Theor Appl Genet (1989) 77:851–856

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

3. Variation in, and the genetics of, seed esterases (*Est-5*)

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Received November 21, 1988; Accepted February 6, 1989
Communicated by F. Salamini

Summary. Isoelectric focusing of seed esterase (*Est-5*) isozymes in 79 *T. tauschii* accessions from diverse sources revealed the presence of six different seed esterase phenotypes. In one of these phenotypes, exclusive to a var. *meyeri* accession (AUS 18989), no detectable enzymatic activity was observed. Segregation in crosses between *T. tauschii* (D^t) accessions confirmed three of the seed esterase phenotypes to be alleles of the designated *Est-*D^t *5* gene locus; the inheritance pattern of these isozymes was not affected by the subspecies differences between the parents. On the bases of variation in *Est-5* and their *Glu-1* and *Gli-1* gene loci (in a previous study in this series), only three *strangulata* accessions showed consistent homology with their prevalent gene expression in the D genome of hexaploid wheat. The implications of these observations for further interpreting the phyletic nature of the D genome donor in natural hexaploid wheat synthesis are also reported.

Key words: Seed esterases – D genome – Isozymes – *T. tauschii* – Phyletic origin

Introduction

At least five sets of homoeologous structural gene loci located on group 3 chromosomes (*Est-1, Est-2* and *Est-5*), the long arm of group 6 chromosomes (*Est-4*) and the short arm of chromosomes 7B and 7D (*Est-3*), have been identified in controlling esterase isozymes in hexaploid wheat (Hart 1987; Ainsworth et al. 1984). Ainsworth et al. (1984) reported four zymogram patterns of seed esterases coded by the *Est-D5* locus which were considered as allelic variants of this locus in the D genome.

Intraspecific variation in seedling (coleoptile, primary leaf and root tissue) esterases occurs in the putative diploid donor of the D genome, *T. tauschii*, but the frequency of some of the allozymes are reflected in their subspecies classification (Jaaska 1980). Three seed esterase phenotypes (types 1, 2 and 3) were reported by Nakai (1979) in *T. tauschii* accessions, and in crosses between them, the 'type 2' and 'type 3' (designated *Est-*D^t *5b* and *c*, respectively) phenotypes of the F_2 seed revealed segregation in ratios consistent with allelic variation at a single locus. However, conclusions on the genetic basis of variation between the 'type 1' (*Est-*D^t *5a*) and 'type 2' phenotypes could be made only on the dominance of a single major band of the 'type 2' isozymes with respect to the null region of the 'type 1' zymogram.

The present study reports on newly identified seed esterase phenotypes and their common types in *T. tauschii* accessions which, in conjunction with their previously reported variation of the *Glu-1* and *Gli-1* gene loci (Lagudah and Halloran 1988), have been used in assessing their homology with the D genome of hexaploid wheat. The implications of these observations in providing evidence for either a mono- or polyphyletic origin of the D genome donor in natural hexaploid synthesis have been discussed. For inheritance studies of seed esterases, the same parental cross made by Nakai (1979) in studying the genetics of the 'type 1' (*Est-*D^t *5a*) and 'type 2' (*Est-*D^t *5b*) phenotypes was repeated in view of the marked differences detected between *Est-*D^t *5a* and *Est-*D^t *5b* isozymes in the present study.

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Materials and methods

Plant material

T. tauschii accessions and hexaploid wheat species examined were the same as previously described (Lagudah and Halloran 1988). Ditelocentric lines of chromosome 3D of 'Chinese Spring' were used in identifying the commonly occurring *Est-D5* isozymes in hexaploid wheat.

F_1 seeds of different crosses using the following accessions from the subspecific taxa of *T. tauschii* were analysed for their seed esterase expression: var. *strangulata,* KUSE 2135, 180-1470, 185-1487-7 AUS 18987; var. *meyeri,* 18-895, KUSE 2144; var. *typica,* 211-1624, 215-1662. F_2 seeds were analysed in two crosses, 185-1487-7 × AUS 18987 and KUSE 2144 × 2135.

Isoelectric focusing (IEF) of seed esterase isozymes

Enzyme extraction was carried out on mature seeds placed overnight on moist filter paper and homogenized in 0.5 ml/seed of 50 m*M* potassium phosphate buffer at pH 7.0. The homogenized mixture was centrifuged at 20,000 rpm for 15 min at 2 °C and the supernatant was stored at −20 °C until ready for use. Isozymes were analysed by IEF in a horizontal polyacrylamide gel (2 mm thick) containing 2% carrier ampholytes (LKB) ~ 1.5%, pH 6–8 and 0.5% pH 7–9 ampholine – tailored to give a pH gradient of 5.5–7.7. Electrode solutions were 1 *M* NaOH and 2% ampholine (pH 4–6) for the cathode and anode, respectively. Filter paper pieces (Whatman 3 mm Paratex) were soaked in thawed samples of enzyme extracts and placed 1.5 cm from the cathode on a prefocused gel. Gels were prefocused at 20 W for 30 min and following sample application, focusing was initially at 4 W for 15 min and then increased to 8 W for another 15 min. The pH gradient was determined using marker proteins with known pI's (Pharmacia, High pI calibration kit, pH 5–10.5).

Esterase activity was visualized by incubating the gels at room temperature in the following mixture: 4 ml of 1% α-naphtyl acetate in 60% acetone and 80 mg fast blue RR in 200 ml of 70 *M* phosphate buffer, pH 7.0. After staining for about 10–15 min, the gel was stored in 7% acetic acid.

Results and discussion

Variation in Est-5 in T. tauschii and in the D genome of hexaploid wheat species

T. tauschii. Isoelectric focusing of seed esterases in the *T. tauschii* accessions (*Est-D*^{t} *5*) revealed polymorphism for this character, with isoelectric points (pI) ranging from pH 5.55 to 7.07 (Fig. 1 A). A total of six esterase phenotype classes were observed (*Est-D*^{t} *5a-f*); the allelic designation for three of the phenotypes have been confirmed (see below) while those of *-d, -e* and *-f* are regarded as tentative. Three zymogram phenotypes, *Est-D*^{t} *5a, -b* and *-c* (Fig. 1 A and B), were grouped in a similar way as Nakai's (1979, 1981) type 1, 2 and 3 phenotypes, respectively, but the nomenclature in the present study was based solely on pI's because slight variations were observed in the thin layer IEF system used. The clear separation between closely migrating bands in this system, otherwise reported as single bands with high activity (Nakai 1979), resulted in an increased number of bands within esterase phenotypic classes.

The other esterase isozymes of *T. tauschii, Est-D*^{t} *5d, -e* (Fig. 1) and *-f,* which are exclusive to three individual accessions, are not known to have been reported in any previous work. The single-banded isozyme, *Est-D*^{t} *5d* (Fig. 1) of the accession D6 (var. *typica*), was geographically located in a predominantly *Est-D*^{t} *5c* phenotypic class. The accession originated in northern Afghanistan in an area that was predominantly var. *typica.* However, neither the plant morphological characters nor the available habitat data (Halloran 1968) revealed any major

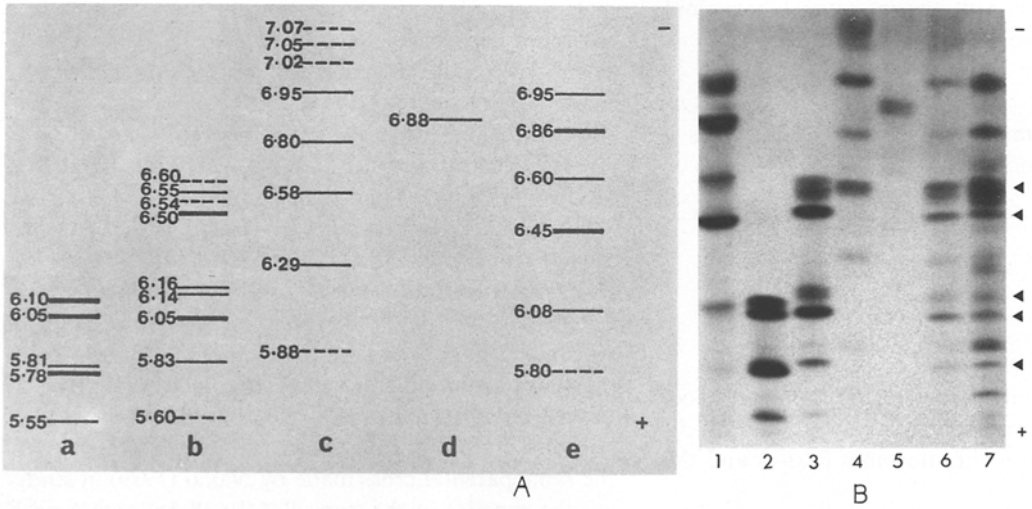

Fig. 1. A Diagrammatic representation of *Est-D*^{t} *5* phenotypes found in accessions of *T. tauschii.* Values against esterase bands refer to their isoelectric points. The frequency (no. of accessions) of the phenotypes are as follows: *a* (2), *b* (37), *c* (37), *d* (1), *e* (1). **B** Seed esterase zymograms of *T. tauschii. 1 – Est-D*^{t} *5e; 2 – EstD*^{t} *5a; 3 – EstD*^{t} *5b; 4 – EstD*^{t} *5c; 5 – EstD*^{t} *5d; 6* – mixture of *EstD*^{t} *5b* and *-c* (1:1); *7* – 'Chinese Spring' pattern. *Arrows* indicate chromosome 3DL coding region

differences between the accession D6 and the others within the region.

In contrast to all the other phenotypes, no enzymatic activity was detected for the *Est-5* assay in the seeds of the accession AUS 18989 var. *meyeri* and, hence, it was considered to be a null phenotype for this character. Null isozyme phenotypes are known to occur either naturally or are able to be induced in some plants (Goodman et al. 1981; Harberd and Edwards 1982) and in some instances, immunological analysis has confirmed the presence of the protein-encoding loci but with a loss of enzymatic activity (McMillin and Scandalios 1982; Brown and Hanson 1983).

Hexaploid wheat species. Seven distinct esterase phenotypes were observed (Fig. 2); six of these variants were found in three accessions of *T. macha* (AUS 10733, 14273, WJR 38548), two in *T. vavilovii* (AUS 10950, 10961) and one in *T. compactum* (AUS 12084), of which the latter was very similar to that of the *Est-A5b* allozyme in hexaploid wheat, described by Ainsworth et al. (1984).

Most of the hexaploid wheat species exhibited the 'Chinese Spring' esterase zymogram pattern and at least five esterase bands (pI 6.60, 6.50, 6.14, 6.05 and 5.83) identified to be controlled by the long arm of chromosome 3D (Figs. 1 B and 2) were found to correspond with the major bands of the *Est-D^t 5b* phenotypes of *T. tauschii*. A few other bands from *Est-D^t 5a* (pI 6.05), *Est-D^t 5c* (pI 6.95, 6.80) and *Est-D^t 5e* (pI 6.95, 6.60) occurred in the 'Chinese Spring' zymogram. Nevertheless, esterase bands with pI 6.95 and 6.80 in 'Chinese Spring' were not under the control of the structural gene on the long arm of chromosome 3D. In addition, none of the other esterase variants of the hexaploid species was found to possess the entire spectrum of any particular zymogram from *Est-D^t 5a, -c, -d* and *-e*. One *T. macha* accession, WJR 38548, lacked all the bands controlled by chromosome 3DL as well as bands with pI 5.55, 5.60, 6.24, 6.57 and 6.68 of 'Chinese Spring' (Fig. 2). The reduction in esterase activity associated with the pI 6.05 band of ditelocentric 3DS (Fig. 2) was attributed to the absence of the structural gene loci on the long arm of chromosome 3D and, hence, the faint band expression at this position in WJR 38548 was considered to be affected by the non-expression of the structural gene locus on 3D coding for the band co-migrating with the pI 6.05 band.

The prevalent 'Chinese Spring' esterase phenotype, with particular reference to isozymes controlled by chromosome 3DL in the hexaploid species, is in agreement with the findings of Nakai (1973, 1979) and Ainsworth et al. (1984). Thus the *Est-D^t 5b* phenotype of *T. tauschii* which possesses esterase bands corresponding to those of the 'Chinese Spring' *Est-D5* allele is more closely related to that in the D genome of hexaploid wheat than are the other *Est-D^t 5* variants. The absence of isozymes at the

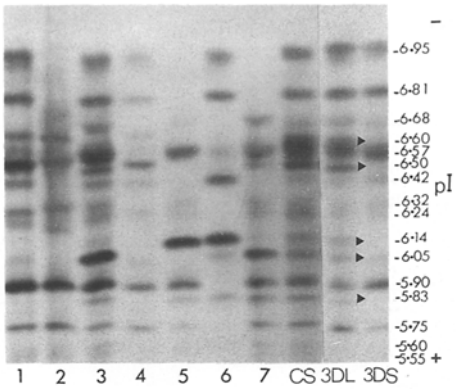

Fig. 2. Seed esterase phenotypes of hexaploid wheat species. *1* – *T. macha*, AUS 10733; *2* – *T. macha*, AUS 14273; *3* – *T. macha* AUS 14519; *4* – *T. macha* WJR 38548; *5* – *T. vavilovii*, AUS 10950; *6* – *T. vavilovii*, AUS 10961; *7* – *T. compactum* AUS 12084; *CS* – 'Chinese Spring'; 3DL and 3DS, ditelocentrics of the long and short arms, respectively, of chromosome 3D. *Arrows* indicate coding region of 3DL

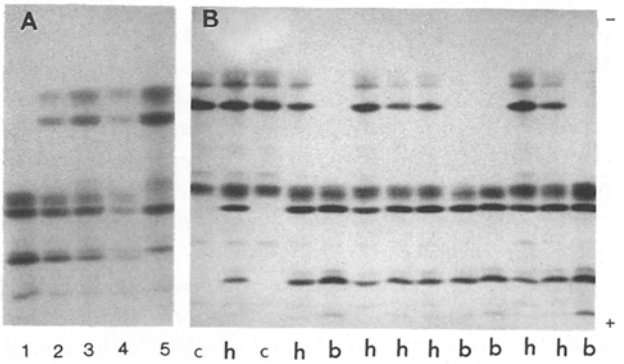

Fig. 3. **A** Esterase in the F_1 seed of *T. tauschii* crosses. *1* – *EstD^t 5a* phenotype; *2* and *3* – reciprocal crosses of KUSE 2135 × 2144 (*EstD^t 5b/a*); *4* – 211-1624 × KUSE 2135 (*EstD^t 5b/a*); *5* – *EstD^t 5b* phenotype. **B.** F_2 segregation of *EstD^t 5* isozymes in the cross of the *T. tauschii* accessions 195-1487-7 × AUS 18987. *b* – *EstD^t 5b; c EstD^t 5c; h* – heterozygous seed

chromosome 3D region of WJR 38548 and the unidentified *T. macha* accession, reported by Ainsworth et al. (1984), could perhaps be attributed to the discovery in *T. tauschii* of the null esterase phenotype (*Est-D^t 5f*) contributing to the variation in the D genome of hexaploid wheat.

Inheritance of Est-D^t 5

The parental esterase phenotypes – *Est-D^t 5a, -b* and *-c* – occurred in all the F_1 seed irrespective of the subspecific taxa of *T. tauschii*. The reciprocal crosses between KUSE 2135 and 2144 were qualitatively similar in their isozyme composition, although the expression of a few bands appeared to differ quantitatively (Fig. 3 A). In general,

Table 1. F_2 segregation of seed esterases (*Est-D^t 5*) in *T. tauschii* crosses

Cross	Progeny			χ^2 value (1:2:1)	*P*
	Est-D^t a	*Est-D^t 5a/b*[a]	*Est-D^t 5b*		
KUSE 2144 × KUSE 2135					
(*Est-D^t 5b*) (*Est-D^t 5a*)	24	39	25	1.16	0.50–0.75
185-1487-7 × 18987	*Est-D^t 5b*	*Est-D^t 5b/c*[a]	*Est-D^t 5c*		
(*Est-D^t 5c*) (*Est-D^t 5b*)	26	46	31	1.66	0.25–0.50

[a] Heterozygotes

three esterase patterns were observed in the F_2 seeds examined: parental phenotypes and F_1 pattern from the *T. tauschii* crosses, KUSE 2144 × KUSE 2135 and 185-1487-7 × AUS 18987 (Fig. 3). The segregation of both crosses fit the ratio (1:2:1) expected for monogenic inheritance (Table 1).

On the basis of the observed ratios of these esterase phenotypes in the F_2 seed, it is inferred that they constitute multiple allelic forms of the *Est-D^t 5* locus. The segregation ratio of the *Est-D^t 5b* and *Est-D^t 5c* phenotypes (1:1) agrees with the results obtained by Nakai (1979). In addition, the pattern of segregation was independent of the varietal form of *T. tauschii;* the parental zymograms of *Est-D^t 5b* and *-c* used in this study were between *strangulata* accessions, while those of Nakai (1979) were between *strangulata* and *typica* accessions.

The isozymes described by Ainsworth et al. (1984), which are coded by *Est-D5a* and *-d* alleles of hexaploid wheat, are similar to the range of isoelectric points of the *Est-D^t 5b* and *-c* allozymes, respectively. Moreover, the source of the *Est-D5d* allele was from a chromosome 3D substitution line of 'Chinese Spring' derived from the *T. tauschii* parent of a synthetic hexaploid. Though the isozyme patterns obtained in the F_2 seed from a cross between the *Est-D5a* and *-d* allele occurred as either of the parental types or their heterozygous form, they deviated from the expected 1:2:1 ratio (Ainsworth et al. 1984).

It was evident from this study that the heterozygotes from the cross between the *Est-D^t 5a* and *-b* phenotypes (KUSE 2144 × 2135) were clearly distinguished from either parental forms. Consequently, there was no ambiguity in establishing the segregation patterns expected for a 1 (*Est-D^t 5a*): 2 (*Est-D^t 5a/b*): 1 (*Est-D^t 5b*) ratio in the F_2 seeds (Table 1). In contrast, Nakai (1979) reported a segregation ratio of 1 ('type 1'): 3 ('type 2') from the same cross as the one re-investigated in this study. In resolving the apparent contradiction, the conclusions of Nakai (1979) needed to be reinterpreted. The higher frequency reported for the 'type 2' (*Est-D^t 5b*) esterase phenotype in the F_2 seeds of his study was, in effect, a combination of both the heterozygote and 'type 2' zymograms, since the 'type 2' seeds were distinguished from the 'type 1' seed by the presence of an extra major band in the former esterase phenotype. Furthermore, the validity of the conclusion of a single dominant gene controlling the extra band in the 'type 2' zymogram can be based only on the assumption that this extra band and its corresponding null region in the 'type 1' phenotype are coded for by a gene locus distinct from the other esterase bands. From the current study, however, isozymes of both *Est-D^t 5a* and *-b,* distinguished by more than one band, segregated as a single compound locus and there was little evidence for the occurrence of recombinant esterase phenotypes.

Phyletic nature of the D genome donor in natural synthesis of hexaploid wheat

On the basis of similarities in seed esterase isozymes between *T. tauschii* (*Est-D^t 5b*) and hexaploid wheat, a monophyletic origin of hexaploid wheat could be deduced, given that the prevalent occurrence of the *Est-D5a* allele of hexaploid wheat could be traced to its corresponding locus, *Est-D^t 5b,* in *T. tauschii.* However, doubt could be cast on this conclusion by the detection of the null (absence of seed esterase expression) variant, *Est-D^t 5f,* reported in this study, which presumably was the source of the rare *Est-D5b* null allele in hexaploids wheat. On the other hand, this possibility may be discounted on the grounds that the *Est-D5b* allele could have evolved independently in hexaploid wheat.

A polyphyletic origin of hexaploid wheat could be proposed in considering the reported variation in high-molecular-weight (HMW) subunits of glutenin and gliadins (*Gli-1*) in hexaploid wheat and their analogous forms in *T. tauschii* (Lagudah and Halloran 1988). It was also apparent from the extensive polymorphism of the *Gli-D^t 1* locus that a limited number of forms were involved in hexaploid wheat synthesis. In this study, *T. tauschii* strains from their easternmost habitats, e.g. Tibet and China, were not included. In further support of a polyphyletic origin, recent claims that some special

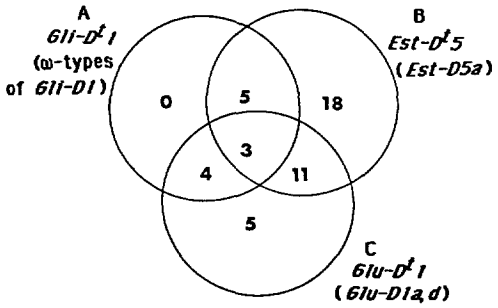

Fig. 4. Venn diagram showing *B:* interrelationships between the number of *T. tauschii* accessions possessing types of seed esterases, *A:* ω-gliadins similar to hexaploid wheats, and *C:* HMW glutenin subunits, corresponding to the prevalent forms in the D genome of hexaploid wheat (shown in *brackets*)

forms of primitive hexaploid wheat, found exclusively in China, were not introduced into China from West Asia have been based on similarities of coleoptile esterase zymograms in *T. tauschii* from areas of its natural distribution in China, as distinct from their sources in Iran and the Caucasus (Yen et al. 1983). Given this possibility, however, no evidence has yet been provided that these primitive hexaploids carry the coleoptile esterase phenotypes identified from the Chinese sources of *T. tauschii.*

In these series of studies, the relatedness of the *Est-D5* and the major forms of the *Glu-D1* and ω-gliadins of the *Gli-D1* loci (Lagudah and Halloran 1988) of hexaploid wheat to their corresponding variants in *T. tauschii* have been documented. By superimposing the variants within these selected groups of enzyme and protein markers, *T. tauschii* accessions with the closest affinity to the D genome of hexaploid wheats can be identified, as illustrated in the Venn diagram (Fig. 4). On these bases, three accessions – 184-1481, AUS 18898 and AUS 18985 – of var. *strangulata* from the 79 accessions of *T. tauschii* examined in these studies showed the closest affinity with the D genome of hexaploid wheats. The accession, 184-1481, was collected from a site 55 km west of Gorgan just southeast of the Caspian Sea (Melbourne University Plant Collecting Expedition, Asia Minor 1975); incidentally, this region has been considered to be the possible origin of the main line of descent of hexaploid wheat on the basis of its ecology and phylogenetic assessments of α- and β-amylases of the D genome (Nishikawa et al. 1980; Nishikawa 1983). However, the Transcaucasus and the area just southwest of the Caspian Sea have also been proposed as areas of the origin of hexaploid wheat (Tsunewaki 1968; Kihara et al. 1965; Nakai 1979; Jaaska 1980).

Conclusions made in the phylogenetic assessments between *T. tauschii* and the D genome of hexaploid wheat, based on specific protein markers, are at best, gene-specific and do not necessarily encompass their entire genomes. Although the present study identified three *strangulata* accessions as possible donors of the D genome (on the basis of three markers associated with two chromosomes), it is possible that other markers may fail to confirm a consistent 'homology' of these accessions with the D genome of hexaploid wheat. To circumvent this problem, the use of markers associated with both chromosome arms of the entire D genome would provide more substantial evidence for nominating the particular source of *T. tauschii* as the putative diploid donor. This approach would have to assume that homologous allelic variation for the genes so chosen would have remained conserved in both the putative diploid donor and the D genome of hexaploid wheat, discounting the possibility for parallel genetic variation occurring in *T. tauschii* and the D genome of hexaploid wheat subsequent to the origin of the hexaploid; thus, the variant forms in the D genome of hexaploid wheat would not be related by descent from *T. tauschii.* The case in point is the null seed esterase phenotypes described previously and also the relatedness of the HMW glutenin subunits in mobility on an SDS-PAGE system but not in their isoelectric points (Lagudah and Halloran 1988).

Acknowledgements. We thank Dr. U. Kushnir, Volcani Institute, Israel, for helpful discussions in the course of this study.

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