Theor Appl Genet (1988) 75:592–598

Phylogenetic relationships of *Triticum tauschii* the D genome donor to hexaploid wheat

1. Variation in HMW subunits of glutenin and gliadins

E. S. Lagudah * **and G. M. Halloran**

School of Agriculture and Forestry, The University of Melbourne, Parkville, Victoria 3052, Australia

Received September 10, 1987; Accepted October 5, 1987
Communicated by F. Salamini

Summary. High-molecular-weight (HMW) subunits of glutenin (*Glu-1*) and gliadins (*Gli-1* and *Gli-2*) were used in assessing phylogenetic relationships between *T. tauschii* and the D genome of hexaploid wheat. The degree of polymorphism in *T. tauschii* (D^t) for these characters occurred in the order: *Glu-*D^t1 < *Gli-*D^t2 < *Gli-*D^t1; although polymorphism for these traits was quite high, only a limited number of variant forms identified with the *Glu-*D^t1 and *Gli-*D^t1 loci were closely matched to their analogous variants in the D genome of hexaploid wheat. Two-dimensional (IEF× SDS-PAGE) analysis revealed differences between one of the prevalent allelic forms – *Glu-D1d* – of hexaploid wheat and its corresponding variant in the putative diploid donor, as evident by a relatively more acidic subunit 10 in the latter compared to the former.

Key words: D genome – *T. tauschii* – HMW glutenin – Gliadin blocks

Introduction

The putative diploid donor of the D genome of hexaploid wheat is generally accepted to be *Triticum tauschii (Aegilops squarrosa)* (Kihara 1944; McFadden and Sears 1946; Riley 1965). However it is known to occur in a number of different morphological forms (Eig 1929; Kihara et al. 1965; Zohary et al. 1969). Eig (1929) recognised two subspecies of *T. tauschii,* ssp. *eusquarrosa* and ssp. *strangulata.* Varietal classes in the former subspecies are var. *typica, meyeri* and *anathera,* while var. *strangulata* is the only member of the latter subspecies.

The wide ecological amplitude and plant morphological diversity of *T. tauschii* and the occurrence of the D genome in bread wheat, as well as in other hexaploid wheats, e.g. *spelta, vavilovii, macha, compactum* and *sphaerococcum* complicates studies aimed at nominating the precise source of the putative diploid donor of the D genome and determining its phylogenetic relationship within wheat using plant morphological characters and/or genetic and cytogenetic attributes based on a few accessions of the species. There are reports of variation amongst *T. tauschii* accessions for isozymes of esterase (Nakai 1979), α-amylase (Nishikawa et al. 1980) and high-molecular-weight (HMW) subunits of glutenin (Lawrence and Shepherd 1980) from whence homology between certain *T. tauschii* strains and the D genome of hexaploid wheat has been established. Despite the conservative genetic status of these markers, a shortcoming in these studies is that each is based on only one biochemical marker. A more accurate interpretation of relative evolutionary affinity between accessions of *T. tauschii* and the D genome of hexaploid wheat are likely to be derived from combined studies of a number of different biochemical markers. From the isozyme studies conducted separately on a varying number of *T. tauschii* accessions by Jaaska (1980, 1981, 1984), the subspecies *strangulata* was found to be the most likely form to have donated the D genome of hexaploid wheat.

We have conducted a series of studies on variation in, and the genetics of, three biochemical markers – HMW glutenin subunits, gliadins and seed esterases in 79 accessions of *T. tauschii,* and compared them with their analogous markers *Glu-D1, Gli-D1* and *Est-D5* in hexaploid wheat species, landraces and cultivated bread wheat. In this approach, using three, rather than one marker, it was considered that the reliability would be enhanced of nominating the particular form of *T. tauschii* with the greatest likelihood of having contributed the D genome to hexaploid wheat. This study reports on the variation in size and charge of HMW glutenin subunits

* Present address: IBPGR, c/o University of Georgia, Coastal Plain Expt. Stn., Tifton, GA 31793, USA

(*Glu-1*) and gliadin (*Gli-1* and *Gli-2*) composition of *T. tauschii.*

Materials and methods

Plant material

Of the 79 *T. tauschii* accessions (41-*typica;* 13-*meyeri;* 16-*strangulata* and 9-intermediate forms) examined, 40 (originating from Turkey, USSR, Iran, Afghanistan, Pakistan) were obtained from the Australian Winter Cereals Collection (AWCC), Tamworth, 21 from The University of Melbourne Plant Collecting Expedition (MUPC) to Asia Minor in 1975 and 7 accessions which were part of a collection made by a British Expedition from the University of Reading Exploration Society (URES) to Afghanistan in 1965 (Halloran 1968). The others were obtained from the Plant Breeding Institute, The University of Sydney and the Laboratory of Genetics, Kyoto University, Japan. Thirty hexaploid wheat species (from the AWCC) used in this study consisted of *T. spelta* (4), *T. macha* (6), *T. vavilovii* (3), *T. compactum* (6) and *T. sphaerococcum* (11). Bread wheat cultivars also examined were 'Chinese Spring', 'Chinese Spring' (Hope chromosome 1D) substitution line and 'Bezostaya 1'.

SDS-PAGE

The composition of HMW glutenin subunits was determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) using 10% acrylamide and 0.13% methylene bis acrylamide according to the methods of Lawrence and Shepherd (1980). A 7.5–15% acrylamide gradient separating gel was also used in conjunction with the uniform (10%) system and their gel solutions were cast in a vertical slab system (LKB 2001/Biorad Protean). Gels were stained in a solution of 0.1% Coomassie Blue R250, 50% methanol, 7% acetic acid for at least 1 h and destained in 20% methanol, 7% acetic acid and 3% glycerol.

Two-dimensional (1EF× SDS-PAGE) electrophoresis

The extraction and isoelectric focusing (IEF) of endosperm proteins in the first dimension (rod gels 2 mm thick) on single grains employed the system of Holt et al. (1981). The ampholytes used in the first dimension consisted of 0.5 and 1.5% ampholine (LKB), pH range 3.5–9.5 and 6.0–8.0, respectively. SDS-PAGE (second dimension) was stopped 1 h after the bromophenol blue layer had run off the plate. Gels were stained overnight and destained as previously described.

Acid polyacrylamide gel electrophoresis (APAGE)

Protein extraction and separation was carried out in 4–15% polyacrylamide gradient gel adapted from the method of du Cros et al. (1983) on to the same electrophoretic apparatus used for the SDS-PAGE. Electrode polarities were reversed and the gels were equilibrated prior to sample loading at a constant voltage of 350 V for 1 h at 20 °C. Subsequently, electrophoresis was maintained at 350 V for five and a half hours to achieve a clear separation of the non-aggregated gliadins (*Gli-1* and *Gli-2* gene loci). The wheat cultivars 'Chinese Spring' and 'Bezostaya 1' were used as standards in all electrophoretic runs.

Nomenclature

Classification of the HMW glutenin subunits (*Glu-1*) is based on the catalogue of alleles described by Payne and Lawrence (1983). With the exception of numerical designations of subunits of the *Glu-D1a* (2 and 12) and *Glu-D1d* (5 and 10) alleles, some of the numerical designations of *T. tauschii* subunits are not the same as those used by Payne and Lawrence (1983). Throughout the study, the superscript -*t*- has been used in distinguishing gene symbols of the D genome of *T. tauschii* from its analogous gene loci in the D genome of hexaploid wheat.

Results and discussion

HMW glutenin subunit composition

1 One-dimensional SDS-PAGE analysis. Fourteen types of HMW subunit combinations (*Glu-D^{t}1)* were observed in the *T. tauschii* accessions, which were generally characterised by a pair of slower and faster mobility subunits (Fig. 1). The group of subunits of lower mobility have been numbered from 2.1 to 5, and the faster mobility group from 10.3 to T_2 in order of increasing mobility. Difficulties were encountered in assigning the HMW subunit 10 belonging to the subunit combinations 2, 10 and 3, 10. This was apparent in a number of electrophoretic runs on either 7.5–10.5% gradient or 10% uniform gels in which subunit 10 appeared to possess a mobility intermediate between subunits 10 and 12.

Within the mobility zones (in SDS-PAGE) which normally contain subunits of the *Glu-D1* locus of bread wheat, the subunit pair 2 and 12 was found in all the hexaploid wheat species, while the allelic subunit pair 5 and 10 occurred in a single accession of *T. macha* and in two accessions of *T. sphaerococcum.* Two other pairs of HMW subunits which possessed the subunit 2.1 (identical in mobility to 2.1 of the *Glu-D^{t}1* locus) in common as the lower mobility band were identified. The HMW subunit combination 2.1 and 10 was observed in both *T. compactum* and *T. macha,* while biotypes characterised by the presence of either the subunit pairs 2.1, 13 or 2, 12 (Fig. 2) were found in an accession of *T. macha* (AUS 14519) at a frequency of 56 and 44% respectively from the screening of 180 seeds of this accession. The F_2 segregation pattern of a cross between 'Chinese Spring' (possessing subunit 2, 12-*Glu-Dla*) and the *T. macha* biotype possessing subunits 2.1 and 13 confirmed the latter subunit pair as an allelic variant of the *Glu-D1* locus (Lagudah and Halloran unpublished results). The absence of subunits usually controlled by the *Glu-D1* locus were observed in two *T. vavilovii* accessions; the somatic chromosome number was found to be 40 in one of the accessions (AUS 10960).

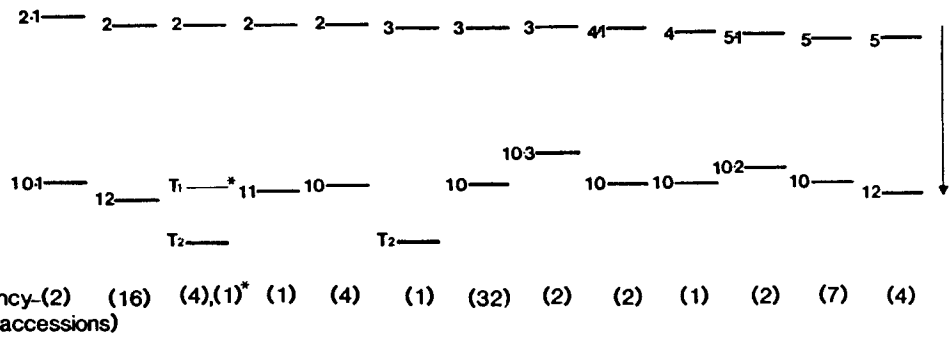

Fig. 1. Diagrammatic representation of all the HMW glutenin subunit types (*Glu-D¹1*) observed in the 79 accessions of *T. tauschii*. The arrow indicates the direction of increasing subunit mobility in SDS-PAGE. *: One accession, AUS 18993, lacked the minor subunits T_1, bringing the total variation to 14

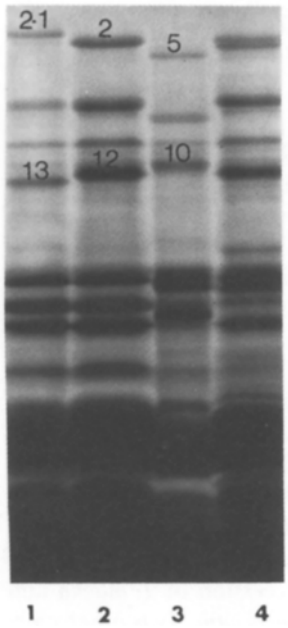

Fig. 2. SDS-PAGE of endosperm proteins from *T. macha* showing the newly-identified HMW subunits 2.1 and 13 and the common *Glu-D1* variants – 2, 12; 5, 10. *Lanes* (*1*) and (*2*) – *T. macha* (AUS 14519) biotypes possessing subunits 2.1, 13, and 2, 12 respectively, (*3*) WJR 38548, (*4*) AUS 17776

The variation in the *Glu-D¹1* subunits reported in this study encompass the range of analogous subunits of the *Glu-D1* locus in the hexaploid wheat species and those from a wide range of cultivated bread wheats and landraces compiled by Payne and Lawrence (1983) and Payne et al. (1984) but it did not possess types with the largest subunit 2.2. Although some of the specific HMW subunit combination pairs of hexaploid wheat could not be unequivocally identified, subunits corresponding to, or closely related to, either of the lower or faster mobility subunits occurred in *T. tauschii*. The rare occurrences of single HMW subunits at the *Glu-D1* locus in hexaploids are not a feature of the pair of subunits commonly found within the variation of *T. tauschii*. In those variants of *T. tauschii* which appeared to possess single subunits, they invariably possessed subunits just outside the recognised HMW range as found in the combination 2, T_2 and 3, T_2 (Fig. 1). Furthermore, most of the accessions with the combination 2, T_2 possessed a minor band, T_1, located in the subunit 10 region (Fig. 1). When several seeds of this latter group of accessions were analysed, a consistent occurrence of a triplet subunit combination – 2, T_1, T_2 occurred in every seed.

In 27 accessions which had details of their precise geographical locations (MUPC and URES sources) the north-eastern region of Iran was observed to have the most diversified range of the *Glu-D¹1* subunits. The HMW glutenins subunits 3 and 10 were common to regions in Afghanistan and Iran while the subunits 2 and 12 were found only in areas around Gorgan and to the west of Bojnurd in Iran.

2 Two-dimensional electrophoresis. Two-dimensional fractionation, combining isoelectrofocusing (IEF) and SDS-PAGE in the first and second dimension respectively, revealed that the group of lower mobility subunits 2.1, 2, 3, 5.1 and 5 were acidic in contrast to the near neutral and/or relatively basic isoelectric points of subunits 10, 10.1, 10.2, 10.3, 12 and T_2. The relative acidic to basic components within the subunit pairs 5, 10 and 2, 12 (Fig. 3) from at least two different *T. tauschii* sources, appeared to be quite similar. However, subunit 2 from the 2, 12 genotypic source was observed to be much more acidic than its counterpart from the 2, T_2 HMW subunit combination (not shown). Comparison of the HMW subunit pairs 2, 12 and 5, 10 from *T. tauschii* were made with their corresponding subunits from the D genome of 'Chinese Spring' and the 'Chinese Spring'/'Hope' chromosome 1D substitution line, respectively (Fig. 3). Similarities were observed in acidic to basic properties of the subunits 2 and 12 respectively from both the putative diploid donor and the D genome (chromosome 1D) of 'Chinese Spring'. In contrast, subunit 10 of the 'Chinese Spring' ('Hope' 1D) substitution line was comparatively more basic than the *T. tauschii* source (Fig. 3). In addition no apparent variability was observed with subunit 10 from four *T. tauschii* sources of the subunit combination 5 and 10, derived from the varietal classes *typica* (AUS

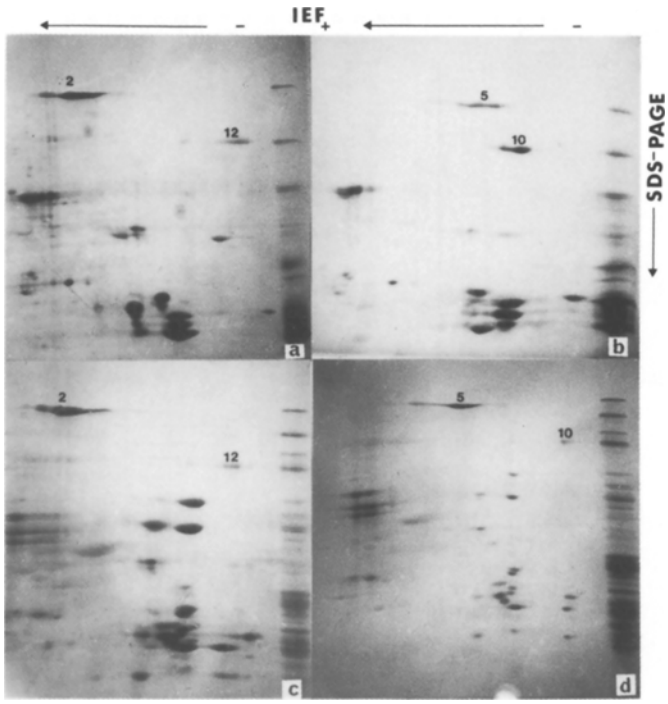

Fig. 3. Two-dimensional (IEF × SDS-PAGE) fractionation of endosperm proteins from *T. tauschii* (*a, b*) and hexaploid wheat (*c, d*). Numbered spots represent their HMW subunits of glutenin. **a** AUS 18898 var. *strangulata; b* AUS 18986 var *strangulata; c* 'Chinese Spring', *d* 'Chinese Spring' ('Hope' 1D) substitution

18990), *meyeri* (AUS 18911) and *strangulata* (AUS 18986, RL 5271).

The faster and lower mobility pair of subunits in *T. tauschii* corresponds closely in position with the subfamily classification of 1Dx and 1Dy subunits in bread wheats, described by Payne et al. (1981). Two-dimensional fractionation (IEF × SDS-PAGE) of these subunits in *T. tauschii* further substantiated the close correspondence of one with 1Dx and the other with 1Dy in their relative acidic to basic components respectively, an observation borne out by the relative isoelectric points for use in subfamily classification by Holt et al. (1981). In spite of the identical electrophoretic mobility of the subunit pair 5 and 10 from different varietal sources of *T. tauschii* and the 'Chinese Spring' ('Hope' 1D) substitution line, a relatively more basic isoelectric point characterised the corresponding 1Dy subunit (subunit 10) in the latter source. Although 'Chinese Spring' ('Hope' 1D) was the only standard source of the subunit pair 5, 10 used in this study, the HMW subunit 10 from different bread wheat sources have been shown to possess similar isoelectric points (Holt et al. 1981; Anderson et al. 1985).

Reasons for the prevalence of subunit pairs, particularly the apparent negative correlation in mobility between the x and y subunit subfamilies in *T. tauschii* (e.g. x, y: 2, 12; 2, 11; 3, 10; 4, 10; 5, 10), have been discussed by Lawrence and Shepherd (1980). Deviations from this general pattern associated with subunit pairs such as 5, 12 and 2.1, 10.1 raises questions on the origin of this latter pattern of subunits. Rare recombination between members of the former pattern of subunits may have given rise to the subunit combination 5 and 12. While *T. tauschii* is a self-pollinating species, it is possible, however, that occasional cross pollination could have taken place in the species. Nevertheless, a rare recombinant source accounting for 5.5% with this subunit combination from the *T. tauschii* accessions examined in this study appears unlikely (the subunit combination 5, 12, which has not been reported in bread wheat, occurred in three of the *meyeri* varietal classes and one intermediate *meyeri-typica* accession). Studies on the inheritance, structure and genetic organisation of the x and y subunits of chromosome 1D have revealed that their encoding genes are related in their DNA sequences with low copy number estimates and are closely linked, although they possess different peptide digest patterns and control different mRNA sizes and DNA restriction fragments (Lawrence and Shepherd 1981; Payne et al. 1981, 1983; Thompson et al. 1983; Galili and Feldman 1985; Harberd et al. 1986). If the x and y subunits are regarded as linked non-allelic genes then the likelihood of an independent origin of the subunit combination 5 and 12 in *T. tauschii* is possible.

Gliadin composition

1 Gli-D^t1. The monomeric gliadins obtained from APAGE have been grouped into blocks and the genetic basis for this classification will be reported in a following paper in this series. Gliadin blocks belonging to the complex *Gli-1* loci are usually found in the ω- and γ-zones, using 'Chinese Spring' and 'Bezostaya-1' as standards (Fig. 4). Seventy two gliadin blocks were identified from the 79 accessions (Fig. 5). In contrast to most of the *Glu-D^t1* variants, each accession could be clearly identified with a specific gliadin block at the *Gli-D^t1* locus, with the exception of blocks 7, 33, 39, 41, 44, 57 and 63. Individual gliadin components were initially ranked as either 1 or 2 or 3 on the basis of their staining intensities – light, medium and dark, respectively. However, in compiling the catalogue of *Gli-D^t1* blocks, their diagrammatic representation (Fig. 5) was simplified, as a number of very faint bands, and those, particularly in the ω-region, were excluded. In addition, only the intensely-staining bands (3) within the γ-zone have been differentiated (darker band, Fig. 5), while the lighter and medium-staining bands (1 and 2) within the γ-zone are represented equally (lighter band, Fig. 5).

Gliadin blocks 11 to 72 lacked ω-bands in the mobility range similar to, or lower than, the slowest moving 'Chinese Spring' ω-band. Protein bands 3 and 7 from the ω-region of blocks 7, 8 and 9 have mobilities equivalent with the pair of slow moving ω-components of 'Chinese Spring' (Figs. 4 and 5). Protein bands with

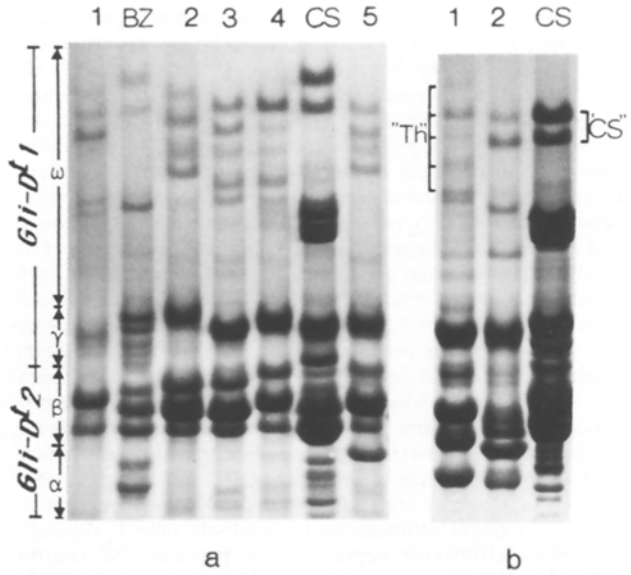

Fig. 4a, b. Gliadin electrophoretic patterns in a number of *T. tauschii* accessions and two bread wheat cultivars (BZ: 'Bezostaya 1'; CS: 'Chinese Spring'). (**a**) *T. tauschii* accessions – *1*) D7, *2*) D8, *3*) D9, *4*) 169-1412-33, *5*) 173-1435. (**b**) *T. tauschii* accessions – *1*) RL 5271, *2*) AUS 18985; 'Th' and 'CS' represent the 'Thatcher' and 'Chinese Spring' group of ω-gliadins

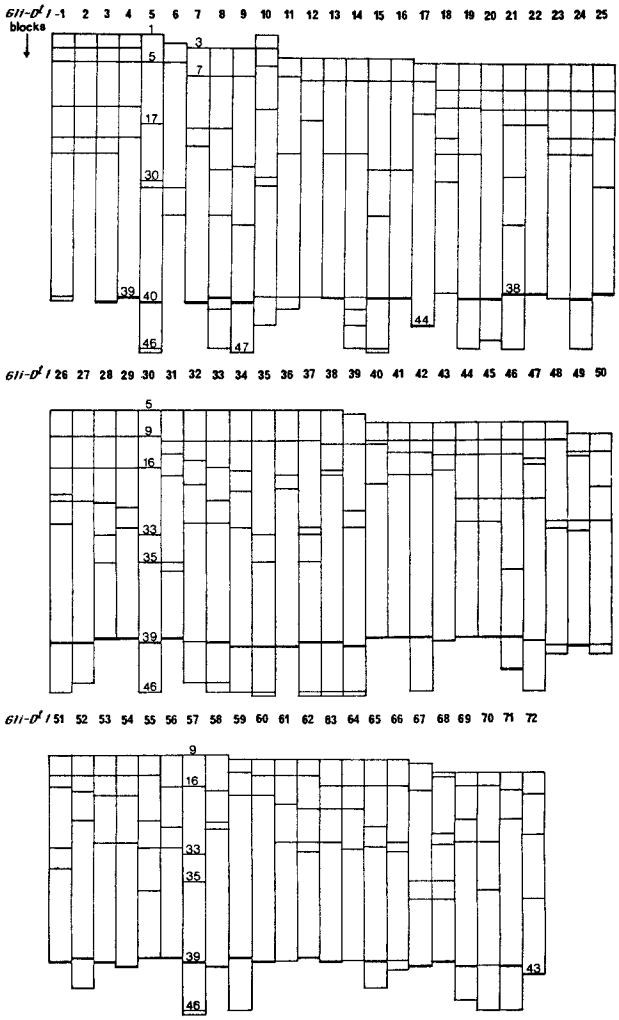

Fig. 5. Diagramatic representation of blocks (1-72) of gliadin components (*Gli-D¹1*) in *T. tauschii.* Numbers within blocks represent gliadin band (component) designations

the lowest mobility were observed in gliadin blocks 1 to 5 and 10; with the exception of block 5 the other five blocks were possessed by var. *strangulata* representatives. In addition, blocks 1 to 4 possessed five ω-gliadin bands in common. This group of five ω-gliadin bands as well as the pair of low-mobility ω-components, associated with blocks 7, 8 and 9, were similar to those of cultivars 'Thatcher' ('Th') and 'Chinese Spring' ('CS') groupings of ω-gliadins, proposed by Wrigley and Shepherd (1974) to be used as the basis for initial identification of gliadin electrophoregrams of hexaploid wheat.

Most of the hexaploid species used in this study possessed the 'CS' group of bands in their ω-components in the *Gli-D1* region, while one *T. compactum* accession carried the 'Th' group of bands. However, the gliadin components from a *T. macha* accession (AUS 14273) occurred in the mobility region below the faster-moving component of the pair of 'CS' group of bands. The absence of the lower mobility member from the pair of 'CS' ω-group of bands was observed in *T. vavilovii,* AUS 10961.

2 Gli-D¹2. The *Gli-D¹2* blocks constituted the range of gliadin components that occurred within the β and α-zones (Figs. 4 and 6). Fifty seven blocks were identified, consisting of 31 components. Most of the components of the *Gli-D¹2* blocks were of higher staining intensity than the *Gli-D¹1* blocks. In contrast to the variation at *Gli-D¹1,* where a maximum of two *T. tauschii* accessions possessed the same components, gliadin block 54 of the *Gli-D¹2* (Fig. 6) group was associated with eleven accessions. In addition, two other gliadin blocks of *Gli-D¹2* (6 and 57) (Fig. 6) were present in more than two accessions.

There was a slight overlap between the *Gli-D¹1* and *Gli-D¹2* groups, e.g. in the region bodering the γ- and β-zones the gliadin band possessing the fastest mobility (band 47) in the *Gli-D¹1* group and the slowest moving band of the *Gli-D¹2* group (band 1) appeared to be of similar mobility which presented difficulties in classifying such groups. In assigning bands to either *Gli-D¹1* or *Gli-D¹2*, consideration was given to the general observation that the most intensely stained bands of the

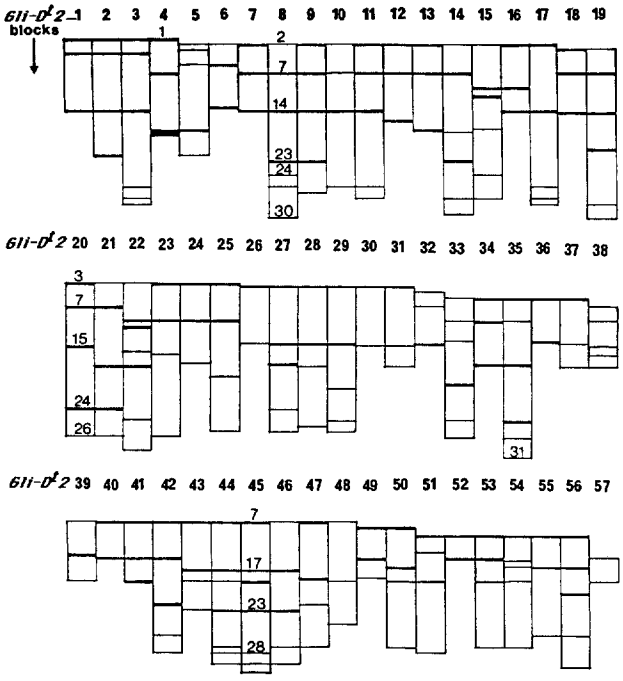

Fig. 6. Diagrammatic representation of blocks (1-57) of gliadin components (*Gli-D¹2*) in *T. tauschii*. Numbers within blocks represent gliadin band (components) designations

γ-zone of the *Gli-D¹1* group occurred at positions 38, 39, 40, 43 and 44 (Fig. 5). Consequently, the lightly stained bands with a marginally slower mobility just within the commencement of the β-zone were included in the *Gli-D¹1* group, while those which were intensely stained and located towards the end of the γ-zone were assigned to *Gli-D¹2* (blocks 1–4).

Unlike most of the *Glu-D¹1* variants, variation for most of the gliadin proteins appeared to be accession-specific, rather than regionally (geographic) distributed. Gliadin polymorphism of the *T. tauschii* accessions revealed that the *Gli-D¹1* blocks were higher than those of *Gli-D¹2*. This observation is consistent with the findings of Sosinov and Poperelya (1982) and Metakovsky et al. (1984) on variation for gliadin blocks associated with groups 1 (*Gli-1*) and 6 (*Gli-2*) chromosomes of hexaploid winter wheat. Although Metakovsky et al. (1984) observed an overall higher variation for the *Gli-1* group (*Gld* 1A, 1B and 1D) than the *Gli-2* loci (*Gld* 6A, 6B and 6D), gliadin variants of the *Gli-D1* locus were one block less than the *Gli-D2* locus. The high specificity of the *Gli-D¹1* blocks in each *T. tauschii* accession confirms their usefulness as a reliable genetic marker for varietal identification as employed in hexaploid wheats (Wrigley et al. 1982).

Gliadin electrophoregrams of bread wheat varieties from the U.S.A. (Jones et al. 1982), the USSR (Metakovsky et al. 1984), Canada (Zillman and Bushuk 1979) and Australia (du Cros et al. 1980) together with the hexaploid wheat species used in this study, constituted a diverse source from which relationships between gliadins of the D genome of hexaploid wheat and *T. tauschii* can be made. A major distinction between tetraploid and hexaploid wheats is the absence in the tetraploid of components of ω-gliadins controlled by chromosome 1D (Wrigley and Shepherd 1974) in the region commencing from the second lowest mobility gliadin band of 'Chinese Spring'. The occurrence of at least two ω-bands in this region occurred in all the hexaploid wheat electrophoregrams of the aforementioned countries. Consequently only 11 gliadin blocks of *T. tauschii* (*Gli-D¹1*, blocks 1–11) found in 12 accessions (7-*strangulata*, 3-*meyeri*, 1 *typica* and 1 intermediate *meyeri/typica*) were observed to possess ω-gliadin components typical of most of the hexaploids. The other components coded by the *Gli-D1* and *Gli-D2* loci overlap with their homoeoallelic components of the A and B genome of hexaploid wheat. Although some hexaploid landraces, particularly those collected from eastern Nepal, have been reported to be deficient in gliadin proteins coded by the *Gli-D1* locus (Payne et al. 1984), most of the other *Gli-D¹1* blocks of *T. tauschii* which lack very low mobility ω-gliadins possess protein bands in both the ω- and γ-gliadin region.

Similarly the relatedness of the major forms of the *Glu-D1* (i.e. *Glu-Dla* and *Glu-D1d*) locus of hexaploid wheat to their corresponding variants in *T. tauschii* occurred in 16 and 7 accessions (found in all the major varietal classes) possessing mobilites in SDS-PAGE equivalent to the HMW subunits 2, 12 and 5, 10, respectively. In all, seven accessions belonging to var. *strangulata* were found to be common to both the *Gli-D¹1* and *Glu-D¹1* groups identified to be closely related to their analogous variants in the D genome of hexaploid wheat. However, it may be argued that the differences in isoelectric point of subunit 10 of the 5, 10 combination between the *Glu-D¹1* and *Glu-D1* sources may not be related by descent. Consequently only six out of the 79 accessions examined in the study appeared to be related to the D genome of hexaploid wheats on the basis of their *Glu-D1* and *Gli-D1* variation, since one of the original seven accessions possessed the subunit pair 5 and 10.

Acknowledgement. E. S. Lagudah was supported by a Melbourne University Postgraduate Scholarship.

References

Anderson NG, Tollaksen SL, Pascoe RH, Anderson L (1985) Two-dimensional analysis of wheat seed proteins. Crop Sci 25:667–674

Cros DL du, Lawrence GJ, Miskelly DM, Wrigley CW (1980) Systematic identification of Australian wheat varieties by laboratory methods. CSIRO Wheat Res Unit Tech No 7

Cros DL du, Joppa LR, Wrigley CW (1983) Two-dimensional analysis of gliadin proteins associated with quality in durum wheat: Chromosomal location of genes for their synthesis. Theor Appl Genet 66:297–302

Eig A (1929) Monographisch-kritische Übersicht der Gattung Aegilops. Reprium Nov Spec Regni Veg 55:1–288

Galili G, Feldman M (1985) Structural homology of endosperm high molecular weight glutenin subunits of common wheat (*T. aestivum* L.). Theor Appl Genet 70:634–642

Halloran GM (1968) Wheat collecting expedition to Afghanistan. Proc 3rd Int Wheat Genet Symp Canberra, Australia, pp 159–160

Harberd NP, Bartels D, Thompson RD (1986) DNA restriction-fragment variation in the gene family encoding high molecular weight (HMW) glutenin subunits of wheat. Biochem Genet 24:579–596

Holt LM, Austin RB, Payne PI (1981) Structural and genetical 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

Jaaska V (1980) Electrophoretic survey of seedling esterases in wheats in relation to their phylogeny. Theor Appl Genet 56:273–284

Jaaska V (1981) Aspartate amino transferase and alcohol dehydrogenase isozymes: Intraspecific differentiation in *Aegilops tauschii* and the origin of the D genome polyploids in the wheat goup. Plant Syst Evol 137:259–273

Jaaska V (1984) NAD-dependent aromatic alcohol dehydrogenase in wheats (*Triticum* L.) and goat grasses (*Aegilops* L.): evolutionary genetics. Theor Appl Genet 67:535–540

Kihara H (1944) Discovery of the DD analyser, one of the ancestors of *T. vulgare*. Agric Hort 19:889–890

Kihara H, Yamashita K, Tanaka M (1965) Morphological, physiological, genetical and cytological studies in *Aegilops* and *Triticum* collected from Pakistan, Afghanistan and Iran. In: Yamashita K (ed) Cultivated plants and their relatives. Koei Printing, Japan, pp 1–118

Lawrence GJ, Shepherd KW (1980) Variation in glutenin protein subunits of wheat. Aust J Biol Sci 23:221–333

Lawrence GJ, Shepherd KW (1981) Inheritance of glutenin protein subunits of wheat. Theor Appl Genet 60:333–337

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37: 81–89, 107–116

Metakovsky EV, Novoselskaya AY, Kopus MM, Sobko TA, Sosinov AA (1984) Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis. Theor Appl Genet 67:559–568

Nakai Y (1979) Isozyme variations in *Aegilops* and *Triticum* IV. The origin of the common wheats revealed from the study on esterase isozymes in synthesized hexaploid wheats. Jpn J Genet 54:175–189

Nishikawa K, Furuta Y, Wada T (1980) Genetic studies on α-amylase isozymes in wheat III. Intraspecific variation in *Aegilops squarrosa* and birth place of hexaploid wheat. Jpn J Genet 55:325–336

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1, Glu-B1* and *Glu-D1* which code for the high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Payne PI, Holt LM, Law CN (1981) Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. 1. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor Appl Genet 60: 229–236

Payne PI, Holt LM, Thompson RD, Bartels D, Harberd NP, Harris PA, Law CN (1983) The high-molecular-weight subunits of glutenin: Classical genetics, molecular genetics and the relationship to bread-making quality. Proc 6th Int Wheat Genet Symp Kyoto, Japan, pp 827–834

Payne PI, Holt LM, Jackson EA, Law CN (1984) Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Philos Trans R Soc London, Ser B304:359–371

Riley R (1965) Cytogenetics and the evolution of wheat. In Hutchinson JB (ed) Crop plant evolution. Cambridge University Press, London, pp 103–122

Sosinov AA, Poperelya FA (1982) Polymorphism of prolamins and variability of grain quality. Qual Plant Plants Foods Hum Nutr 31:243–249

Thompson RA, Bartels D, Harberd NP, Flavell RB (1983) Characterisation of the multigene family coding for HMW glutenin subunits in wheat using cDNA clones. Theor Appl Genet 67:87–96

Wrigley CW, Shepherd KW (1974) Identification of Australian wheat cultivars by laboratory procedures: examination of pure samples of grain. Aust J Exp Agric Anim Husb 14: 796–804

Wrigley CW, Autran JC, Bushuk W (1982) Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Pomeranz Y (ed) Adv Cereal Sci Technol. AACC, St Paul, Minnesota, pp 211–259

Zillman R, Bushuk W (1979) Wheat cultivar identification by gliadin electrophoregrams III. Catalogue of electrophoregram formulas of Canadian wheat cultivars. Can J Plant Sci 59:287–298

Zohary D, Harlan JR, Vardi A (1969) The wild diploid progenitors of wheat and their breeding value. Euphytica 18:58–65