinese Spring (Metakovsky 1991), with two major ω-gliadins of slow mobility named ω-3 and ω-5. The allele *b* of the *Gli-D1* locus of Egret (Metakovsky 1991) basically differed from the one in the

Table 3 Recombination percentage and map distance between the loci studied on chromosome 1D/1D^t

Loci	Linkage		Conclusion	Recombination (%)	Map distance (cM)
	χ^2 (9:3:3:1)	χ^2 (6:3:3:2:1:1)			
Glu-D1 Gli-D1	–	4.41 n.s.	No linkage	38.0	53.38
Glu-D1 Gli-DT1	–	11.51*	Linkage	33.3	40.20
Gli-D1 Gli-DT1	25.79***	–	Linkage	12.9	13.18

cM=centiMorgan

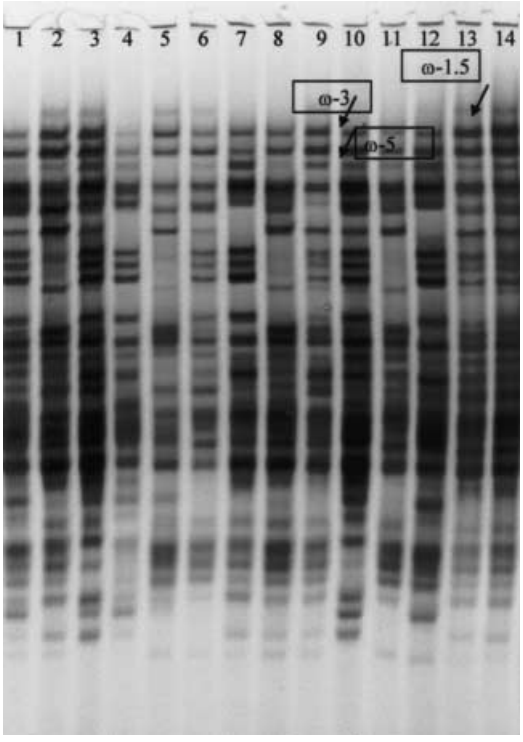

Fig. 4 A-PAGE separation of gliadins of F_2 progeny from the cross of the synthetic hexaploid wheat L/18913× cv Egret (*lanes 1 to 14*). *Arrows* indicate ω-gliadin components encoded at the *Gli-D1* locus

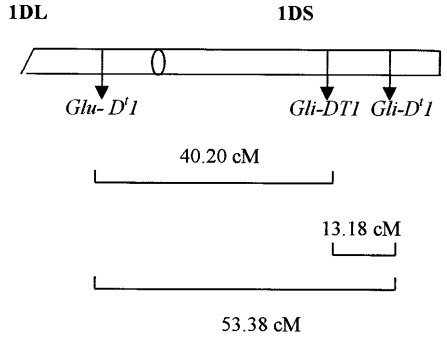

Fig. 5 Linkage map of endosperm protein loci (*Glu-D^{t}1* and *Gli-D^{t}1*) and the *Gli-DT1* locus on chromosome 1D of *T. tauschii*

synthetic wheat due to the presence of an extra ω-gliadin, named in this study ω-1.5 (Fig. 4). Therefore, the variants of the *Gli-D1* locus in F_2 grains were unambiguously scored as the presence or absence of ω-1.5 gliadin. Some gliadin analyses of segregating material are also shown in Fig. 4. Segregation data for the presence or absence of ω-gliadin 1.5 agreed with the expected ratio 3:1 (χ^2=3.63). Although A-PAGE was used to identify *Gli-D1* alleles, the identification of ω-gliadin T1 in the F_2 was extremely difficult due to the fact that other co-migrating bands occurred in the region where ω-gliadin T1 is present. Hence, the identification of ω-gliadin T1 was performed by SDS-PAGE analysis. The segregation data for the presence and absence of ω-gliadin T1 from *T. tauschii* followed the expected 3:1 ratio (χ^2=1.01). The locus for the ω-gliadin T1, designated *Gli-DT1*, segregated independently of the *Gli-D1/D^{t}1* locus. However, *Gli-DT1* was linked to the *Gli-D1/ D^{t}1* locus with a recombination estimate of 12.9% (13.18 cM, Table 3). Recombination between *Gli-DT1* and *Glu-D1* was estimated at 33.3%. The *Glu-D1* locus was not significantly linked to *Gli-D1*, showing a percentage of recombination of 38% (see Table 3). Therefore the new *Gli-DT1* locus has been located on the short arm of chromosome 1D, between the *Glu-D1* locus on the long arm of chromosome 1D and the *Gli-D1* locus on the short arm of the same chromosome (Fig. 5).

Discussion

Among gliadins, the ω-gliadins encoded at the *Gli-B1* locus, in both *T. aestivum* and *T. turgidum* var. *durum*, have the highest molecular weight (Kasarda et al. 1983; Tatham and Shewry 1995), and no gliadins have been reported previously with molecular weights similar to those of HMW glutenin subunits. The molecular weight of T1 protein is very similar to subunit Dy-10 which has been estimated to be between 91600 to 95000 Da by SDS-PAGE (according to results published by Ng and Bushuk 1988 and Bunce et al. 1985, respectively). In contrast, the largest ω-gliadins reported in hexaploid wheats have been estimated to be around 75000 Da (Kasarda et al. 1983). Although, over-estimation of the molecular weight of wheat endosperm proteins by SDS-PAGE has been reported, comparisons of the relative differences in sizes between both types of proteins are reasonably valid. This wide difference in terms of molecular weight also represents a wide difference in terms of the number of amino acids, with about 180 to 200 amino-acid residues more for ω-gliadin T1 than for the com-

mon ω-gliadin (Kasarda et al. 1983; Tatham and Shewry 1995). The unique features of the ω-gliadin T1 to functional properties and its potential to modify wheat quality is a subject of further investigation.

Additional data based on N-terminal sequencing presented in this paper confirmed that the T1 protein was an ω-gliadin. The first seven amino acids of the protein had 100% homology with an ω-gliadin reported by Tatham and Shewry (1995) present in *T. monococcum*. The non-repetitive N-terminal sequence of ω-gliadins and their homoeologous proteins (C-hordeins and ω-secalins present in barley and rye) consists of a small region of only 12 amino acids whereas the central repetitive domain represents around 90% of their structure (Tatham and Shewry 1995). On the basis of N-terminal amino-acid sequences, three different types of ω-gliadins can be found in hexaploid wheats and *Triticum* species. These types are the "SRL-type" sequence, typical of the largest ω-gliadins encoded by chromosome 1B, and the "KEL-type" that is found in the species *T. tauschii*, *T. durum* and *T. aestivum*, corresponding to ω-gliadins encoded on the 1B and 1D chromosomes (Kasarda et al. 1983). The third sequence type corresponds to the "ARQ-type", which is widely distributed in species of *Triticum* and *Aegilops*, as well as barley and rye. The sequence of ω-gliadin T1 represents a modification of the latter sequence with T (threonine) at the beginning of the N-terminal region. Although less frequent than ARQ-, the TRQ- sequence has been previously reported by Tatham and Shewry (1995) in *T. monococcum*.

According to Lagudah and Halloran (1988a), the majority of gliadins in *T. tauschii* are encoded by two loci, *Gli-D^{t}1* and *Gli-D^{t}2*, the former coding for ω- and γ-gliadins and the latter for α-gliadins and β-gliadins. In those accessions with the T1 protein, four gliadin bands were present in the area of ω-gliadins; two with lower electrophoretic mobility named ω-3 and ω-5, possessed identical mobility to those in the hexaploid wheat cv Chinese Spring (allele *a* at the *Gli-D1*) and two with faster mobility, termed ω-7 and ω-9 gliadins. However, ω-gliadins encoded at the *Gli-D1* locus with similar electrophoretic mobility to those of the faster mobility have not been reported in *T. aestivum* (Metakovsky 1991). The ω-gliadin, named ω-7, has been shown in this study to be the T1 protein encoded by the new locus designated as *Gli-DT1*. There is no evidence about the location of the gene encoding gliadin ω-9, and it remains to be shown whether it is part of the new *Gli-DT1* locus. Four seed-storage protein loci have been mapped previously on chromosome 1D of *T. tauschii* (Lagudah et al. 1991) and *T. aestivum* (Van Deynze et al. 1995; Dubcovsky et al. 1997), one on the long arm of chromosome 1D encoding HMW glutenin genes (*Glu-D1/GluD^{t}1*) and three on the short arm of the same chromosome. These correspond to low-molecular-weight glutenin genes (*Glu-D3/GluD^{t}3*), ω- and γ-gliadin genes (*Gli-D1/Gli-D^{t}1*) and triticin genes (*Tri-1D/Tri-1D^t*). In this study, we have mapped an additional locus on the short arm of chromosome 1D. Analysis of progenies from the cross between the synthetic hexaploid wheat L/18913 and the wheat cv Egret has allowed determination of the chromosomal location of the new locus named *Gli-DT1* on the short arm of chromosome 1D. As expected, subunits 5+10 from *T. aestivum* and 2^t+T2 from *T. tauschii* were confirmed as allelic variants at the *Glu-D1/Glu-D^{t}1* locus. These results also corroborate the previous result reported by Lagudah and Halloran (1988b, 1989) about the spatial relationship between the *Glu-D^{t}1* and *Gli-D^{t}1* loci in *T. tauschii* and their corresponding loci in *T. aestivum*. The genes at the *Glu-D1* locus segregated independently of those of the *Gli-D1* (χ^2=4.41). The recombination values of 38% between both loci on chromosome 1D from the cross studied indicated that the *Glu-D1/Glu-D^{t}1* and *Gli-D1/Gli-D^{t}1* loci are widely separated. The recombination percentage was similar to the 42.7% obtained by Lagudah and Halloran (1988b) in *T. tauschii* and 44.4% reported by Jones et al. (1990) in *T. aestivum*. Contrary to the results reported by Lagudah and Halloran (1988b), the T1 protein is not inherited in a block with subunits 2^t and T2, which are typical HMW glutenin subunits in terms of solubility and electrophoretic mobility. This inconsistency could be the result of lack of pairing in the cross analyzed by Lagudah and Halloran (1988b), where probably the whole 1D chromosome was inherited without segregation of its traits. Thus the proteins encoded by the 1D^t chromosome of *T. tauschii* in their study were presumably inherited together, without segregation of subunits 2^t and T2 at the *Glu-D1* locus and ω-gliadin T1 at the *Gli-DT1* locus.

Payne et al. (1982) and Lagudah and Halloran (1988b) have previously reported that the *Gli-D^{t}1/Gli-D1* loci occur towards the end of the short arm of chromosome 1D in both *T. tauschii* and *T. aestivum*. The results in the present study showed a recombination of 12.9% between the new *Gli-DT1* locus and *Gli-D^{t}1*; therefore it was assumed that the locus controlling the synthesis of this protein is also situated on the short arm of chromosome 1D between the *Glu-D^{t}1* and *Gli-D^{t}1* loci. In homoeologous group 1, the major components of ω- and γ-gliadins are encoded at the *Gli-1* loci at the distal ends of the maps of the 1AS, 1BS and 1DS chromosome arms. Several loci controlling ω-gliadins have been described on the short arms of chromosomes 1A and 1B, between the *Gli-1* and *Tri-1* loci (Galili and Feldman 1984; Sobko 1984; Jackson et al. 1985; Dachkevitch et al. 1993; Ruiz and Carrillo 1993). These ω-gliadins are encoded at the *Gli-3* and *Gli-4* loci located between *Gli-1* and the centromere. Although examples of ω-gliadins encoded by these loci have been reported on chromosomes 1A and 1B, no examples of the *Gli-3* locus have been reported on the short arm of chromosome 1D in *T. aestivum* or *T. tauschii*. The *Gli-DT1* locus appears to differ from the *Gli-3* loci in terms of the recombination distance to the *Gli-D1* locus. Thus, the 12.9% recombination distance between *Gli-DT1* and *Gli-D1* is smaller than the 28.1 cM calculated by Galili and Feldman (1984) between *Gli-B1* and *Gli-B3*, or the 22.1 cM obtained by Dachkevitch et al. (1993) for the same

loci. Although recombination rates vary in different crosses, it appears that the *Gli-DT1* locus may be more related to the locus *Gli-4*. The *Gli-A4* locus (Redaelli et al. 1992) has been mapped at 10 cM from the *Gli-A1* locus on chromosome 1 A or at a recombination distance of 5% from *Gli-A1* according to Metakovsky et al. (1986). Recently, Rodriguez-Quijano and Carrillo (1996) reported a new locus, *Gli-D4*, located on the short arm of the 1D chromosome of *T. aestivum*, controlling one γ-gliadin gene, with a recombination of 9.9% with the *Gli-D1* locus. Recombination between *Gli-DT1* and the *Gli-D^{t}1*, estimated at 12.9%, is similar to those mentioned above for *Gli-A4* and *Gli-D4*, and are also comparable to those observed in rye between the *Gli-R1* and a gliadin locus (R=12.04%) reported by Carrillo et al. (1992). It is also similar to the value in *Aegilops longissima* between *Gli-S^{l}4* and *Gli-S^{l}1* reported by Rodriguez-Quijano et al. (1996). Therefore, all these loci may represent part of a series of *Gli-4* homoeologous genes.

Conclusion

Protein T1, previously considered as a HMW glutenin subunit, has now been characterized as an ω-gliadin with an unusually high-molecular-weight. The ω-gliadin T1 is encoded by a new locus located proximally to the *Gli-D1* locus on the short arm of chromosome *1D*. This locus may be considered part of a series of homoeologous genes, *Gli-4*, and present in *Triticum* and *Aegilops* species.

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