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Genetic characterisation of a further homoeoallelic series of grain esterase loci, *Est-6*, in wheat

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Summary. Isoelectric focussing in alkaline pH gels has permitted the identification of a new homoeoallelic series of genes, *Est-6*, encoding grain esterases in bread wheat, *Triticum aestivum*. Nullisomic analysis located these genes to the short arms of the homoeologous group 2 chromosomes. A search for polymorphism within *Est-6* revealed null alleles at each of *Est-A6*, *Est-B6* and *Est-D6*. A further homoeolocus, *Est-M6*, is present on chromosome arm *2MS* of *Aegilops comosa*.

Key words: Grain esterase – Wheat – Isoelectric focussing – Isozymes

Introduction

Esterases are a common class of non-specific enzyme found in both plant and animal tissue. Activity is generally assayed by the cleavage of naphthyl acetate in the presence of an azo dye. In wheat, five homoeoallelic sets of esterase genes have been described (*Est-1*: Barber et al. 1969; *Est-2*, *Est-3*, *Est-4*: Jaaska 1980; *Est-5*: Ainsworth et al. 1984). The chromosomal locations of these genes cover both arms of the homoeologous group 3 chromosomes (*Est-1*, *Est-2*, *Est-5*), the long arms of the group 6 chromosomes (*Est-4*) and the short arms of the group 7 chromosomes (*Est-3*). The present paper reports a new series located on the short arms of the group 2 chromosomes.

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Materials and methods

Plant materials

The following genotypes were analysed: bread wheat cv Chinese Spring (CS) and its nullisomic-tetrasomic (Sears 1966) and ditelosomic aneuploids (Sears and Sears 1978). A sample of over 60 diverse cultivars of hexaploid wheat was taken from the collection maintained at the Institute of Plant Science Research (IPSR), Cambridge Laboratory. Single chromosome substitutions involving the homoeologous group 2 chromosomes of cultivar Favorits substituted into Carmen (developed by A. Giura, Fundulea, Romania), and those of a *Triticum dicoccum* × *Aegilops squarrosa* amphiploid (McFadden and Sears 1946), 'Synthetic' (IPSR 1190903), substituted into CS (C. N. Law and A. J. Worland, unpublished data). The amphiploid CS × *Ae. comosa* (*Ae. comosa* IPSR 2110001) and its derived homoeologous group 2 addition and substitution lines (Riley et al. 1966).

Electrophoresis

Isoelectric focussing was carried out following the method of Koebner (1987), using as ampholyte Isolyte 6–10 (Isolabs), and as electrolytes 1 *M* phosphoric acid (anode) and 0.5 *M* sodium hydroxide (cathode). Samples were extracted by incubating the crushed endosperm half of a mature grain in 0.1 ml of distilled water at room temperature for about 1 h, and were loaded onto the gel about 10 mm from the anode via paper applicators (Pharmacia). Gels were stained following Ainsworth et al. (1984), with the exception that the substrate and dye were predissolved in dimethyl formamide instead of acetone.

Results and discussion

Esterase activity was resolved into two zones. The first, close to the anode, was recognisable as the products of the *Est-5* genes, while the second, in the central portion of the gel, focussing in a pH range of 7.5–8.3, represents the products of a new series, here designated *Est-6* (Fig. 1). Like EST-5, EST-6 activity is present in the endosperm of mature grains.

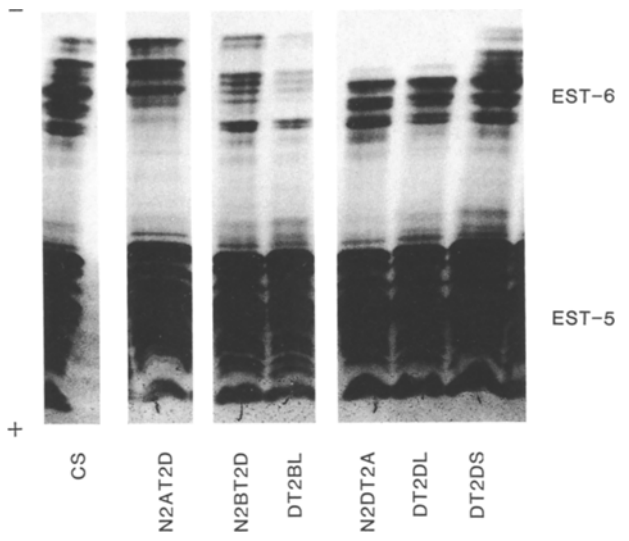

Fig. 1. EST-6 patterns of CS and its homoeologous group 2 aneuploids, showing chromosomal location of *Est-6* genes. N2AT2D = nullisomic *2A* tetrasomic *2D*, *DT2BL* = ditelosomic *2B* (long arm). EST-5 activity is visualised at the acidic end of the gel

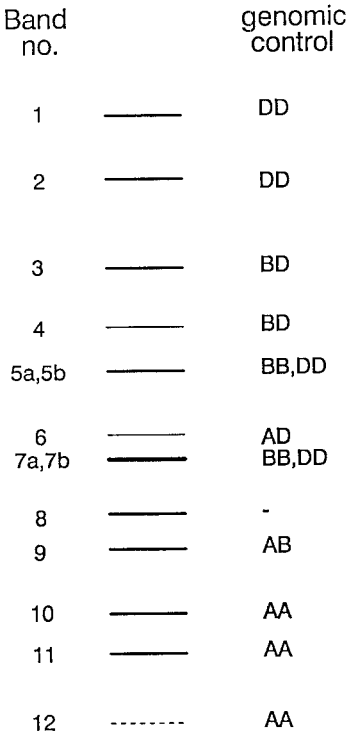

Fig. 2. Ideogram of the EST-6 pattern of CS, showing genomic control of individual isozymes

Chromosomal location of Est-6 genes

The EST-6 pattern of CS consists of 14 isozymes (Figs. 1 and 2). The same pattern was observed in all the nullisomic-tetrasomic combinations other than those involving homoeologous group 2, the deficient patterns of which allowed the chromosomal location of the genes controlling the production of 13 of the 14 isozymes to be determined. Thus, the isozymes numbered 6, 9, 10, 11, 12 were absent in stocks lacking chromosome *2A* or its short arm; 3, 4, 5b, 7a, 9 were absent in those lacking *2BS*; and 1, 2, 3, 4, 5a, 6, 7b were absent in those lacking *2DS*. Isozyme 8 was present in all lines and, therefore, probably represents a complex of products, co-focussing at a common pH. Thus, the presence of a homoeoallelic series *Est-A6, Est-B6, Est-D6,* present on the short arms of the wheat group 2 chromosomes, is proposed. The simultaneous control of some of these enzymes (e.g. isozymes 3 and 4 by *Est-B6* and *Est-D6*; Fig. 2) by two loci indicates that EST-6 isozymes are dimeric in structure. Thus, for example, isozyme 1 represents a D + D homodimer, while isozyme 3 is a B + D heterodimer.

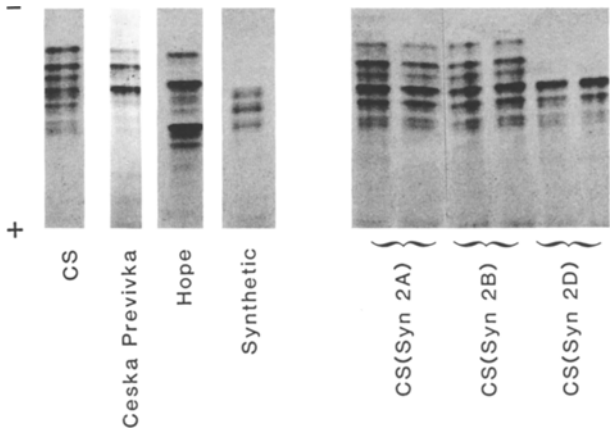

Fig. 3. Intraspecific polymorphism for EST-6 and demonstration, by intervarietal chromosome substitution analysis, that the variant pattern of Synthetic is due to an allelic difference at *Est-D6*. CS (Syn *2A*) = substitution of CS chromosome *2A* by its homologue of Synthetic

Intervarietal polymorphism

In the survey of wheat cultivars, the majority showed the same EST-6 pattern as CS. However, three variant patterns were observed. In the cultivar Ceska Previvka, the pattern resembled that of CS aneuploids lacking chromosome *2A*; in five genotypes (Hope, Favorits, RL4137, C591, R1), the pattern resembled that of CS aneuploids lacking chromosome *2B*; while in Synthetic, the pattern resembled that of CS aneuploids lacking *2D* (Fig. 3). Substitution line analysis showed that the second variant indeed was due to an allelic difference between *Est-B6* in Carmen (allele *a*) and Favorits (*b*) (data not shown). Similarly, the pattern of Synthetic was identical to that of CS (Synthetic *2D*), where CS chromosome *2D* is replaced by its homologue from Synthetic, while those of CS (Synthetic *2A*) and CS (Synthetic *2B*) were indistinguishable

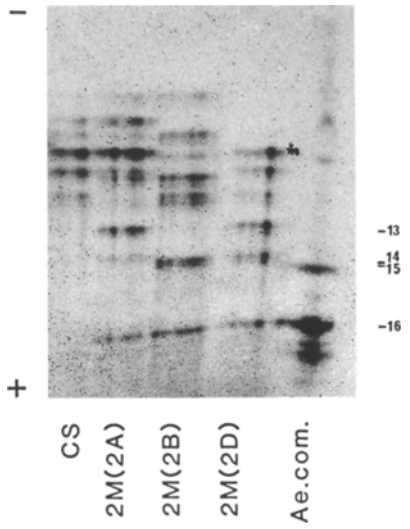

Fig. 4. Products of the alien homoeolocus *Est-M6* of *Ae. comosa*. Non-wheat isozymes numbered at *left*. *2M(2A)* = substitution of CS chromosome *2A* by its homoeologue *2M* from *Ae. comosa*. Ae. com. = *Ae. comosa*

from CS (Fig. 3). This analysis demonstrates the presence of alleles *Est-D6a* in CS and *Est-D6b* in Synthetic. The chromosomal location of the Ceska Previvka variant was not directly possible, as no intervarietal substitution lines are available involving this cultivar. However, it is likely to reside on chromosome *2A*, by analogy with the null variants for *2B* and *2D*.

Alien homoeoloci

A number of wheat/alien amphiploids were analysed in a search for alien homoeoloci of *Est-6*. However, only the CS × *Ae. comosa* hybrid produced any EST-6 isozymes that were distinguishable from those of CS. The *Ae. comosa* homoeolocus, *Est-M6*, produces two major EST-6 isozymes, which focus at pHs lower than those of CS. These two isozymes (14, 16 – Fig. 4) are also expressed in the amphiploid and in each of the three lines, *2M(2A)*, *2M(2B)* and *2M(2D)*, which represent the substitutions by *Ae. comosa* chromosome *2M* for, respectively, chromosomes *2A*, *2B* and *2D*. Heterodimers formed by the products of *Est-M6* and two of the homoeoloci of CS can be recognised (A + M isozyme 15, B + M 13); D + M heterodimers were not recognised, but probably co-focus with other wheat isozymes.

Conclusions

EST-6 is presently the first isozyme marker located to the short arm of the group 2 chromosomes to show intervarietal polymorphism. All three variants observed appear to represent "silenced" genes. Null alleles of this type have been noted in other wheat systems (Ainsworth et al. 1984; Chojecki and Gale 1982), and may be the result either of mutations causing frame shift or stop signals within the coding region, or of micro-deletion events not detectable cytologically. The *Est-B6* variation is currently being exploited to help map the group 2 chromosomes with DNA restriction fragment length loci and morphological markers.

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