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# Characterisation of high- and low-molecular weight glutenin subunits associated to the D genome of *Aegilops tauschii* in a collection of synthetic hexaploid wheats

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**Abstract** Synthetic hexaploid wheats (2n=6*x*=42, AABBDD) involving genomes from *Triticum turgidum* (2n=4*x*=28, AABB) and *Aegilops tauschii* (2n=2*x*=14, DD) have been produced as a means for introducing desirable characteristics into bread wheat. In the present work we describe the genetic variability present at the *Glu-D$^t$1* and *Glu-D$^t$3* loci, encoding high- (HMW) and low-molecular-weight (LMW) glutenin subunits respectively, derived from *Ae. tauschii*, using electrophoretic and chromatographic methods, in a collection of synthetic hexaploid wheats. A wide variation both in mobility and surface hydrophobicity of HMW glutenin subunits was observed between different accessions of *Ae. tauschii* used in the production of the synthetic hexaploids. A combination of electrophoretic and chromatographic methods improves the identification of HMW glutenin subunits; in fact subunits with identical apparent mobility were revealed to have a different surface hydrophobicity by reversed-phase high performance liquid chromatography. None of the Dx5$^t$ subunits present in *Ae. tauschii* showed the presence of the extra cysteine residue found in the HMW glutenin subunit Dx5 of *Triticum aestivum*, as revealed by selective amplification with polymerase chain reaction (PCR). The wide variability and the high number of subunits encoded by the *Glu-D$^t$3* locus suggests that *Ae. tauschii* may be a rich source for enhancing the genetic variability of glutenin subunits in bread wheat and improving bread-making properties.

**Keywords** *Aegilops tauschii* · Synthetic hexaploids · HMW-GS · LMW-GS · Electrophoresis

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## Introduction

*Aegilops tauschii* (2n=2*x*=14, DD), syn. *Triticum tauschii; Ae. squarrosa* is accepted as the D-genome donor to cultivated bread wheat (*Triticum aestivum* L., 2n=6*x*=42, AABBDD) (Kihara 1944; Mc Fadden and Sears 1946). However, the origin of hexaploid wheat has been related to an apparently small number of *Ae. tauschii* genotypes of restricted geographic origin (Lagudah et al. 1991), resulting in a narrow genetic diversity for the D genome in *T. aestivum*. Therefore, other accessions of this wild wheat relative may be further utilized as sources of new genes for bread wheat improvement.

Common wheat carries the D genome still largely unchanged, so that *Ae. tauschii* forms a natural extension of the common wheat gene pool (Kimber et al. 1981). Because of the close relationship between the D genome of *T. aestivum* and *Ae. tauschii* genetic recombination barriers are greatly reduced facilitating the introduction of new genes into cultivated wheat using standard breeding techniques (Appels and Lagudah 1990).

Bread wheat can be crossed directly with *Ae. tauschii* (Gill and Raup 1987; Cox et al. 1991, 1995) or the tetraploid wheat *Triticum turgidum* ssp. *durum* Desf. em. M.K. can be used as a bridging species, crossed with *Ae. tauschii*, for indirect gene transfer to *T. aestivum* (Mujeeb-Kazi et al. 1996).

Accessions of *Ae. tauschii* have been evaluated for a wide range of agronomically important traits, including disease and insect resistance (Dyck and Kerber 1970; Harvey et al. 1980; Pasquini 1980; Hatchett et al. 1981; Cox et al. 1986; Gill et al. 1986), endosperm proteins af-

fecting end-use quality (Lagudah and Halloran 1988; Peña et al. 1996) and physiological traits (Zohary et al. 1969; Le et al. 1986).

There is evidence that *Ae. tauschii* has a greater genetic variability for endosperm proteins, gliadins and glutenins, compared to *T. aestivum*. Variation in HMW glutenin subunit composition is greatly responsible for differences in the bread-making quality among bread wheat cultivars. Genes encoding HMW glutenin subunits are present at the *Glu-1* loci located on the long arm of the group-1 chromosomes (1A, 1B and 1D) of bread wheat; each locus contains two tightly linked genes corresponding to a subunit of high- and a subunit of low-molecular-size, termed x- and y-types, respectively. Allelic variation at the *Glu-D1* locus has a strong influence on bread-making quality than variation at other *Glu-1* loci. The positive effect of the pair of subunits 5+10, compared to 2+12, on dough strength is well documented (Payne et al. 1981; Lagudah et al. 1988; Gupta and MacRitchie 1994). The number and position of cysteine residues present on these subunits are considered to be of prime importance in determining the observed differences. It has been suggested that subunits 5+10 exert a greater effect on dough strength by producing a greater proportion of larger-size glutenin polymers than subunits 2+12 (Gupta and MacRitchie 1994). This effect on polymer size is attributed to the presence of an extra cysteine residue present in subunit 5 compared to subunit 2 (Lafiandra et al. 1993).

Invariably on the short arm of the group-1 chromosomes, genes corresponding to the low-molecular-weight glutenin subunits (*Glu-3* loci) are present tightly linked to those encoding gliadin components (*Gli-1* loci) (Singh and Shepherd 1988). The LMW glutenins have been subdivided into the B- C- and D-group based on differences in size and isoelectric points (Jackson et al. 1983).

The high crossing ability of the synthetic hexaploids with bread wheat indicates that the former could be utilized for introducing new allelic variation present at the *Glu-D$^t$1* and *Glu-D$^t$3* loci of *Ae. tauschii* into cultivated bread wheat.

In this work we describe the genetic variability at the *Glu-D$^t$1* and *Glu-D$^t$3* loci derived from *Ae. tauschii*, and characterise the subunits encoded by the *Glu-D$^t$1* locus by electrophoretic and chromatographic methods, in a collection of synthetic hexaploid wheats produced at CIMMYT from crosses between various cultivars of durum wheat and *Ae. tauschii* accessions. This characterisation was made using sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) and reversed-phase high performance liquid chromatography (RP-HPLC). Specific primers for subunit Dx5 of *T. aestivum*, were used to elucidate if the extra cysteine residue characteristic of this subunit is also present in subunit Dx5$^t$.

## Materials and methods

### Plant material

A collection of synthetic hexaploids, derived from the cross among different genotypes of *T. turgidum* cv durum with diverse accessions of *Ae. tauschii*, produced at CIMMYT was used.

One-hundred and twenty eight synthetic hexaploids derived from the cross of the durum cultivars Laru, Altar 84, Ceta, Dverd 2, Sora, Croc 1 and Doy 1 with 92 different accessions of *Ae. tauschii* were used in this study.

### Protein extraction

Gliadins were extracted from single seeds by grinding them in a mortar with 1.5 M dimethyl formamide (DMF). The solvent volume to seed weight ratio was 5:1 (µl : mg). The solution was centrifugated for 10 min at 14,000 g and the supernatant was stored at –20°C for subsequent gliadin analysis.

The pellet remaining after gliadin extraction was treated with 1 ml of 0.08 M Tris-HCl buffer (pH 8.5) containing 1% SDS for 30 min with intermittent vortexing followed by centrifugation (8 min at 14,000 g). This procedure was repeated twice discarding the supernatant. The pellet was used to extract glutenin subunits, using 0.08 M Tris-HCl buffer (pH 8.5) containing dithiotreitol (DTT) (1.5% w/v) and SDS (1% w/v). The extraction was carried out for 30 min at 60°C with occasional vortexing followed by centrifugation (10 min at 14,000 g). Two-hundred microliters of each supernatant were used for alkylation, employing 2.8 µl of 4-vinylpyridine. Samples were incubated at 60°C for 30 min with occasional vortexing. Acetone (1 ml) was added to each sample to precipitate glutenins. Samples were mantained overnight at –20°C and then centrifuged (10 min at 14,000 g), discarding the supernatant. Pellets were re-suspended with 0.25 M Tris-HCl buffer (pH 6.8) containing 1.5% DTT [the solvent volume to seed weight ratio was 5:1 (µl:mg)].

For two-dimensional electrophoresis, the pellet remaining after gliadin extraction was washed twice with 50% propan-1-ol (1 ml) for 30 min at 60°C; after centrifugation, the supernatant was discarded. The pellet was then re-suspended in 250 µl of 50% propan-1-ol containing 1% of dithiothreitol (DTT).

The samples were incubated for 30 min at 60°C. After centrifugation, 200 µl of supernatant was transferred to a new tube with 2.8 µl of 4-vinylpyridine. The samples were incubated for 30 min at 60°C. Glutenin subunits were precipitated with cold acetone (1 ml) for 1 h and 30 min at –20°C, and centrifuged at 14,000 g for 15 min. The dried pellet was re-suspended in 6 M urea, 0.142% acetic acid and 1% DTT.

The selective precipitation of HMW glutenin subunits for RP-HPLC analysis was based on the method of Marchylo et al. (1989). Samples of ground grain (40 mg) were extracted sequentially by shaking in 0.5 M NaCl during 1 h at room temperature. The extract was then clarified after centrifugation (4,000 g) at 20°C for 15 min and the supernatant containing albumins and globulins was discarded. The pellet was washed three times with water for 30 min and centrifuged each time (4,000 g) at room temperature. The pellet was then extracted twice with 50% (v/v) propan-1-ol, 1% (w/v) DTT at 60°C for 30 min, with regular stirring, and centrifuged for 30 min at 4°C. Both supernatants were pooled. The HMW-GS were precipitated adding propan-1-ol containing 1% (w/v) DTT to bring the final concentration of propan-1-ol to 60% (v/v). The precipitate was collected after centrifugation for 20 min at 4°C. The samples were divided into two subsamples one of which was alkylated with 4-vinylpiridine according to Margiotta et al. (1993).

### Electrophoretic methods

Gliadins were separated by A-PAGE (aluminium lactate buffer, pH 3.1) according to Khan et al. (1985), in 140×160×1.5-mm gels.

Electrophoresis was performed at a constant current of 25 mA/gel, and the electrode buffer was cooled by circulating water at a constant temperature of 20°C.

Glutenin subunits were analyzed by SDS-PAGE in 8% acrylamide gel or in a 7.5–13% linear acrylamide gradient gel with 1.28% cross-linker concentration. Electrophoretic separations were carried at 30 mA/gel.

Glutenin subunits were also analyzed by two-dimensional A-PAGE×SDS-PAGE, according to Morel (1994). HMW glutenin subunits were designated on the basis of the numbering system of Lagudah and Halloran (1988) and Peña et al. (1995) for *Ae. tauschii*. Superscript t has been used to distinguish gene symbols of the D genome of *Ae. tauschii* from its homologous gene loci of bread wheat.

### RP-HPLC-analysis

RP-HPLC analysis was carried out on a Waters HPLC system using a Supelcosil LC-308 column with a 300 Å pore size and a 5-µm particle size, 250×4.6 mm id. Samples were run at a solvent flow rate of 1 ml/min using a column temperature of 50°C and the effluents were monitored at 210 nm. The solvent consisted of water and acetonitrile, each containing 0.1% (v/v) trifluoroacetic acid. A linear gradient of 23–28% acetonitrile was used for the first 23 min, followed by a second linear gradient of 28–31% acetonitrile from 23 to 88.5 min.

### PCR-analysis

DNA extraction was carried out using part of the endosperm from a single seed following the procedure reported by Benito et al. (1993) as modified by D'Ovidio and Porceddu (1996). Amplification reactions were carried out using primers and conditions described by D'Ovidio and Anderson (1994).

## Results and discussion

### Characterisation of HMW-GS from *Ae. tauschii*

#### *SDS-PAGE analysis*

A wide variation in the HMW glutenin subunits was observed between different accessions of *Ae. tauschii* used as parents for the synthetic hexaploid collection under study. Nineteen types of HMW glutenin subunit combinations were found at the *Glu-D$^t$1* locus (Fig. 1). They were characterised by a pair of subunits (x- and y-type). None of these alleles showed null-forms. These subunit combinations are indicative of a larger polymorphism in *Ae. tauschii* than the one reported at the *Glu-D1* locus of bread wheat (Payne et al. 1983).

Although the alleles 2+12 and 5+10 are the most common in bread wheat, their frequency in the *Ae. tauschii* accessions was much lower. The different allelic forms associated with the 92 accessions of *Ae. tauschii* used as parents of the synthetic hexaploids studied are reported in Table 1.

Seven x-type subunits were identified: 2.1*$^t$, 2.1$^t$, 1.5$^t$, 2$^t$, 3$^t$, 4$^t$ and 5$^t$ in order of ascending mobility. Subunit named 2.1*$^t$, with a lower mobility than 2.1, is first reported in this study, and its mobility is slightly higher than subunit 1 of hexaploid wheat, coded by the *Glu-A1* locus.

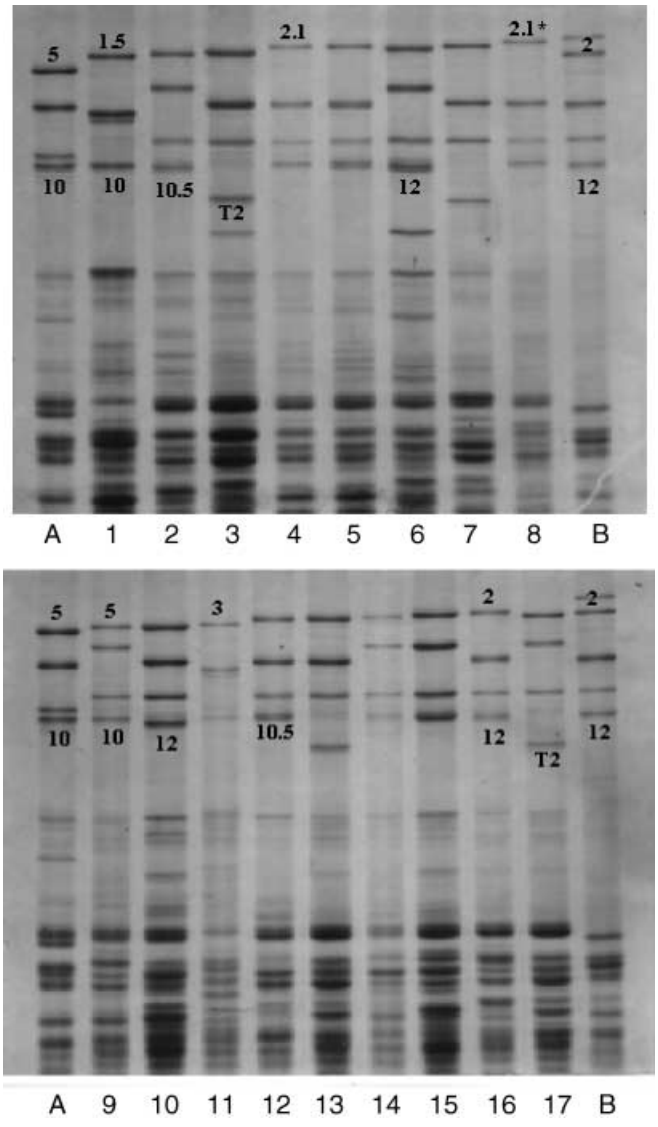



**Fig. 1** SDS-PAGE of *Glu-D$^t$1* variants from synthetic hexaploids. *Lanes A*: bread cultivar 'Pegaso' (7+9, 5+10); *lanes B*: bread line 'N11' (1, 7+8, 2+12); *lanes 1 to 17*: some of the *Glu-D$^t$1* allelic variations observed in synthetic hexaploids

Five diverse y-type subunits were detected, named 10$^t$, 10.5$^t$, 12$^t$, 12*$^t$ and T2 (according to the nomenclature of William et al. 1993).

In bread wheat, a tight linkage between genes encoding subunits 5+10 and 2+12 has been observed, but in *Ae. tauschii*, subunit 5$^t$ was found along with subunit 10$^t$ or 12$^t$. Furthermore, subunit combinations 2$^t$+10$^t$; 2$^t$+12$^t$, 2$^t$+10.5$^t$ and 2$^t$+T2 were present in *Ae. tauschii* accessions. Rare recombination between subunits may have given rise to the subunit combinations 5$^t$+12$^t$ and 2$^t$+10$^t$. These combinations must also have been present in nature, but were not utilized in the evolutionary synthesis of bread wheat. However, it is not possible to affirm that subunit 2 or 5 of *Ae. tauschii* are the same in the different combinations. They could be different subunits with the same electrophoretic mobility and, therefore, un-

**Table 1** Different *Glu-D$^t$1* allelic forms of *Aegilops tauschii* and the number of accessions in which were detected

| *Glu Dt 1* variants | Number of accessions |
|---|---|
| 5+10 | 9 |
| 5+12 | 2 |
| 4+10 | 1 |
| 3+10.5 | 1 |
| 3+T2 | 4 |
| 2+10 | 20 |
| 2+10.5 | 6 |
| 2+12 | 8 |
| 2+T2 | 2 |
| 1.5+12 | 3 |
| 1.5+10 | 2 |
| 1.5+10.5 | 1 |
| 1.5+T2 | 7 |
| 2.1+12 | 6 |
| 2.1+10 | 14 |
| 2.1+10.5 | 1 |
| 2.1+T2 | 3 |
| 2.1*+10.5 | 1 |
| 2.1*+12* | 1 |

detectable by this methodology. In fact, Lagudah and Halloran (1988) have demonstrated that subunit 2$^t$ from 2$^t$+12$^t$ was much more acidic than its counterpart from 2$^t$+T2.

Furthermore, workers employing different metodologies, have determined that 2+12 and 5+10 subunit pairs of *Ae. tauschii* are not the same as those present in *T. aestivum* (Lagudah and Halloran 1988; Mackie et al. 1996a, b; Gianibelli et al. 2000).

## RP-HPLC

Given the very close homology between *Ae. tauschii* and the D genome of *T. aestivum*, it may be argued that although HMW glutenin subunits of *Ae. tauschii* may be identical with those of *T. aestivum* in terms of size (mobility in SDS-PAGE) they may be heterogeneous with respect to charge.

Examples of subunits with identical electrophoretic mobility but different charge were described by Lagudah and Halloran (1988). A relatively more basic isoelectric point characterised subunit 10 (from 5+10 of *T. aestivum*) compared to subunit 10$^t$ (5$^t$+10$^t$ pair).

Reversed-phase high performance liquid chromatography (RP-HPLC) separates proteins according to their surface hydrophobicity, thus complementing information provided by electrophoresis, which separates proteins according to size or charge differences.

Results of RP-HPLC analysis of the HMW-GS are given in Tables 2 and 3. Three bread wheat cultivars (Chinese Spring, Cheyenne and Melchior) were included as controls. For each subunit, four values are supplied: the elution time of the reduced HMW glutenin subunit, the elution time of the reduced and pyridylethylated subunit, the difference in elution times between both values, and the latter parameter expressed as a percentage. The

**Table 2** Comparison of the retention times by RP-HPLC of x-type HMW glutenin subunits reduced or reduced and alkylated

| Genotype | Subunit | Reduced (min) | Reduced and alkylated (min) | Time difference (min) | Time difference (%) |
|---|---|---|---|---|---|
| Syn 146 | 1.5$^t$ | 81.2 | 34.5 | 46.7 | 57.5 |
| Syn 318 | 1.5$^t$ | 81.5 | 34.5 | 47.0 | 57.7 |
| Syn 424 | 1.5$^t$ | 82.2 | 34.9 | 47.3 | 57.5 |
| Chinese Spring | 2 | 81.5 | 35.2 | 46.3 | 56.8 |
| Syn 20 | 2$^t$ | 81.4 | 35.1 | 46.3 | 56.9 |
| Syn 266 | 2$^t$ | 81.5 | 34.8 | 46.7 | 57.3 |
| Syn 398 | 2$^t$ | 80.8 | 34.5 | 46.3 | 57.3 |
| Syn 426 | 2.1$^t$ | 81.2 | 34.5 | 46.7 | 57.5 |
| Syn 11 | 2.1$^t$ | 81.3 | 34.9 | 46.4 | 57.1 |
| Syn 38 | 2.1$^t$ | 81.7 | 34.8 | 46.9 | 57.4 |
| Syn 187 | 2.1$^t$ | 81.3 | 34.7 | 46.6 | 57.3 |
| Syn 396 | 2.1$^t$ | 82.8 | 35.3 | 47.5 | 57.4 |
| Syn 71 | 2.1*$^t$ | 81.0 | 34.9 | 46.1 | 56.9 |
| Melchior | 3 | 81.1 | 34.3 | 46.8 | 57.7 |
| Syn 429 | 3$^t$ | 81.7 | 34.5 | 47.2 | 57.8 |
| Cheyenne | 5 | 82.5 | 33.9 | 48.6 | 58.9 |
| Syn 96 | 5$^t$ | 81.8 | 35.7 | 46.1 | 56.4 |
| Syn 131 | 5$^t$ | 81.5 | 35.2 | 46.3 | 56.8 |

**Table 3** Comparison of the retention times by RP-HPLC of y-type HMW glutenin subunits reduced or reduced and alkylated

| Genotype | Subunit | Reduced (min) | Reduced and alkylated (min) | Time difference (min) | Time difference (%) |
|---|---|---|---|---|---|
| Cheyenne | 10 | 48.6 | 21.3 | 27.3 | 56.2 |
| Syn 38 | 10$^t$ | 48.2 | 21.4 | 26.8 | 55.6 |
| Syn 131 | 10$^t$ | 54.3 | 22.8 | 31.5 | 58.0 |
| Syn 146 | 10$^t$ | 49.3 | 21.7 | 27.6 | 56.0 |
| Syn 426 | 10$^t$ | 48.1 | 21.0 | 27.1 | 56.3 |
| Syn 429 | 10$^t$ | 49.2 | 21.0 | 28.2 | 57.3 |
| Syn 71 | 10.5$^t$ | 50.0 | 22.0 | 28.0 | 56.0 |
| Syn 187 | 10.5$^t$ | 49.3 | 20.9 | 28.4 | 57.6 |
| Syn 398 | 10.5$^t$ | 46.0 | 20.7 | 25.3 | 55.0 |
| Chinese Spring | 12 | 48.2 | 21.3 | 26.9 | 55.6 |
| Syn 20 | 12$^t$ | 48.0 | 21.1 | 26.9 | 56.0 |
| Syn 96 | 12$^t$ | 54.2 | 22.8 | 31.4 | 57.9 |
| Syn 396 | 12$^t$ | 47.9 | 21.1 | 26.8 | 55.9 |
| Syn 424 | 12$^t$ | 47.6 | 20.8 | 26.8 | 56.3 |
| Syn 11 | T2 | 49.5 | 21.8 | 27.7 | 56.0 |
| Syn 266 | T2 | 49.1 | 21.5 | 27.6 | 56.2 |
| Syn 318 | T2 | 49.5 | 21.1 | 28.4 | 57.4 |

effect of alkylation with 4-vinyl pyridine on the HMW glutenin subunits was assessed by comparing the difference in elution time between the reduced and reduced-and-pyridilethylated subunits. The subunits have been divided into x-types and y-types for ease of comparison.

Subunits of the x- and y-type were eluted at two clearly different times, with y-type subunits appearing at lower elution times. Among the x-type subunits, elution times for unalkylated subunits were quite similar, ranging from 80.8 to 82.8 min. Reduced glutenin subunit Dx5 from the bread wheat cultivar Cheyenne and subunit Dx2.1$^t$ from the synthetic hexaploid 396 (with *Glu-D$^t$1* pair 2.1$^t$+12$^t$) had the highest values of surface hydroph-

obicity, with elution times of 82.5 and 82.8 min, respectively. Alkylation of the sulphydryl groups with 4-vynil pyridine results in decreased surface hydrophobicity, as revealed by the great reduction in elution times of reduced and alkylated subunits as compared with elution times for reduced subunits only.

Subunit Dx5 from the bread wheat cultivar Cheyenne showed a greater reduction in surface hydrophobicity upon alkylation than subunit Dx2 from the cultivar Chinese Spring. This is consistent with previous reports that the effect of alkylation on the HMW glutenin subunits is related to the number of cysteine residues present. In fact, subunit Dx5 contains five cysteine residues, whereas Dx2 contains four cysteine residues (Margiotta et al. 1993). In particular, subunits with less reduction in elution time upon alkylation were Dx2, $Dx2^t$ (from the $2^t$+$12^t$ pair) and $Dx5^t$ (from the $5^t$+$10^t$ and $5^t$+$12^t$ pairs), suggesting that also subunit $Dx5^t$ from *Ae. tauschii* could contain four cysteine residues.

Within the y-type subunits a great variation in elution times was observed, with values ranging between 46.0 to 54.3 min for reduced subunits. Subunits $Dy10^t$ and $Dy12^t$ (from the pairs $5^t$+$10^t$ and $5^t$+$12^t$) had the highest values of surface hydrophobicity with elution times of 54.3 and 54.2 min, respectively. The other subunits designated $10^t$ or $12^t$ present in other combinations had elution times similar to those of subunits 10 and 12 present in bread wheat cultivars.

Subunits $Dy10^t$ (from $5^t$+$10^t$ pair) and $Dy12^t$ (from the $5^t$+$12^t$ pair) showed a greater reduction in hydrophobicity after alkylation (58 and 57.9%, respectively). As indicated by Margiotta et al. (1993), an initial explanation could be that these subunits differ in the number of cysteine residues, but the unalkylated subunits also had distinct differences in elution times from the other subunits, indicating that they have quite different surface hydrophobicities. T2 (from $1.5^t$+T2 pair) also showed a high reduction in elution time (57.4%) and was different to the other subunits named T2 from other combinations pairs.

Some glutenin subunits with identical apparent mobilities on SDS-PAGE (i.e. $10^t$ from the pair $5^t$+$10^t$, and $10^t$ from the other combination pairs) were revealed to have different surface hydrophobicities. But in other cases, subunits having different mobilities in SDS-PAGE, such as $1.5^t$, $2.1^t$ and $3^t$, have similar hydrophobicities, and it was not possible to differentiate them by RP-HPLC. A combination of electrophoretic and chromatographic methods improves the identification of HMW glutenin subunits.

Mackie et al. (1996a) considered that it may not be valid to compare the elution times of subunits from *T. aestivum* whith those from *Ae. tauschii* because alkylated and unalkylated *Ae. tauschii* y-subunits had differences in elution times with those from hexaploid wheat. These authors have indicated that the y-type HMW glutenin subunits from *Ae. tauschii* have a higher surface hydrophobicity than those from *T. aestivum*. But in their study they analysed subunits $10^t$ and $12^t$ only in the combination pairs $5^t$+$10^t$ and $5^t$+$12^t$. Accordingly with their results, in our study, subunits $10^t$ and $12^t$ from the combination pairs $5^t$+$10^t$ and $5^t$+$12^t$ are also much more hydrophobic, whereas subunits $10^t$ and $12^t$ from other subunit pairs (i.e. $1.5^t$+$10^t$; $2.1^t$+$10^t$; $1.5^t$+$12^t$;

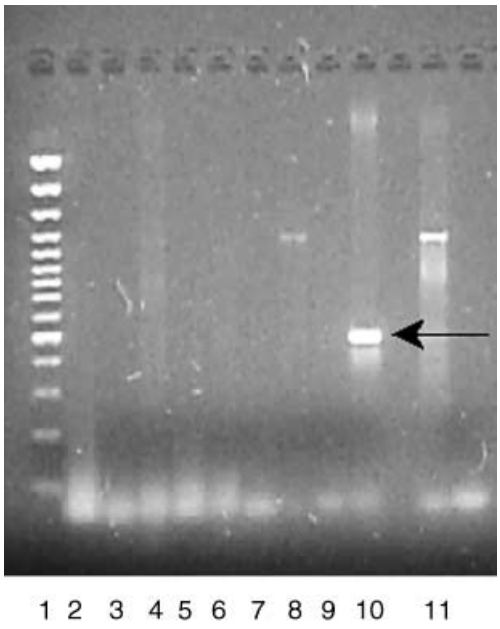



**Fig. 2** PCR analysis using specific primers for the *Dx5* gene of *T. aestivum*. *Lane 1*: molecular-weight-marker; *lanes 2 to 9*: synthetic hexaploids with $Dx5^t$; *lane 10*: bread cultivar 'Cheyenne' (check for *Dx5*); *lane 11*: bread cultivar 'Chinese Spring' (check for *Dx2*). The *arrow* indicates the 450-bp specific of the *Dx5* gene

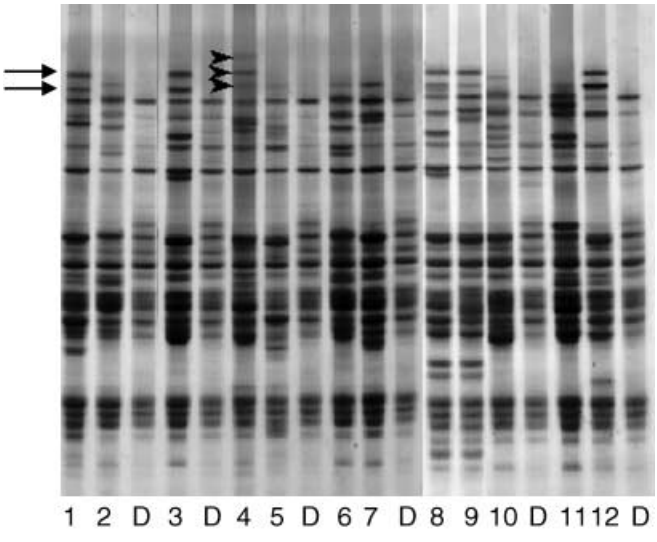



**Fig. 3** Gliadin electrophoretic patterns in some synthetic hexaploids. *Lanes D*: durum wheat cultivar 'Doy 1' (progenitor of these hexaploids), *lanes 1 to 12*: synthetic hexaploids. *Lane 1*: Syn 395; *lane 2*: Syn 338; *lane 3*: Syn 349; *lane 4*: Syn 401; *lane 5*: Syn 403; *lane 6*: Syn 426; *lane 7*: Syn 398; *lane 8*: Syn 6; *lane 9*: Syn 447; *lane 10*: Syn 383; *lane 11*: Syn 373; *lane 12*: Syn 395. *Arrows and arrowheads* indicate 'Chinese Spring' and 'Cheyenne' types of ω-gliadins

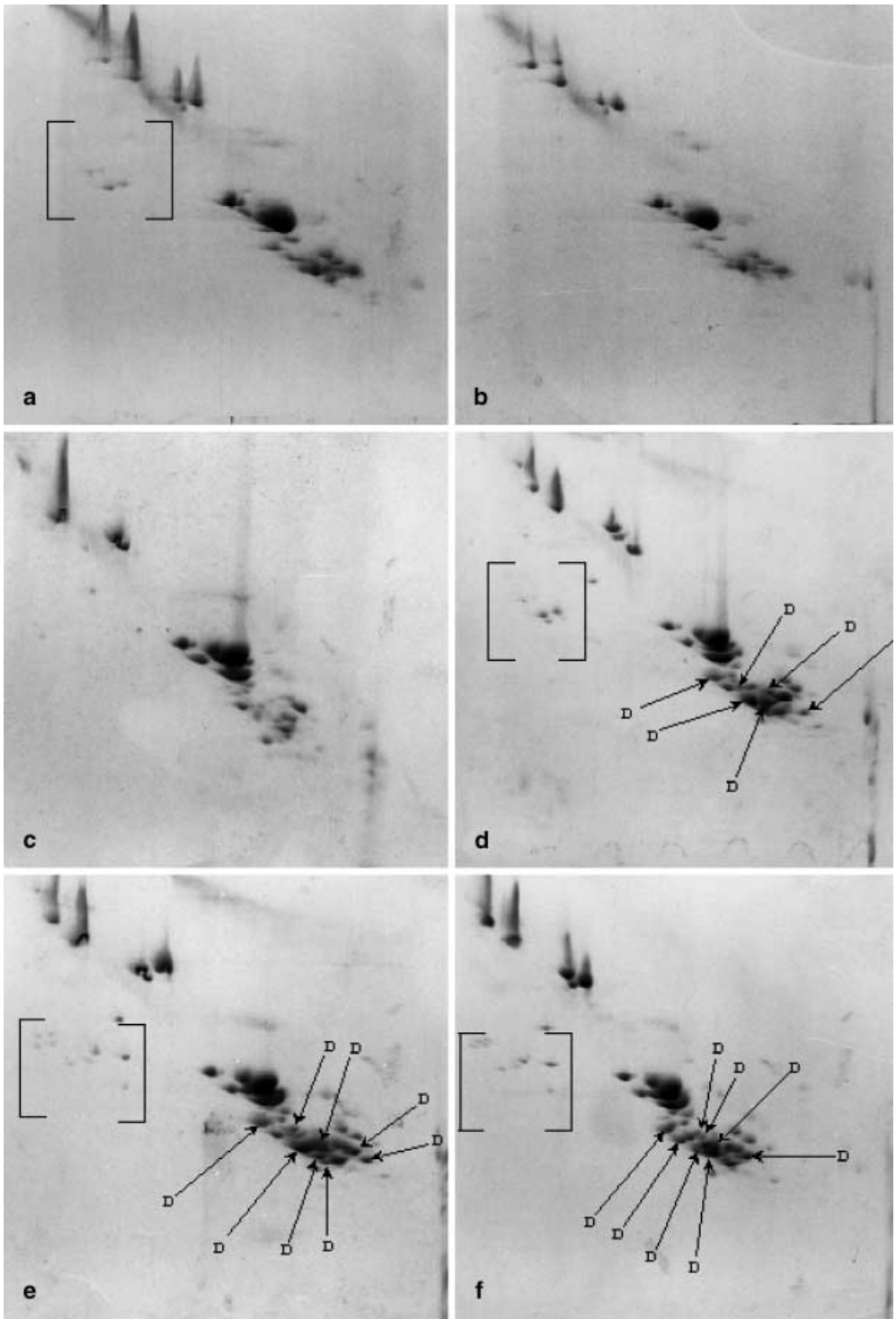


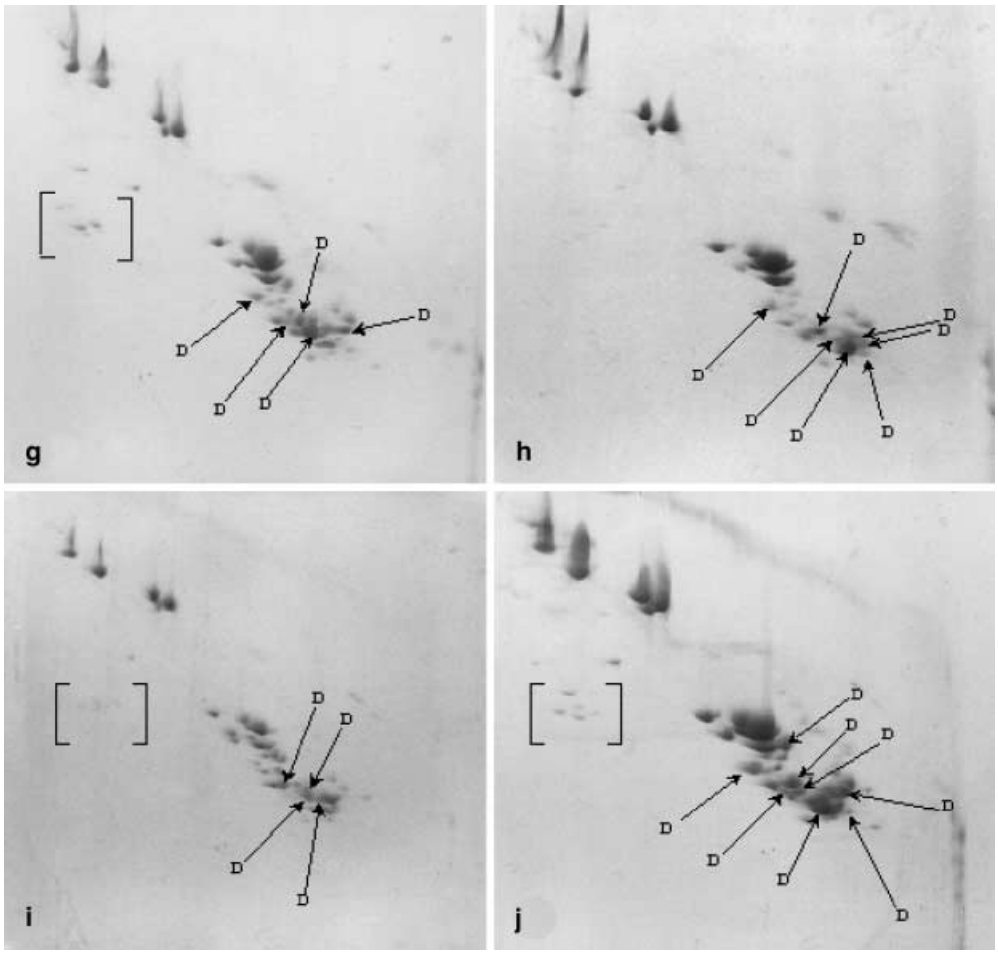

**Fig. 4** Two-dimensional A-PAGE×SDS-PAGE of glutenin subunits from: *a* cv 'Newton' Chinese Spring type; *b* cv 'Newton' Cheyenne type; *c* durum wheat cultivar 'Doy 1'; *d to j* synthetic hexaploids, *d* Syn 447; *e* Syn 6; **f**: Syn 349; *g*: Syn 396; *h*: Syn 403; *i*: Syn 426; *j*: Syn 398. *Arrows* indicate B and C-LMW-Gs of *Ae. tauschii*. D-LMW-Gs are shown in *brackets*

$2^t+12^t$; $3^t+12^t$) have elution times similar to those of Dy10 or Dy12 from *T. aestivum*.

## PCR analysis of Dx $5^t$

Eight synthetic hexaploids (with subunit Dx$5^t$ from the pairs $5^t+10^t$ and $5^t+12^t$) were analysed by PCR by using the primers identified by D'Ovidio and Anderson (1994). These were developed using differences in the nucleotide sequences of the Dx2 and Dx5 genes, and particularly with a nucleotide difference corresponding to a cysteine residue present only in the Dx5 subunit at the beginning of the repetitive region. Results of PCR amplification of the material including bread wheat cultivars Cheyenne (Dx5) and Chinese Spring (Dx2) used as checks are reported in Fig. 2. Only subunit Dx5 from the cultivar Cheyenne showed the presence of the expected

**Fig. 4** (continued)




amplified fragment of 450 bp, considered typical for cultivars possessing this particular subunit. Chinese Spring and the synthetic hexaploids analysed did not show the amplification band of 450-bp confirming that Dx5$^t$ subunits do not possess the extra cysteine residue of Dx5 from *T. aestivum*.

These results are in concordance with the different reduction in elution times found between Dx5 from *T. aestivum* and Dx5$^t$ of *Ae. tauschii* by RP-HPLC.

## Characterisation of gliadins and LMW-GS from *Ae. tauschii*

In bread wheat, a limited variation is present for omega gliadins encoded by genes present at the *Gli-D1* locus and the D-, B- and C-group of LMW glutenin subunits encoded by the linked genes at the *Glu-D3* locus. In fact, Masci et al. (1991) observed that in all the bread wheats studied two main types were present according to the electrophoretic patterns of the chromosome 1D-encoded omega gliadins and LMW glutenins. These resembled analogous patterns found in the bread wheat cultivars Chinese Spring (CS) and Cheyenne (CNN), and therefore indicated as CS- and CNN-type wheats. Masci et al. (1991) also found that the D-group of LMW glutenin subunits were present only in those bread wheats with CS-type omega gliadins, whereas they were absent in those cultivars possessing CNN-type omega gliadins.

In the synthetic hexaploids studied, a high variability for gliadin components is present (Fig. 3). Some of the accessions of *Ae. tauschii* used as parents of the synthetic hexaploids possess the CS-type of bands in their omega components, while five accessions carried the CNN-type of bands.

For the characterisation of the LMW glutenin subunits associated with the *Glu-D$^t$3* locus of different accessions of *Ae. tauschii*, a group of synthetic hexaploids, whose durum parent was the cultivar Doy 1, were selected. Glutenin subunits present in 16 different genotypes have been analysed by two-dimensional electrophoretic separation (A-PAGE×SDS-PAGE), and compared with those present in the durum wheat cultivar Doy1 and the two biotypes detected in the bread wheat cultivar Newton showing gliadins and LMW glutenin subunits associated to the *Gli-D1/Glu-D3* loci of the CS- and CNN-type, respectively. Only two genotypes (synthetic 6 and 131) showed the same electrophoretic pattern for *Gli-D1*/*Glu-D3* associated proteins.

Although a reduced number of genotypes was analysed, a wide variability was present at the *Glu-D$^t$3* locus. Considering the B- and C-group of LMW glutenin

subunits contributed from *Ae. tauschii*, from four to eight spots were unequivocally assigned to *Glu-D$^t$3* in the different synthetics analysed, whereas a maximum of five subunits was found associated to the *Glu-D3* allele in bread wheat. The D-group of LMW-GS were absent in synthetics having Cheyenne-type omega gliadins, similar to what was observed in bread wheat. They were also absent in a genotype whose omega gliadins were different from those found in bread wheat (synthetic hexaploid 403, Fig. 3 lane 5, Fig. 4h). The remaining synthetics all showed the D-group of LMW glutenin subunits, but there was a variability in the number and position of their spots.

Allelic differences in LMW glutenin subunits have been shown to be related to flour quality in durum and bread wheat. Autran et al. (1987) have demonstrated that in durum wheat the variation in dough properties is most probably determined by the different relative amounts of LMW glutenin subunits, these being considerably greater in the γ-gliadin type 45 cultivars.

Accessions with a high number of subunits associated with the *Glu-D$^t$3* locus of *Ae. tauschii* have been detected. This could have significant effects on bread-making properties as these novel alleles could be used to improve technological properties of bread wheat flour.

## Conclusions

A wide variability of storage proteins present at the *Glu-D$^t$1* and *Glu-D$^t$3* loci was detected in the accessions of *Ae. tauschii* used as parents of the synthetic hexaploids. A combination of SDS-PAGE and RP-HPLC was necessary to detect the large variability in HMW glutenin subunits encoded at the *Glu-D$^t$1* of *Ae. tauschii*.

HPLC and selective amplification by PCR indicated the absence in subunit Dx5$^t$ of the extra cysteine residue present in Dx5 of bread wheat.

More attention should be paid to the contribution of *Glu-D$^t$3* on bread-making quality. The wide variability and the high number of subunits encoded by this locus suggests that *Ae. tauschii* accessions may be a rich source for enhancing the genetic variability of bread wheat and for improving bread-making quality.

The expression of genes affecting quality characteristics may be very different when the genes are introgressed into common wheat. With the purpose of evaluating their effects in a background of bread wheat, a backcross program is currently in progress.

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