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# Seed protein and isozyme variations in *Triticum tauschii* (*Aegilops squarrosa*)

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**Abstract.** Sixty *Triticum tauschii* (*Aegilops squarrosa*, 2n = 2x = 14, DD) accessions were evaluated for the variability of high-molecular-weight (HMW) glutenins, gliadins and isozymes of seed esterase, β-amylase and glucose-phosphate isomerase. Wide variability was observed for HMW-glutenins and gliadins. The implications of unique HMW-glutenin alleles for quality parameters are discussed. Isozyme evaluations indicated more variability for the *Est-D$^t$5* locus as compared to the *Est-D5* of bread-wheat. The polymorphism for *β-Amy-D$^t$1* was less than that of *β-Amy-D1*. Similar to the bread-wheat situation, *Gpi-D$^t$1* showed no polymorphism. The variability observed with the traits evaluated can be readily observed in *T. turgidum* × *T. tauschii* synthetic hexaploids (2n = 6x = 42, AABBDD) suggesting that *T. tauschii* accessions may be a rich source for enhancing the genetic variability of *T. aestivum* cultivars.

**Key words:** *Triticum tauschii* – Genetic variability – HMW-glutenins – Gliadins – Isozymes

## Introduction

*Triticum tauschii* (*Aegilops squarrosa*; 2n = 2x = 14, DD) is unequivocally accepted as the D genome donor to *T. aestivum* (Kimber and Feldman 1987) and the importance of some of the *Glu-D1*-encoded proteins on quality parameters has also been well established (Kerber and Tipples 1969; Orth et al. 1973). The origin of hexaploid wheat (AABBDD) is apparently ascribed to a small number of *T. tauschii* genotypes of restricted geographic origin (Lagudah et al. 1991) implying a narrow genetic diversity for the D-genome in *T. aestivum*. Hence, other wild-form D genome accessions may be further utilized as sources of new genes for bread-wheat improvement (Appels and Lagudah 1990). The wide ecological adaptability, the diploid status, and the D genome proximity to that of *T. aestivum* should permit utilization of *T. tauschii* accessions for enhancing the adaptability of bread-wheat cultivars into areas considered marginal or non-profitable for wheat.

This study reports the variability associated with 60 *T. tauschii* accessions for seed storage proteins (high-molecular-weight subunits of glutenins and gliadins) and three isozyme loci (seed esterase, EST-5; β-amylase, β-AMY; and glucose-phosphate isomerase, GPI). These accessions have been used in the production of synthetic hexaploids (Mujeeb-Kazi et al. 1993). A sample of these synthetic hexaploids was also utilized to evaluate whether the variability for seed proteins and isozymes observed in the *T. tauschii* accessions was also expressed in the synthetics derived from them.

In wheat, genes for HMW-glutenins are located in the long arms of chromosomes 1A (Lawrence and Shepherd 1981), 1B and 1D (Bietz et al. 1975). Genes for GPI are located in the short arms of group 1 chromosomes (Hart 1979) while those for β-amylase are in chromosomes 4AL, 4DL and 5AL (Ainsworth et al. 1983). Grain EST genes are located in the long arms of homoeologous group 3 chromosomes (Ainsworth et al. 1984), while gliadin genes are in the short arms of homoeologous group 1 and group 6 chromosomes (Wrigley and Shepherd 1973; Payne et al. 1982). The proteins and isozymes thus evaluated cover homoeologous group 1 (HMW glutenins, gliadins and GPI), group 3 (EST-5), group 4 and/or 5 (β-AMY), and group 6 (gliadins).

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**Table 1.** Enzyme system, pH gradients of IEF gels, and cathode and anode buffer solutions used

| Enzyme | Extraction buffer | pH gradient of gel | Cathode | Anode |
|---|---|---|---|---|
| GPI | 0.05 M Tris-Hcl (pH 7.4) | 3.5–9.5 | 1 M NaOH | 1 M $H_3PO_4$ |
| EST | 0.5 M $NaH_2PO_4$ (pH 7.0) | 3.5–9.5 | 1 M NaOH | 1 M $H_3PO_4$ |
| β-AMY | 0.05 M Tris-Hcl (pH 7.4) 0.1 M β-mercaptoethanol | 4.0–6.5 | 0.5 M β-Alanine | 0.5 M $H_3PO_4$ |

## Materials and methods

A total of 490 *Triticum tauschii* accessions are maintained in the CIMMYT wheat wide-crossing programs working collection. The collection is also maintained in CIMMYT's germplasm bank at El Batan, Mexico. Sixty accessions were employed in this study. These *T. tauschii* accessions are being utilized in the production of synthetic hexaploids (Mujeeb-Kazi et al. 1993) as well as for effecting direct transfers of some biotic and abiotic stresses to *T. aestivum* cultivars. Some of these synthetic hexaploids, togehter with their *T. turgidum* and *T. tauschii* parents, were also analyzed for seed protein and isozyme variations.

In keeping with previously reported work on HMW-glutenins and seed esterase (Lagudah and Halloran 1988, 1989) the superscript 't' has been used in assigning gene symbols to the D genome of *T. tauschii* to distinguish them from homologous gene loci in the D-genome of hexaploid wheat. Polymorphisms observed among 60 different accessions for the proteins and enzymes mentioned above are reported. *T. tauschii* accessions were analyzed, along with bread-wheat cultivars of known allelic combinations used as references, to identify the allelic combinations present for the *Glu-D$^t$1* locus.

### *SDS-PAGE*

High-molecular-weight subunits of glutenin were separated by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) on a Hoeffer vertical electrophoresis unit. Acrylamide concentration in the running gel was 8.5% or 10% with a 3% stacking gel being used in the discontinuous SDS-PAGE. Sample extraction and electrophoretic conditions were adopted from Payne et al. (1980). The gel dimensions were 14.5 × 16.5 × .15 cm. Gels were run at a constant current of 10 mA per gel for 2 h, followed by 15 mA per gel until the dye front reached the bottom of the gel. Gels were rinsed in 30% ethanol to remove SDS and stained in a solution of 0.1% coomassie brilliant blue R-250, 50% methanol and 10% acetic acid. Destaining was in a 40% methanol, 10% acetic acid solution. HMW-glutenin subunit identification was according to the classification of Payne and Lawrence (1983) and Lagudah and Halloran (1988).

### *Acid-PAGE*

Ethanol-soluble gliadins were extracted from endosperm portions and separated using polyacrylamide-gel electrophoresis on an acid medium (Acid-PAGE). The extraction procedure and the electrophoretic conditions were according to Ng et al. (1988) as modified by William et al. (1992). Gels were stained in 10% trichloroacetic acid (TCA) containing 0.1% coomassie brilliant blue G and destained in 10% TCA.

### *Separation of isozymes*

Isozymes of seed esterase (EST-5, E.C.3.1.1.1), glucose-phosphate isomerase (GPI, D-glucose 6-phosphate ketol-isomerase, E.C.5.3.1.9) and β-amylase (β-AMY, α-1,4-glucan maltohydrolase E.C.3.2.1.2) were separated using isoelectric focusing (IEF). Pharmacia PAG plates were utilized for IEF. The pH gradients of the gels, the buffer solutions used and the extraction solutions are given in Table 1.

Mature grains were ground and extracted with 300 µl of the respective extraction buffer solutions. The GPI staining solution was adopted from Chojecki and Gale (1982) while that for EST and β-AMY was similar to that reported by William and Mujeeb-Kazi (1992).

## Results and discussion

Polymorphisms among different *T. tauschii* accessions for esterase (Nakai 1979), α-amylase (Nishikawa et al. 1980), and for high-molecular-weight (HMW) glutenin subunits (Lawrence and Shepherd 1980; Lagudah and Halloran 1988) have been observed. Some of these have been studied further.

### *High-molecular-weight glutenin subunits*

A wide variation in HMW-glutenin subunit composition was observed among different accessions of *T. tauschii* (Fig. 1 a). The *Glu-D1* locus is characterized by the presence of a pair of subunits, one of faster mobility as compared to the other (Payne et al. 1981 a). Generally the *Glu-D$^t$1* locus is also characterized by a pair of bands with differential mobilities similar to the *Glu-D1* locus. Six alleles for *Glu-D1* have been reported in bread-wheat (Payne and Lawrence 1983). Fourteen *Glu-D$^t$1* subunit combinations were observed among different *T. tauschii* accessions (Fig. 1 a). Although we did not observe all six allelic forms reported for bread-wheat (Payne and Lawrence 1983) in the *T. tauschii* accessions, the 14 subunit combinations of *Glu-D$^t$1* are indicative of more polymorphism in *T. tauschii* than was reported for the *Glu-D1* locus of bread-wheat.

A number, 2.1, has been assigned for a *Glu-D$^t$1* subunit with a slower mobility than subunit 2 (Lagudah and Halloran 1988). We observed two different bands with slower mobilities as compared to subunit 2. One of these, corresponded to the 2.1 subunit, while the new subunit with a slightly faster mobility than 2.1, and not reported in either bread-wheat or *T. tauschii*, is identified as 1.5 (Fig. 1 a). The *Glu-D1* 2.2, 4 and 11 subunits of bread-

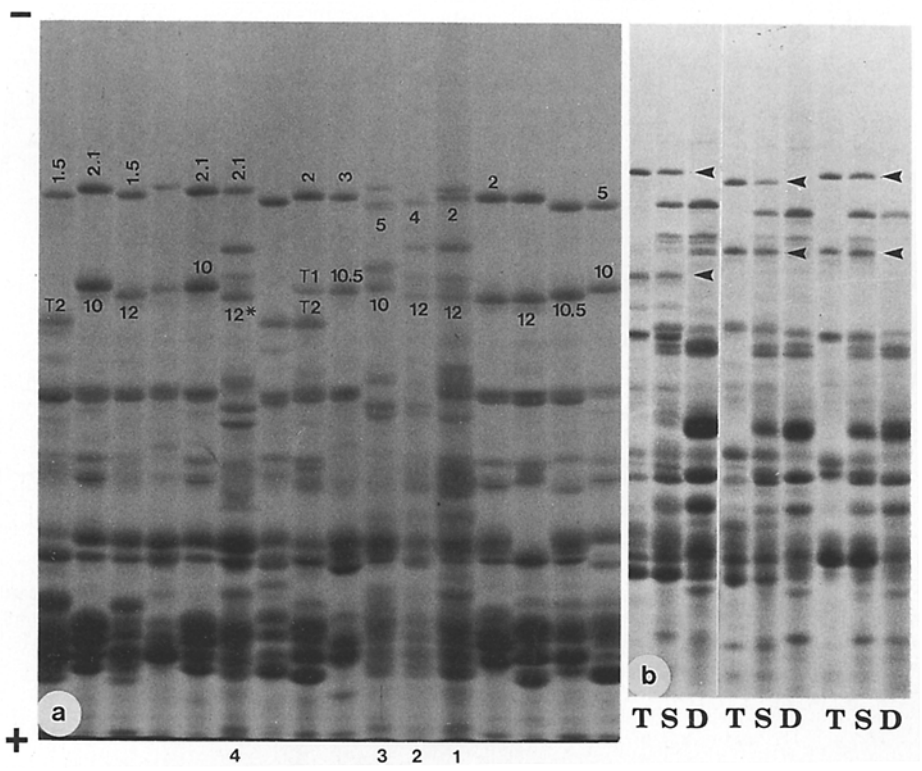


**Fig. 1 a, b.** High-molecular-weight subunits of glutenin on SDS-PAGE using 10% polyacrylamide gels. **a** Some of the *Glu-D$^t$1* allelic variations observed in *Triticum tauschii* accessions together with standards. The standards included are (*1*) 'Pitic' (1, 7+8, 2+12), (*2*) 'Champlin' (4+12), (*3*) 'L86-69' (1, 17+18, 5+10) and (*4*) *T. macha* biotype (2+12*), respectively. HMW-glutenins were separated on 8.5% polyacrylamide gels. **b** The allelic forms observed in the combinations of *T. tauschii* (*T*), the synthetic hexaploid *T. turgidum* × *T. tauschii* (*S*) and *T. turgidum* (*D*). The subunits specific to *T. tauschii* are *arrowed*

**Table 2.** Different *Glu-D$^t$1* allelic forms of *Triticum tauschii* together with the number of accessions that belonged to each group

| *Glu-D$^t$1* variants | No. of accessions |
|---|---|
| 3+10 | 11 |
| 5+10 | 4 |
| 5+12 | 1 |
| 2.1+T1+T2 | 1 |
| 2+12 | 8 |
| 3+12 | 1 |
| 2+T1+T2 | 7 |
| 3+T1+T2 | 1 |
| 1.5+T1+T2 | 1 |
| 1.5+10 | 3 |
| 2.1+10.5 | 1 |
| 1.5+12 | 4 |
| 2.1+10 | 3 |
| 3+10.5 | 7 |

wheat were not observed among the *T. tauschii* accessions examined in this study. Failure to observe subunit 2.2 among accessions of *T. tauschii* in the present study, as well as by Lagudah and Halloran (1988), adds evidence to the hypothesis of Payne et al. (1983) that subunit 2.2 arose within a hexaploid wheat by a rare unequal crossing over with another gene which coded for high-molecular-weight subunits. Most of the *Glu-D$^t$1* locus variants in *T. tauschii* accessions had a pair of subunit combinations (Fig. 1 a), but some possessed three i.e., 2, $T_1$ and $T_2$. Subunit $T_2$ had a faster mobility than subunit 12 which is the fastest moving subunit in bread-wheat. Though the occurrence of subunit $T_1$ was vague in some individual seeds of a given accession that possesses a $T_1+T_2$ combination, we have assumed that subunits $T_1$ and $T_2$ occur together. Hence the bands $1.5+T_2$ (Fig. 1 a) can also be interpreted as bands $1.5+T_1+T_2$ (Table 2). In addition to the newly reported 1.5 HMW-glutenin subunit, another subunit (designated as 10.5) was observed in some accessions with a slightly faster mobility than subunit 10 (Fig. 1 a). Whenever observed, this subunit occurred in 2.1+10.5 and 3+10.5 combinations. However, we do not discount the possibility that subunit 10.5 may be the same as subunit 11 reported earlier (Lagudah and Halloran 1988). All these *T. tauschii* accessions with diverse subunit combinations have been utilized in the production of synthetic hexaploids to examine the association between storage protein diversity and bread-making related parameters (Peña et al. 1991). Payne et al. (1981 b) established that the subunit 5+10 combination had a superior quality effect over the 2+12,

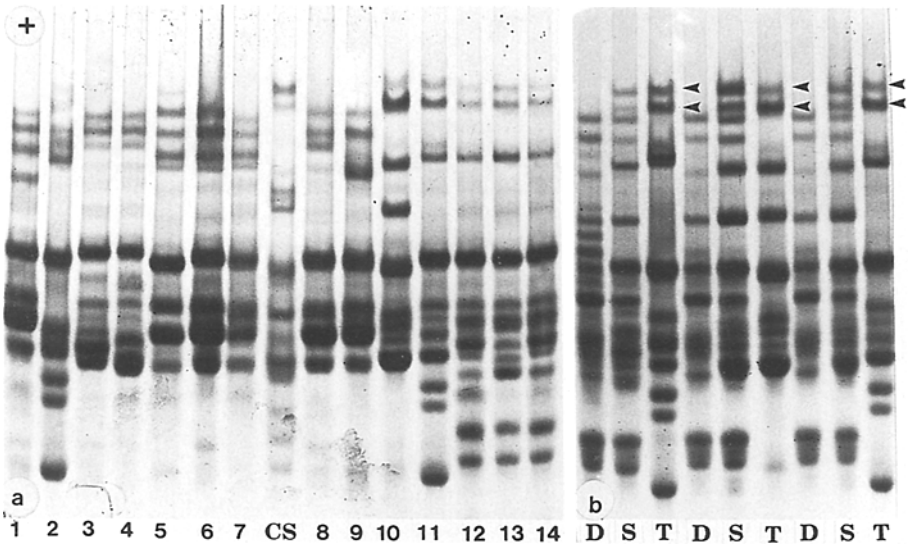


**Fig. 2a, b.** Gliadin banding profiles on Acid-PAGE. **a** A sample of different gliadin banding patterns observed in *Triticum tauschii* (*T*) accessions together with *T. aestivum* cv 'Chinese Spring' (*CS*). The *numbers 1–14* indicate banding patterns specific to 14 different accessions of *T. tauschii*. **b** The gliadin banding patterns observed in *T. turgidum* (*D*), synthetic hexploid (*S*) and *T. tauschii* (*T*). The bands specific to *T. tauschii* are *arrowed*

3+12 or 4+12 combinations, with the differential effects of the HMW-glutenins being strongest for subunits coded by chromosome 1D. Identification of some allelic combinations in the synthetic hexaploids equal or better than subunit 5+10 should lead to their being introduced into *T. aestivum* by breeding. A tight subunit 5+10 and 2+12 linkage in bread-wheat has been reported by Payne et al. (1981 a). Our analysis shows that subunit combinations 5+12, 3+10, 2.1+10 and 1.5+10 are present in *T. tauschii*. These combinations must also have been present in nature but presumably were not utilized in the evolutionary synthesis of bread-wheat. It is also possible that rare recombinations between tightly-linked different allelic forms may have resulted in accessions with subunits such as 5+12. A sample of synthetics was further evaluated for the HMW-glutenin subunits together with the durum and respective *T. tauschii* parents (Fig. 1 b). This demonstrated that the *T. tauschii* and *T. turgidum* allelic combinations were present in the synthetic hexaploids. Though we have not made crosses to establish the allelic nature of the HMW-glutenin subunit combinations present in *T. tauschii*, the expression of the HMW-glutenin subunits in the synthetic hexaploids is indicative of the allelic nature of *Glu-D$^t$1* locus variants. The number of *T. tauschii* accessions (53) with different HMW-glutenin subunit combinations are listed in Table 2.

*Gliadins*

In bread-wheat, gliadins are coded by genes on the short arms of homoeologous group 1 and 6 chromosomes (Wrigley and Shepherd 1973; Payne et al. 1982). Similar to the situation of *Glu-D1*, recombination events among allelic forms of gliadins have not been frequently observed (Sozinov and Poperelya 1980), and the prevalence of tightly-linked genes in the gliadin coding loci has been suggested (Kasarda 1980). Consequently, gliadins, are inherited as blocks or linked groups (Mecham et al. 1978) and multiple allelism of their coding loci in the two homoeologous groups of wheat has been established (Sozinov and Poperelya 1980; Metakovsky et al. 1984). We observed 56 different gliadin patterns among the *T. tauschii* accessions (Fig. 2 a) with polymorphisms for *Gli-D$^t$1* being greater than those for the *Glu-D$^t$1* locus. Quality parameters associated with the presence of individual gliadins are not as well defined as they are for glutenins; however, their impact on quality is well established and different gliadin blocks have been found to produce differential quality (Sozinov and Poperelya 1980; Payne et al. 1984). Metakovsky et al. (1984) reported that the polymorphism associated with chromosomes 1D and 6D, and hence the D genome of bread-wheat, was not as intense as the gliadin polymorphism of the A and B genomes. Hence, the incorporation of diversity from *T. tauschii* may increase the gliadin variation in bread-wheat, and the polymorphisms expressed in the synthetic hexaploids provides support for this concept (Fig. 2 b). Gliadins in the $\omega$-gliadin region of the *T. tauschii* accessions were always expressed in the synthetic hexaploids. There were cases, however, where not all of the $\alpha$- and $\beta$-gliadins of *T. tauschii* were present in the synthetic hexaploids.

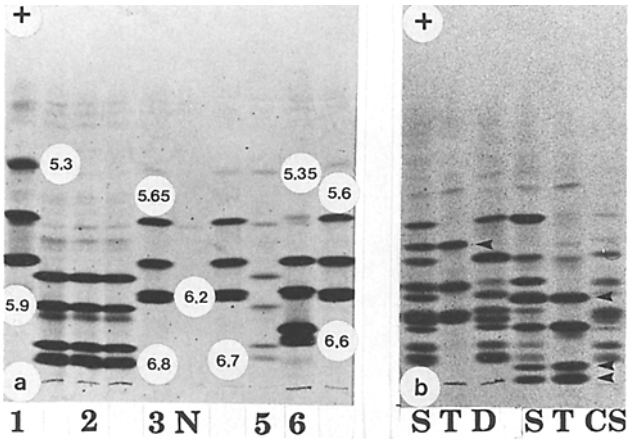


**Fig. 3a,b.** Separation of seed esterase isozymes on IEF (pH 3.5–9.5) polyacrylamide gels. **a** Different phenotypic groups of *Est-D$^t$5* are identified by numbers (*1, 2, 3, 4 and 6*) and the null phenotype is indicated by *N*. Isoelectric points of different bands in phenotypes are indicated. **b** The banding patterns observed in the combinations of *Triticum turgidum* (*D*), synthetic hexaploid (*S*) and *T. tauschii* (*T*). The bands specific to *T. tauschii* are *arrowed*. 'Chinese Spring' (*CS*) is included as a standard

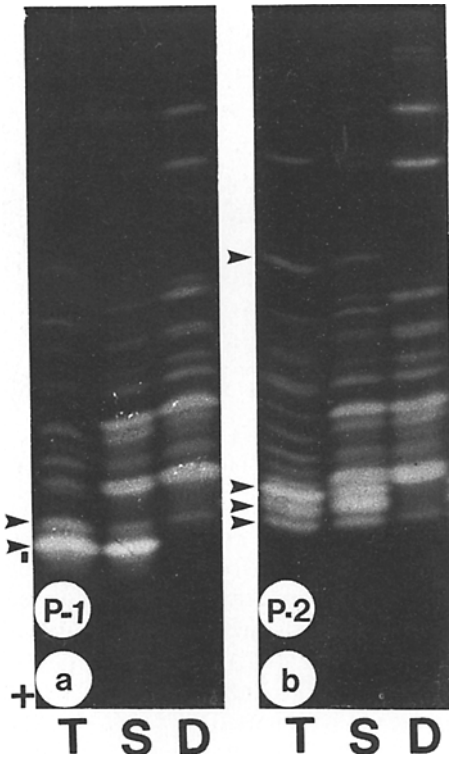


**Fig. 4a,b.** $\beta$-Amylase isozymes separated on IEF (pH 4–6.5) polyacrylamide gels. The two $\beta$-AMY phenotypes observed among different *Triticum tauschii* accessions are identified as *P-1* and *P-2* in **a** and **b** respectively. $\beta$-AMY phenotypes in the combinations of *T. tauschii* (*T*), the synthetic hexaploid (*S*) and *T. turgidum* (*D*). The bands specific to *T. tauschii* are *arrowed*

*Seed esterase (EST-5)*

A total of six *Est-D$^t$5* phenotypic classes were found among different accessions of *T. tauschii* (Fig. 3a). One particular accession (INTERVER 329) did not have a detectable IEF banding profile and may be considered as a null (N) phenotype (Fig. 3a) as reported by Lagudah and Halloran (1989) for seed esterase. Two different *Est-A5*, four *Est-B5* and four *Est-D5* alleles have been reported for the *Est-5* locus (Ainsworth et al. 1984). Polymorphism among different phenotypes suggests that more variability is present for the *Est-D$^t$5* locus than for *Est-D5* in bread-wheat. In the analysis of EST banding patterns, vague bands at the acidic end of the gel have been excluded as was also done in the genetic analysis of grain EST genes in bread-wheat (Ainsworth et al. 1984). Thirty four accessions belonged to the *Est-D$^t$5* phenotype 2, having a total of seven bands of which five were sharp and two had reduced intensity in the pI range of 5.65–6.8 (Fig. 3a). The two most basic bands in phenotype 2 had more basic pI values as compared to the most cathodal band of 'Chinese Spring'. The second most abundant *Est-D$^t$5* phenotype, 3, was characterized by five bands; four sharp and one of low intensity in the 6.2–5.35 pI range. Nineteen accessions belonged to *Est-D$^t$5* phenotype 3. Three accessions belonged to *Est-D$^t$5* phenotype 1 possessing four bands in the pI range of 5.9–5.3, three being sharp and one of reduced intensity. *Est-D$^t$5* phenotype 5 had six bands within pI values of 6.7–5.35, lighter in intensity as compared to the other phenotypes. Only one accession (INTERVER 368) belonged to this phenotype. Two accessions (INTERVER 216, 365) belonged to phenotype 6 with a pattern of six bands within the 6.6–5.6 pI range. One of the bands was light with reduced intensity. The two most cathodal bands of *Est-D$^t$5* phenotype 5 corresponded with those of 'Chinese Spring'; this is intriguing, since these bands are known to be coded by genes on chromosome 3AL (Ainsworth et al. 1984). Our results indicate that the D genome of *T. tauschii* possesses more variability for *Est-5* than is present in the D genome of bread-wheat. All the phenotypic classes observed in the *T. tauschii* accessions were expressed in the synthetic hexaploids, of which two are shown in Fig. 3b.

*Beta-amylase ($\beta$-AMY)*

Mainly two different *$\beta$-Amy-D$^t$1* phenotypes were present in different accessions of *T. tauschii* (Fig. 4). Phenotype 1 possessed a banding profile of 12 bands. Phenotype 2 had a profile of 13/14 bands. The major difference between them was that phenotype 1 had two high intensity bands in the more acidic region (Fig. 4a, P-1). A majority of *T. tauschii* accessions (55) belonged to phenotype 2 (Fig. 4b, P-2). Both *T. tauschii* phenotypes were expressed in the synthetic hexaploids (Fig. 4). In bread-

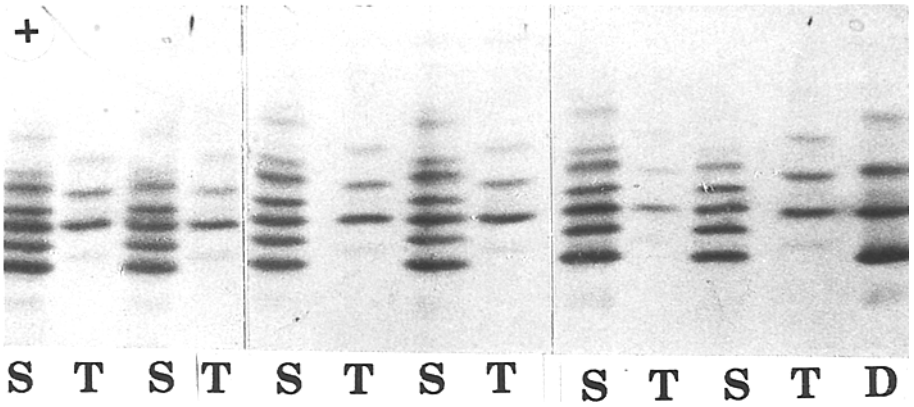


**Fig. 5.** Seed GPI banding profiles on IEF (pH 3.5–9.5) polyacrylamide gels. Banding patterns of different synthetic hexaploids (*S*), *Triticum tauschii* (*T*) and *T. turgidum* (*D*) are shown

wheat, Ainsworth et al. (1983) reported 11 phenotypic groups among 46 hexaploid wheat varieties evaluated, but the majority of the wheat varieties tested belonged to two phenotypic groups. Our study indicates less phenotypic variation for *β-Amy-D$^t$1* in the *T. tauschii* accessions tested in comparison to that reported for *β-Amy-D1*. It is possible that in bread-wheat (an allopolyploid), as compared to *T. tauschii*, allelic variations result from possible mutational events and are better tolerated.

*Glucose-phosphate isomerase (GPI)*

There was no polymorphism in *T. tauschii* accessions for GPI (Fig. 5) indicating a conservation of the molecular nature of GPI. Chojecki and Gale (1982) reported very little polymorphism among different bread-wheats for seed GPI and, since GPI is an important enzyme in the glucose metabolic pathway, it is possible that there was selection against mutations. Durum wheat had a banding profile with 4/5 bands (Fig. 5). When the D genome of *T. tauschii* was combined with the A and B genomes of durum wheats in the form of synthetic hexaploids, a GPI banding profile with a multitude of bands similar to the banding profiles of bread-wheats was observed (Fig. 5). This resulted from the interaction of GPI-D$^t$1 subunits with those of the A and B genome in forming active GPI dimers. Although bread-wheats may have naturally evolved from a small number of *T. tauschii* accessions (Lagudah et al. 1991) GPI-D$^t$1 subunits of any *T. tauschii* accession are capable of interacting with the A and B genome subunits to give rise to the bread-wheat banding profile. The possibility of interaction of the GPI-D$^t$1 subunits with those of the A and B genomes which result in the bread-wheat banding profile further indicates a close homoeology between GPI subunits from different genomes, thus adding more evidence to the phylogenetic relationship of *T. tauschii* as the D-genome donor to hexaploid wheat.

## Conclusions

Extensive unique variation for high-molecular-weight glutenins and gliadins was observed among different accessions of *T. tauschii*. These polymorphisms were readily expressed in synthetic hexaploids. Since both HMW-glutenins and gliadins are implicated in bread-making quality, exploiting the synthetic hexaploids in conventional wheat breeding programs is an important means of increasing genetic variability. Though polymorphism associated with the low-molecular-weight glutenin genes that occur at the *Glu-D$^t$3* locus was not evaluated, there is a strong possibility that *Glu-D$^t$3* in *T. tauschii* may also show polymorphism.

Much greater variation has been found in storage proteins of wild wheat relatives such as diploid *Triticum* and *Aegilops* species (Law and Payne 1983). Incorporation of wide variability into bread-wheat from *Aegilops* species poses difficulties due to the presence of the "pairing homoeologous" *Ph* gene on chromosome 5B, although efforts have been made to transfer *Glu-1* genes from *Ae. umbellulata* to wheat (Law et al. 1984). The procedure involved in using *T. tauschii* are less complicated because the synthetic hexaploids can be directly used in conventional breeding programs. There are also additional options of effecting direct transfers from *T. tauschii* to *T. aestivum* (Alonso and Kimber 1984; Gill and Raupp 1987; Mujeeb-Kazi et al. 1993).

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