Theor Appl Genet (1990) 79:781–784

Est-7, a set of genes controlling green tissue esterases in wheat and related species

C. J. Liu and M. D. Gale*

Cambridge Laboratory, Colney Lane, Norwich NR4 7UJ, UK

Received January 5, 1990; Accepted February 9, 1990
Communicated by J. W. Snape

Summary. Analysis using isoelectric focusing of "Chinese Spring" wheat genetic stocks revealed a set of coleoptile and leaf esterase loci, designated *Est-7*, on the long arms of the group 2 chromosomes. A survey of 38 other hexaploid genotypes revealed onyl a single variant, at *Est-D7*. Homoeoloci were found on chromosome (arm) *2HL* of *Hordeum vulgare*, *2RL* of *Secale cereale*, $2R^{m}\alpha$ of *S. montanum*, *2U* of *Aegilops umbellulata*, *2E* of *Agropyron elongatum* and *2V* of *Dasypyrum villosa*.

Key words: Esterase – Isozymes – Wheat – Triticeae

Introduction

In hexaploid wheat, *Triticum aestivum* (2n = 6x = 42), six sets of loci encoding esterase (E.C.3.1.1.1) isozyme production have been identified; *Est-1*, on the short arms of homoeologous group 3 chromosomes; *Est-2* and *Est-5*, on the long arms of group 3; *Est-3*, on the short arms of group 7; *Est-4*, on the long arms of group 6 chromosomes (all references in McIntosh 1988); and *Est-6*, recently identified on the short arms of group 2 chromosomes (Petchey et al. 1990). Isozymes encoded by some of these loci have been demonstrated to be tissue-specific. *Est-2*, encodes coleoptile-specific isozymes (Jaaska 1980), *Est-4* encodes leaf-specific isozymes (May et al. 1973; Nakai 1976; Jaaska 1980) and *Est-5* encodes mature grain-specific isozymes (Ainsworth et al. 1984). Isozymes encoded by *Est-6* are dimeric, while the remaining five sets of gene encode monomeric isozymes. In this paper we report another set of esterase loci, *Est-7*, which encode monomeric, green tissue-specific isozymes.

* To whom correspondence should be addressed

Materials and methods

Genetic stocks

The following genetic stocks were examined: (a) All the available nullisomic-tetrasomic and the homoeologous group 2 ditelosomic lines of "Chines Spring" ("CS") (Sears 1954, 1966 a, b). (b) Thirty-nine diverse hexaploid wheat varieties (Liu et al. 1990) maintained by A. J. Worland (Institute of Plant Science Research, Cambridge Laboratory), including "Synthetic 6x" (IPSR 1190903), the *T. dicoccum* × *Aegilops squarrosa* amphiploid (McFadden and Sears 1946). (c) Intervarietal single chromosome substitution lines of "CS" ("Synthetic") developed by C.N. Law and A.J. Worland (IPSR Cambridge Laboratory). (d) Wheat-alien additions and/or substitution lines: "CS"/*Hordeum vulgare* cv "Betzes" additions (Islam et al. 1981), "CS"/*Secale cereale* cv "Imperial" additions (Driscoll and Sears 1971) and substitutions (Sears 1968), "Holdfast"/*S. cereale* var. "King II" substitutions (Chapman and Miller 1979), "CS"/*S. montanum* additions (Miller 1974), "CS"/*Ae. longissima* additions (Feldman 1975), "CS"/*Ae. umbellulata* additions (Kimber 1967), "CS"/*Agropyron elongatum* additions (Dvorak and Knott 1974) and "CS"/*Dasypyrum villosum* additions, kindly provided by Professor E.R. Sears, University of Missouri, Columbia.

Enzyme analysis

Four- to six-day-old seedlings were used for analysis. Sample extraction and isoelectric focusing (IEF) in the high pH range, using 17 cm gels, were carried out as described for peroxidase (Liu et al. 1990), with extraction as for PER-1 and IEF as for PER-2. Isozyme activity was visualized using a mixture of 50 mg α-naphthyl acetate and 100 mg. Fast Blue RR salt in 100 ml of 0.1 *M* Tris-HCl buffer (pH 8.0–8.8). Gels were stained in this solution in the dark for 30 min.

Results

Chromosomal location of Est-7 in "CS"

The "CS" EST-7 pattern comprised three isozymes with isoelectric points around pH 8, which focused within the

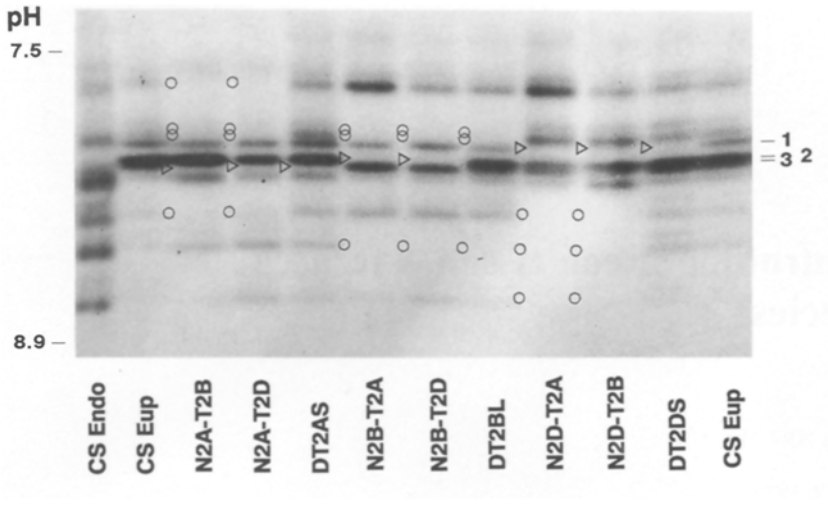

Fig. 1.

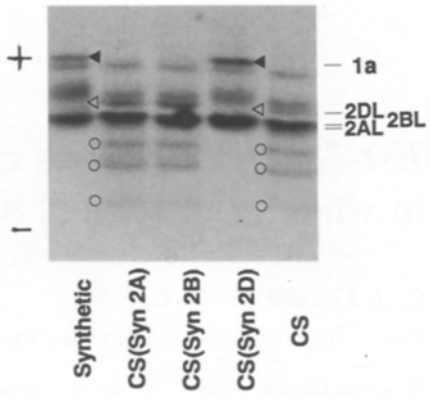

Fig. 2.

Figs. 1 and 2. **1** Nullisomic analysis of EST-7 production in seedling extracts of "CS". The three EST-7 isozymes are indicated at the right and their absence is marked by ▷. The absence of EST-6 isozymes is marked by ○. An endosperm extract of "CS" is included in track 1 for comparison. **2** Intervarietal single chromosome substitution analysis of an Est-D7 variant in "Synthetic". Isozymes not present in "CS" are marked by ▶, the absence of CS EST-7 isozymes is marked by ▷ and the absence of EST-6 isozymes marked by ○.

region of the gel spanned by EST-6 isozymes (Fig. 1). Nullisomic analysis indicated that each of these three isozymes is controlled by gene(s) on each of the homoeologous group 2 chromosomes: isozyme 1 by *2D*, 2 by *2B* and 3 by *2A*. Isozymes 1 and 3 were also absent in DT2AS and DT2DS, respectively, and isozyme 2 was present in DT2BL, thus the controlling genes, designated *Est-A7*, *Est-B7* and *Est-D7*, are located on the long arms of the respective chromosomes. Another isozyme, which was focused at a slightly higher pI than isozyme 3, was also shown to be the product of a gene on chromosome *2B*. However this isozyme was not consistently observed and was thus excluded from the analysis. As no hybrid bands were observed, it was concluded that EST-7 isozymes are monomeric.

Seedling extracts also showed other esterase activity apart from the three EST-7 isozymes. Although these were only weakly expressed, Fig. 1 clearly shows that genes encoding these isozymes are located on the short arms of homoeologous group 2 chromosomes and that all these isozymes were expressed by sample from endosperm extracts. These are EST-6 isozymes as reported by Petchey et al. (1990).

Allelic variation

All the hexaploid wheat varieties surveyed expressed the same EST-7 pattern to that of "CS", except "Synthetic". The isozyme pattern produced by "Synthetic" differed from the "CS" type in that isozyme 1 was replaced by a isozyme, designated "1 a" (Fig. 2). Analysis of intervarietal substitution lines showed that this difference was due to an allelic variant, designated *Est-D7b*, on chromosome *2D*. The null phenotype due to *Est-D6b* in "Synthetic" reported by Petchey et al. (1990) is also clearly seen in Fig. 2.

Homoeoloci in alien species

Except for *Ae. longissima*, all the other homoeologous group 2 wheat-alien additions or substitutions analysed, including "CS"/*H. vulgare* cv "Betzes", "Holdfast"/*S. cereale* var. "King II", "CS"/*S. cereale* var. "Imperial", "CS"/*S. montanum*, "CS"/*Ae. umbellulata*, "CS"/*Ag. elongatum* and "CS"/*D. villosum* (data not shown) expressed EST isozymes not present in their background wheat genomes. Moreover in the cases of *H. vulgare* and *S. cereale*, genes encording the "alien" isozymes were located on the respective long arms by the analysis of telocentric additions, for in both cases the long arm telocentric additions produced the same EST-7 patterns as the respective whole chromosome additions. Similarly *Est-R^{m}7* was located to the α arm of chromosome *2R^m* (Fig. 3). Telocentric additions for chromosomes *2U* and *2E* were not available, so arm locations of genes encoding the isozymes expressed by these two chromosomes were not established. However, they are very likely to be members of the *Est-7* set, for samples from endosperm extracts did not express these isozymes (E.M. Petchey, R.M.D. Koebner and M.D. Gale, unpublished data). Thus, these loci have been designated *Est-H7*, *Est-R7*, *Est-R^{m}7*, *Est-U7*, *Est-E7* and *Est-V7*, respectively.

Where wheat-alien substitutions were used, the isozyme patterns always lacked those wheat isozymes

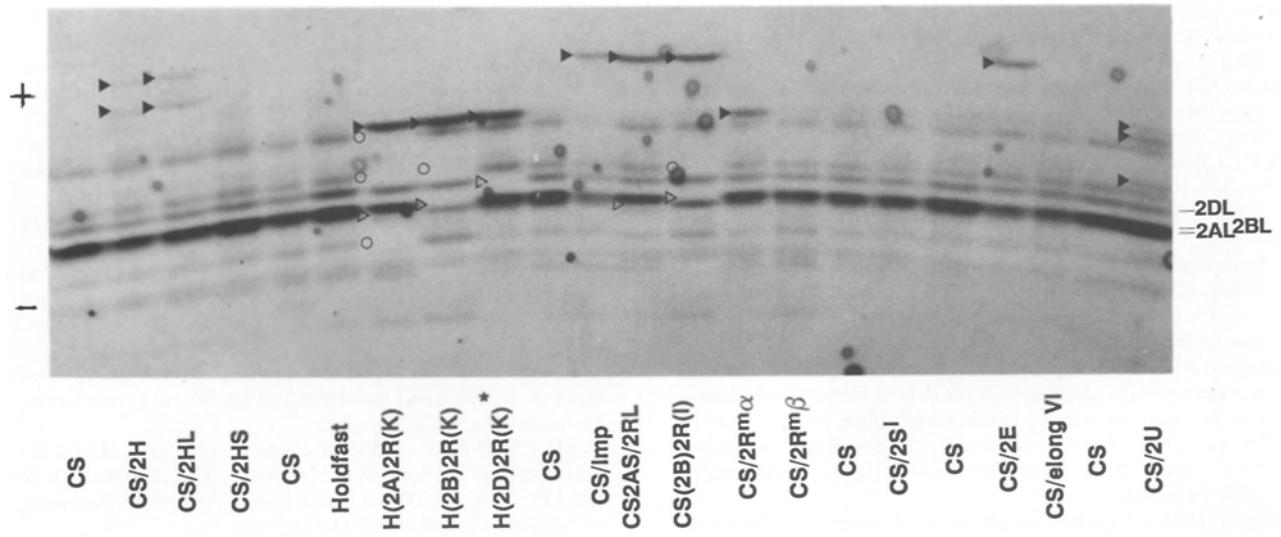

Fig. 3. Alien EST-7 isozymes: H = Barley "Betzes"; R = Rye; I = "Imperial"; and "K = King II"; R^m = *S. montanum;* S^1 = *Ae. longissima;* U = *Ae. umbellulata* and E = *Ag. elongatum.* Alien isozymes are marked by ▸, the absence of wheat EST-7 isozymes is marked by ▷ and the absence of EST-6 isozymes marked by ○. * See text

controlled by the absent wheat chromosomes, except for that currently identified as Holdfast(*2D*)*2R*(King II) (Chapman and Miller 1979). This line expressed the King II EST-7 isozyme and lacked isozyme 1, which had been showed to be encoded by a gene on chromosome arm *2DL*. However, it expressed the *Est-D6* isozymes controlled by the short arm of chromosome *2D* (Fig. 3). Thus, it is possible that this line is actually a Holdfast *2DS*/King II *2RL* translocation line. Further study is needed to clarify its identity.

Unlike the CS/*Ag. elongatum 2E* addition, no alien isozymes were expressed by CS/*Ag. elongatum* VI addition (Fig. 3), which is believed to carry one chromosome arm of *2E* (Hart and Tuleen 1983). This indicates that this lines must carry the short arm of this chromosome.

Discussion

Until recently, the group 2 chromosomes of wheat were devoid of biochemical marker loci. *Est-7* is now, however, the fifth to be found, together with *Sod-1,* also on long arms of group 2 (Neuman and Hart 1986) and *Est-6, Per-2* (Bosch et al. 1986) and *Per-5* (Liu et al. 1990) on the short arms. Except for *Sod-1,* all the other four sets of loci have allelic variants reported in wheat and thus may be precisely mapped intrachromosomally. In addition, the *Est-R7* isozyme from "King II" rye differs from that of "Imperial" and thus *Est-R7* can also be mapped in rye.

Although the *Est-6* and *Est-7* products have similar pIs, this should not hamper their use as marker loci because the individual isozymes from each set can be easily recognised, and because the *Est-7* products are not observed in mature endosperm extracts.

Acknowledgements. C.J. Liu is grateful to the Agricultural Genetics Company for the financial support through a studentship. We also wish to thank C.N. Law, A.J. Worland, T.E. Miller and S.M. Reader for supplying the genetic stocks, T.E. Miller for his critical reading of the manuscript and R.M.D. Koebner for his advice in this study.

References

Ainsworth CC, Gale MD, Baird S (1984) The genetic control of grain esterases in hexaploid wheat. 1. Allelic variation. Theor Appl Genet 68:219–226

Bosch A, Figueiras AM, Gonzalez-Jaen MT, Benito C (1986) Leaf peroxidases – a biochemical marker for the group 2 chromosomes in the Triticinae. Genet Res Camb 47:103–107

Chapman V, Miller TE (1979) Alien chromosome addition and substitution lines. Annu Rep Plant Breeding Institute, 1978:124

Driscoll CJ, Sears ER (1971) Individual addition of the chromosomes of "Imperial" rye to wheat. Agron Abstr 1971:6

Dvorak J, Knott DR (1974) Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum.* Can J Genet Cytol 16:399–417

Feldman M (1975) Alien addition lines of common wheat containing *Triticum longissimum* chromosomes. In: Proc 12th Int Bot Chongr, Leningrad, p 506

Hart GE, Tuleen NA (1983) Chromosomal locations of eleven *Elytrigia elongata* (= *Agropyron elongatum*) isozyme structural genes. Genet Res Camb 41:181–202

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterisation of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

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

Kimber G (1967) The addition of the chromosomes of *Aegilops umbellulata* to *Triticum aestivum* (var. Chinese Spring). Genet Res Camb 9:111–114

Liu CJ, Chao S, Gale MD (1990) The genetical control of tissue-specific peroxidases, *Per-1, Per-2, Per-3, Per-4* and *Per-5* in wheat. Theor Appl Genet 79:305–313

May CE, Vickery RS, Driscoll CJ (1973) Gene control in hexaploid wheat. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, University of Missouri Co, pp 843–849

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

McIntosh RA (1988) A gene catalogue in wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Institute of Plant Science Research, Cambridge, pp 1225–1323

Miller TE (1974) Alien chromosome additions and substitutions. Annu Rep Plant Breeding Institute, Cambridge, 1973:143–144

Nakai Y (1976) Isozyme variations in *Aegilops* and *Triticum*. 3. Chromosomal basis of the esterase isozyme production in different organs of Chinese Spring wheat. Bot Mag 89:219–234

Neuman PR, Hart GE (1986) Genetic control of the mitochondrial form of superoxide dismutase in hexaploid wheat. Biochem Genet 24:435–446

Petchey EM, Koebner RMD, Gale MD (1990) Genetic characterisation of a further homoeoallelic series of grain esterase loci, *Est-6*, in wheat. Theor Appl Genet 79:294–296

Sears ER (1954) The aneuploids of common wheat. Agric Exp Stn Res Bull, University of Missouri, 572:1–58

Sears ER (1966 a) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulation and plant genetics. Oliver and Boyd, London, pp 29–45

Sears ER (1966 b) Chromosome mapping with the aid of telocentrics. In: McKey J (ed) Proc 2nd Int Wheat Genet Symp, Hereditas [Suppl 2]:370–381

Sears ER (1968) Relationship of chromosomes 2A, 2B and 2D with their rye homoeologue. In: Finlay KW, Shepherd KW (eds) Proc 3rd Int Wheat Genet Symp, Australian Academy of Science, Canberra, pp 53–61