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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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*') accessions confirmed three of the seed esterase phenotypes to be alleles of the designated *Est-D5* 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-D5b* and *c*, respectively) phenotypes of the F₂ 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-D5a*) 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-D5a*) and 'type 2' (*Est-D5b*) phenotypes was repeated in view of the marked differences detected between *Est-D5a* and *Est-D5b* isozymes in the present study.
Materials and methods

Plant material

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

F₁ 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. F₂ 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 mM 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) 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-D₅) revealed polymorphism for this character, with isoelectric points (pI) ranging from pH 5.55 to 7.07 (Fig. 1A). A total of six esterase phenotype classes were observed (Est-D₅ 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-D₅ 5a, -b and -c (Fig. 1A 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-D₅ d, -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-D₅ d (Fig. 1) of the accession D6 (var. typica), which was located in a predominantly ester-D₅ c 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-D₅ 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-D₅ 5c; 2 - Est-D₅ 5a; 3 - Est-D₅ 5b; 4 - Est-D₅ 5c; 5 - Est-D₅ 5d; 6 - mixture of Est-D₅ 5b and -e (1:1); 7 - 'Chinese Spring' pattern. Arrows indicate chromosome 3DL coding region.](image-url)