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The genetical control and tissue-specificity of esterase isozymes in hexaploid wheat

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Abstract A comparative genetic analysis of esterase (E.C.3.1.1.1) isozymes of wheat cultivar 'Chinese Spring' in endosperm, embryo, coleoptile, leaf and root tissues revealed eight sets of isozymes characterised by different tissue specificities, pI ranges and the chromosomal locations of their controlling genes. This data was considered together with previously published work, resulting in a proposed rationalization of nine sets of wheat esterase isozymes. Although this classification included two sets of isozymes controlled by genes on the short arms of homoeologous group 3 chromosomes and three sets on the long arms of the same chromosomes, for which no recombination evidence of genetic distinctness has been obtained among either group, it is argued that the different characteristics of the various sets warrant retention of separate set nomenclatures. Previously unreported esterase genes include *Est-9*, a low pI, monomeric, embryo-specific group with controlling genes on chromosomes 3BS and 3DS and two further members of *Est-1*, *Est-H1* in *Hordeum vulgare* and *Est-S^{l}1* in *Aegilops longissima*.

Key words Wheat · Esterase · Isozymes
Isoelectric focusing

Introduction

Carboxylic ester hydrolase (E.C. 3.1.1.1), known more simply as esterase, is one of the most complicated isozyme systems in hexaploid wheat. The complications arise from the number of controlling loci, the possibility that some "loci" comprise multigene families or compound loci, the tissue specificity of the various isozymes and the confounding effects of developmental stage on expression within a tissue.

Eight sets of genes, all supposedly independent, have been reported to date. The first set, now recognised as *Est-1*, was reported by Barber et al. (1968) and located on the short arm of chromosome 3A and on chromosomes 3B and 3D. These genes were shown to be expressed in all of the different plant tissues investigated, including root, leaf sheath, leaf blade and developing grain, and to encode dimeric isozymes. The two loci on chromosomes 3B and 3D were subsequently found also to be on the respective short arms (Bergman 1972). Another three sets of *Est* loci, subsequently given standard nomenclatures in the wheat gene catalogue, were revealed in the work of Jaaska (1980): *Est-2* located on each of the three long arms of the homoeologous group 3 chromosomes and encoding coleoptile-specific isozymes; *Est-3* on the short arms of chromosomes 7B and 7D, encoding isozymes that appeared to be expressed in more than one plant tissue; and *Est-4*, carried on the long arms of the group 6 chromosomes, encoding leaf-specific isozymes. The *Est-5* gene set was described by Ainsworth et al. (1984). These loci were shown to be present on the three long arms of the group 3 chromosomes and to encode endosperm esterases. The *Est-6* gene set, which encodes dimeric esterase isozymes in endosperm, was located on the short arms of group 2 chromosomes by Petchey et al. (1990). Another set of loci, also designated *Est-6*, encoding monomeric leaf esterases, was located on homoeologous group 3 chromosomes by Jouve and Diaz (1990). These isozymes are likely, for reasons described below, to be those described by May et al. (1973) for which the controlling loci were located on the three long arms of the same chromosomes. In addition, another set of loci controlling green tissue esterases, designated *Est-7*, were located on the long arms of group 2 chromosomes (Liu and Gale 1990).

Other reports of esterase genes, not yet designated gene symbols, include that by Rebordinos and Perez de la Vega (1989) who observed a set of genes encoding dimeric endosperm esterases on the short arms of chromosomes 3A and 3D, and that by Ainsworth et al. (1984) who noted the

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existence of a single locus on the short arm of chromosome 7A that encodes three esterase isozymes in immature grains.

It may be expected that this complexity is in part an artifact and has arisen because of the low substrate specificity of this enzyme (Walker and Macknees 1983). Indeed, the esterases picked out with the generally used azo-dye coupled stains may represent a series of different proteins. Nevertheless, the relationship between these different groups of esterase isozymes is not clear and the unique designations for some of the existing loci are also not entirely convincing. Some of the loci, especially the five "sets" located on the homoeologous group 3 chromosomes, have been detected in analyses of different plant tissues with the use of different electrophoretic separation methods by different workers. Few attempts have been made to co-relate the various findings, and thus the possibility of multiple designations for one or more sets exists.

Moreover, the same arm locations for more than one set of loci encoding esterase isozymes have also raised the question as to whether these loci are dispersed on the same chromosome arm or whether they comprise a closely linked multiple gene family. The answer will indicate whether, when used as genetic markers, the controlling genes can be treated as single loci.

It is possible that a gene can be differentially expressed at different stages of plant development or in different plant tissues. This is why the guidelines for protein gene symbolisation in wheat (Hart and Gale 1988) insist that a new gene symbol may be assigned to a second gene, which has been shown to be carried on the same chromosome arm as the first encoding a similar protein, only when recombination evidence is available to demonstrate that the two genes are spatially separated on that arm. On the other hand, it is probably safe to conclude that isozymes from different plant tissues are controlled by the same locus if they show a similar electrophoretic pattern and their controlling genes are located to the same chromosome arms.

The experiments described here were carried out to rationalize and clarify the relationships between the various reported genes encoding esterases. The enzymes have been extracted from different plant tissues and analysed with a single separation technique. Where appropriate segregating populations have been employed to distinguish genes carried on a single chromosome arm, the smallest chromosome unit that can be resolved with standard aneuploid genetic stocks.

Materials and methods

Genetic stocks

The following genotypes were employed: (1) hexaploid wheat accessions of 'Chinese Spring' ('CS') and 'Synthetic', (2) all of the available nullisomic-tetrasomic and ditelosomic lines of the hexaploid wheat cv 'CS' developed by Sears (1954, 1966a,b), (3) the homoeologous group 3 intervarietal substitution lines of 'CS' ('Synthetic') developed by C.N. Law and A.J. Worland at the Cambridge Laboratory, (4) 50 random F_3 lines from the cross of 'CS' × 'Synthetic', (5) barley cv 'Betzes' and rye cv 'Imperial' and (6) the homoeologous group 3 wheat-alien addition lines of 'CS'/*H. vulgare* cv 'Betzes' (Islam et al. 1981), 'CS'/*S. cereale* cvs 'King II' (Miller 1973) and 'Imperial' (Driscoll and Sears 1971) and 'CS'/*Ae. longissima* (Feldman 1975).

Enzyme analysis

Five different tissues were analysed: the embryo plus scutellum, endosperm, coleoptile, leaf and root. The embryo ends or the endosperm ends (about 1/4 of the grain) of individual mature dry grains were crushed in a microhammer mill and incubated in 50 µl of 20% sucrose solution at room temperature for 1 h and centrifuged briefly prior to application to the gel. The coleoptile, primary leaf or roots from individual 4- to 8-day-old seedlings were ground by a pestle in a mortar with 40 µl of 20% sucrose solution at room temperature. The macerate was used immediately for electrophoresis. For the segregational analysis, the endosperm ends of single F_3 grains were used for analysing endosperm esterases, and the other parts were germinated and used for analysing green tissue esterases.

The gel width, composition of ampholyte, electrode buffer and sample loading positions were different for different groups of isozymes, and details are given in Table 1. Enzyme 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 8.5). Gels were stained in this solution in the dark for 30 min.

Results

The esterase isozyme patterns, as seen on wide pH range gels, of extracts from different tissues of 'CS' are shown in Fig. 1. To facilitate their description, these isozymes are grouped, each marked with a number which represents the

Table 1 Gel width, ampholyte, electrolyte and sample loading position

Figure	Gel width	Ampholyte	Electrolyte	Loading end
1; 7B	17 cm	Isolyte 3–7	1 *M* H_3PO_4 1 *M* NaOH	Anode
2; 3	12 cm	Isolyte 3–5: Pharmalyte 4.2–4.9 = 1:2	1 *M* H_3PO_4 0.1 *M* NaOH	Cathode
4; 6; 7A	17 cm	LKB 3.5–10: LKB 5–7 = 1:1	1 *M* H_3PO_4 1 *M* NaOH	Anode
5	12 cm	Isolyte 3–5: Pharmalyte 4.2–4.9	0.04 *M* L-glutamic acid 0.1 *M* NaOH	Cathode

Table 2 Locations of the encoding loci and tissue-specificity of esterases in hexaploid wheat

2S	*Est-A6, B6, D6*[b]		Dimeric[b]	Endosperm[b]
2L	*Est-A7, B7, D7*[c]		Monomeric[c]	Green tissue[c]
3S	*Est-A1, B1, D1*	[*EstA, B, D*[d]]	Dimeric[d]	Root[d], leaf[d], young grain[d], embryo[a]
	Est-B9, D9[a]		Monomeric[a]	Embryo[a]
Endosperm specific isozymes controlled by genes on 3AS and 3DS have been reported[e], but not confirmed[a].				
3L	*Est-A2, B2, D2*	[*Est-2A, B, D*[f]]	Monomeric[a, f]	Coleoptile[f]
	Est-A5, B5, D5[g]		Monomeric[g]	Grain[g]
	Est-A8, B8, D8[a]	[*Est-A6, B6, D6*[h]]	Monomeric[a, h, i]	Leaf[h, i], coleoptile[a], root[a]
A further embryo specific isozyme controlled by a gene on 3AL was identified[a].				
6L	*Est-A4, B4, D4*	[*Est-4A, 4B, 4D*[f]]	Monomeric[f, i]	Leaf[f, i]
7S	*Est-A3, B3, D3*	[*Est-3A, 3B, 3B*[f]]	Monomeric[f]	Seedling[f]
A further 7AS locus controlling immature grain esterase isozymes was reported[g]. The genes on group 7 chromosomes were not detected with IEF methods[a].				

[a] This paper, [b] Petchey et al. (1990), [c] Liu and Gale (1990), [d] Barber et al. (1968), [e] Rebordinos and Perez de la Vega (1989), [f] Jaaska (1980), [g] Ainsworth et al. (1984), [h] Jouve and Diaz (1990), [i] May et al. (1973)

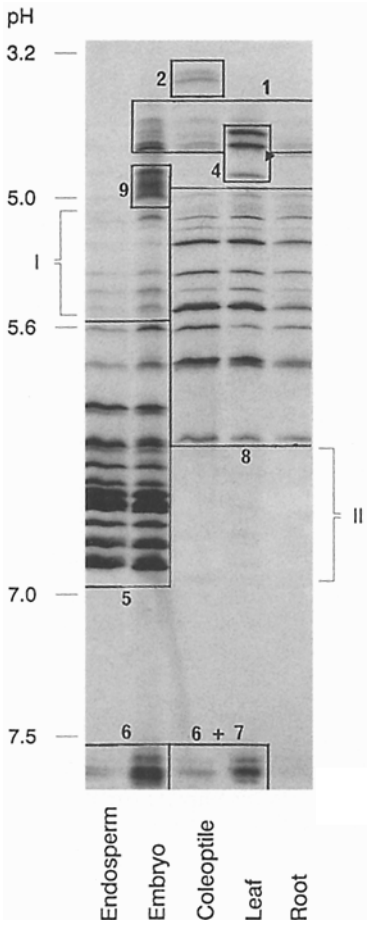

Fig. 1 The esterase isozyme patterns of extracts from coleoptile, leaf, root, endosperm and embryo plus scutellum, showing the relative position of those isozymes expressed by different tissues. The root-specific isozyme is marked by a *solid triangle*

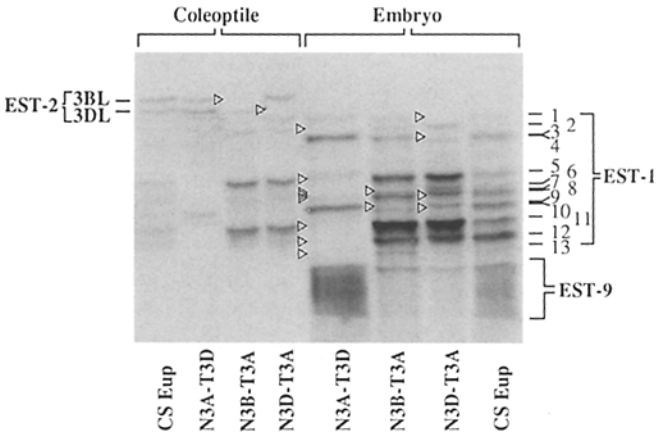

Fig. 2 The EST-1 and EST-2 phenotypes of the aneuploid homoeologous group 3 lines of 'CS'. Samples were from coleoptile or embryo plus scutellum extracts, as indicated. Absence of 'CS' isozymes encoded by *Est-1* is marked in embryo plus scutellum extracts and those encoded by *Est-2* are marked in the coleoptile extracts (▷)

respective gene set symbol, as proposed in our rationalization summarized in Table 2. In addition, genetical control of some other isozymes has not been determined. These include the isozymes expressed by embryo and endosperm extracts, focusing between pH 5.0–5.5 (zone I), the isozymes expressed by green-tissue extracts focusing between pH 6.0–7.0 (zone II) and the single root-specific isozyme focused at the cathodal side of EST-1 isozymes (marked by a solid triangle in Fig. 1).

Chromosomal control

Est-1

This set of loci is believed to control the production of the isozymes focusing around pH 4. This group of isozymes was expressed in all of the tissues but endosperm (Fig. 1) and contained at least 13 isozymes. Nullisomic (Fig. 2) and ditelosomic analysis (not shown) indicated that isozymes 2, 5, 7, 11, 12 and 13 were encoded by a gene(s) on chromosome arm 3AS, isozyme 9 by 3BS and isozymes 1, 3 and 10 by 3DS. Isozyme 6 was found to be encoded by both 3AS and 3BS and isozyme 8 by 3AS and 3DS. Thus, these are almost certainly the dimeric EST-1 isozymes first reported by Barber et al. (1968).

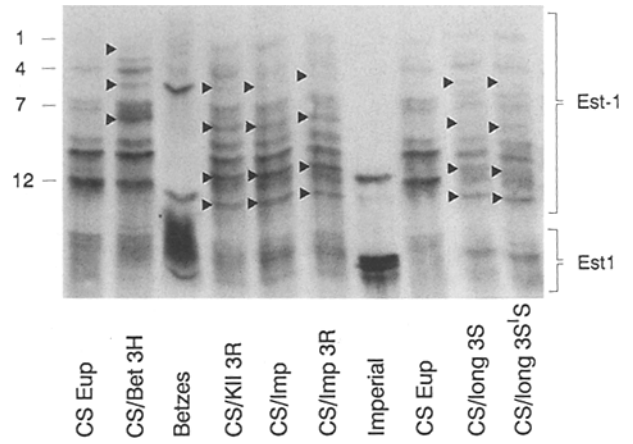

Fig. 3 Alien EST-1 isozymes: (*H* barley 'Betzes'; *R* rye; *Imp* 'Imperial', *K* 'King II' and S^{l} *Ae. longissima*). Isozymes not present in 'CS' are marked (▶)

To further confirm the dimeric nature of this set of genes, 4 sets of homoeologous group 3 wheat-alien addition lines were analysed, with a view of confirming the location of *Est-1* homoeoloci in related species and detecting alien-wheat heterodimers.

All of the 4 group 3 wheat-alien addition lines analysed, including 'CS'/*H. vulgare* cv 'Betzes' 3H, 'CS'/*S. cereale* cv 'King II' 3R, 'CS'/*S. cereale* cv 'Imperial' 3R and 'CS'/*Ae. longissima* 3S^{l}, expressed isozymes not present in 'CS' in this region (Fig. 3). The ditelosomic additions of 'CS'/'Imperial' (not shown) and 'CS'/*Ae. longissima* were also analysed. In both cases the short arm additions expressed the same isozyme patterns as the respective whole chromosome addition, while the long arm additions failed to express these non-wheat isozymes. This indicates that, as in wheat, genes encoding these alien isozymes are located on the short arms. The locus on 3RS is likely to be that reported by Barber et al. (1968). The two loci encoding *H. vulgare* and *Ae. longissima* isozymes were designated *Est-H1* and *Est-S^{l}1*, respectively.

Two alien parents, barley 'Betzes' and rye 'Imperial', were also analysed. This revealed that both 'CS'/'Betzes' 3H and 'CS'/'Imperial' 3R additions expressed esterase isozymes not present in either 'CS' or the respective alien parents. As shown in Fig. 3, 'CS'/'Betzes' 3H expressed three EST-1 bands not present in 'CS', one is a barley homodimer focusing below isozyme 4 and the other two are barley-wheat heterodimers, one below isozyme 1 and the other below isozyme 7. It was thus confirmed that this set of genes encode dimeric isozymes.

Est-2

The two coleoptile-specific isozymes are likely to be controlled by *Est-2*, a set of genes previously described by Jaaska (1980). Of all the esterases detected in extracts from different tissues these 2 isozymes showed the lowest pIs. The one with higher pI was absent in lines lacking chromosome 3B while present in ditelosomic 3BL lines and the other was absent in lines lacking chromosome 3D and ditelosomic 3DS lines while present in ditelosomic 3DL line (Fig. 2). Their controlling loci were thus located on chromosome arms 3BL and 3DL, respectively. The chromosomal location of the encoding genes and their tissue-specificity indicate that these are likely to be some of the EST-2 isozymes reported by Jaaska (1980).

Est-3

This symbol was assigned to the set of genes located on the short arms of group 7 chromosomes that encode monomeric isozymes in young seedlings (Jaaska 1980); this is supported by the location of a homoeolocus in barley chromosome 7H (Hart et al. 1980). In this study none of the esterase isozymes was found to be controlled by genes on any of the group 7 chromosomes. Possible explanations included that focusing for the isozymes encoded by this set of genes was not adequate on the iso-electric focusing (IEF) gels used, or that the isoelectric points of these isozymes were not covered by the pH range selected here.

Est-4

Three esterase isozymes were distinctive in leaf esterase zymograms (Fig. 1). Nullisomic analysis showed that the one with the highest pI was controlled by a locus on chromosome arm 6BL and the one with middle pI by 6DL. The one with the lowest pI seemed to be controlled by a locus on chromosome 6A (not shown). In view of their tissue-specificity and the controlling gene location, these are likely to be the EST-4 isozymes reported by Jaaska (1980).

Est-5

This set of loci controls the production of the grain isozymes with pIs ranging from pH 5.5 to 7.0 (Fig. 1). There are more than 20 isozymes in this group, and they account for more than two-thirds of the total grain esterase activity. Nullisomic analysis (not shown) indicated that these isozymes are encoded by genes on the long arms of group 3 chromosomes: 5 by genes on 3AL, 7 by genes on 3BL and another 7 by genes on 3DL. These isozymes were absent in the respective nullisomic-tetrasomic combinations and the available short arm ditelosomic lines but present in the long arm ditelosomic lines. This group of isozymes are EST-5 as reported by Ainsworth et al. (1984).

Est-6 and Est-7

As previously reported, two sets of loci, one located on the short arms and the other on the long arms of group 2 chromosomes are responsible for the production of the group of isozymes with the highest pIs on the wide pH range gel (Fig. 1). These loci were designated *Est-6* (Petchey et al. 1990) and *Est-7* (Liu and Gale 1990), respectively. Narrow range high pH gels which allow EST-6 and EST-7 to be discriminated are shown in Liu and Gale (1990).

Est-8

This symbol is proposed here for the genes encoding the production of a group of isozymes that focus between pH 4.5 and 6.5 and are expressed in coleoptile, leaf and root extracts (Fig. 1). Some of these isozymes showed similar pIs to EST-5 isozymes, while most focused in a lower pH range. Aneuploid analysis indicated that some of these isozymes are encoded by genes on the long arms of the homoeologous group 3 chromosomes. Isozymes numbered 6 and 9 were absent in lines lacking chromosome 3B, isozyme 8 was absent when chromosome 3D was removed and isozyme 13 and most of isozyme 14 were absent in lines lacking chromosome 3A (Fig. 4). These isozymes were also absent in the available short arm ditelosomic lines (DT3BS was not available) but present in the respective long arm ditelosomic lines. They are likely to be the isozymes reported by May et al. (1973) and Jouve and Diaz (1990). The homoeologous locus in rye was also located on chromosome 6R in this study (not shown). Jouve and Diaz (1990) designated the encoding genes *Est-6*. However, a new symbol is required, and *Est-8* is suggested for this set of loci.

Est-9

There is one group of esterase isozymes, identified as 9 in Fig. 1, which are active only in the embryo. Aneuploid analysis indicated that at least 2 of these isozymes are controlled by genes on chromosomes 3BS and 3DS (Fig. 5). The symbol of *Est-9* is proposed here for these 2 genes. A third isozyme, the one with the highest pI in this group, is encoded by a locus on 3AL because it was not expressed by the ditelosomic 3AS (Fig. 5). However, a method providing consistent separation for this group of isozymes is not yet available and attempts to demonstrate that this third isozyme is also absent in the 2 lines lacking 3A chromosomes (N3A-T3B and N3A-T3D) were not successful. Intervarietal variation was not detected for any of these loci.

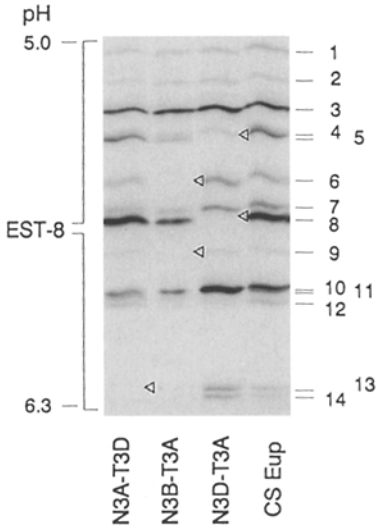

Fig. 4 The EST-8 isozyme patterns of the aneuploid homoeologous group 3 lines of 'CS'. Samples came from six-day-old seedlings. Absence of 'CS' isozymes is marked (◁)

Other esterase isozymes

The group of isozymes expressed in grain tissues which focus between EST-1 and EST-5 (identified as zone I in Fig. 1) may be the products of yet further genes. These isozymes showed a very similar pattern to some of the EST-8 isozymes on wide pH range gels (Fig. 1). However, the patterns of EST-8 isozymes and those in Zone I could be seen to differ on high pH range gels (Fig. 6). The *Est-8* products were sharply focused but the isozymes in Zone I were not well resolved. An protocol for more efficient separation of this group of esterase isozymes is not yet available.

Another group of isozymes for which we were unable to locate the encoding genes were those identified as Zone II and expressed weakly by green tissue extracts. They focused at the cathodal side of those EST-8 in a similar pH range occupied by EST-5 isozymes in grain tissue. These isozymes were most active in leaf tissue from very young seedlings (4 days old), and some were shown clearly to be encoded by genes on the long arms of group 3 chromosomes. The relationship between these isozymes and those of EST-5 and EST-8 is not clear.

In addition, a root-specific esterase isozyme was also observed that located on the cathodal side of those EST-1 isozymes (Fig. 1). The genetical control of this isozyme has not yet been determined.

Overall, loci encoding esterase isozymes in hexaploid wheat have been detected on six different sets of wheat chromosome arms. These include chromosome arm sets 2S, 2L, 3S, 3L, 6L and 7S. The locations of these loci and the tissue-specificity of esterase isozymes encoded by them are summarized in Table 2.

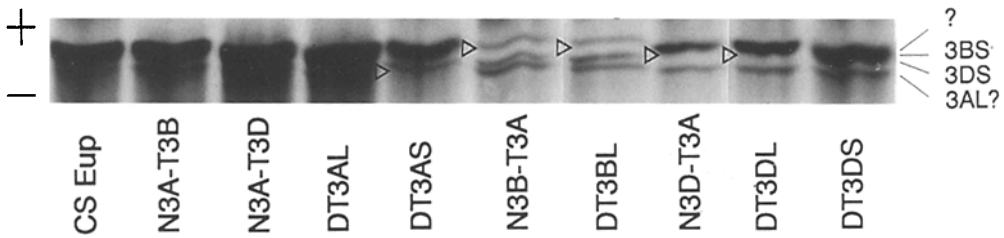

Fig. 5 The EST-9 phenotypes of the aneuploid homoeologous group 3 chromosomes. Samples were from embryo plus scutellum extracts. Absence of 'CS' isozymes is marked (▷)

Developmental variation in green tissue esterase isozymes

As shown in Fig. 1, different esterase phenotypes were produced by different plant tissues. Similarly, developmental changes of green tissue esterase isozymes are also clearly observed. The three leaf-specific isozymes (EST-4) and the isozymes in zone II showed stronger activity when younger seedlings were used for sample extraction, but the activity of the EST-8 isozymes was increased in older seedling extracts. However, contrary to the findings of Rebordinos and Perez de la Vega (1989) on starch gels, no unique esterase isozymes were detected in etiolated seedlings (not shown).

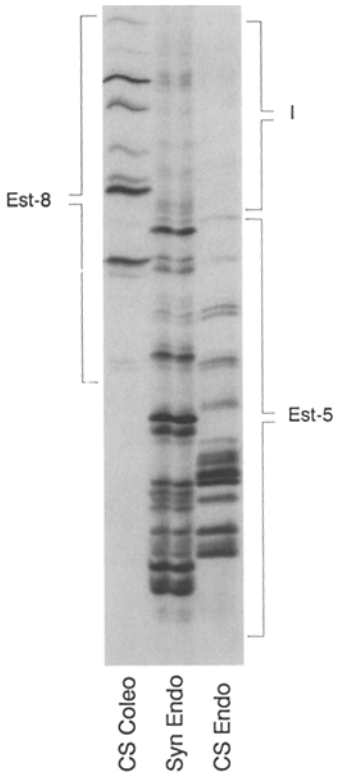

Fig. 6 The esterase isozyme patterns of extracts from endosperm of 'CS' and 'Synthetic' and coleoptile of 'CS', showing the difference between EST-8 isozymes and those in zone I

The relationship between Est-2, Est-5 and Est-8

The three sets of isozymes EST-2, EST-5 and EST-8 are all controlled by genes carried on the long arms of the group 3 chromosomes and beg the question as to whether they are encoded by the same or different sets of genes. Analysis of the 'CS'('Synthetic') intervarietal chromosome substitution lines indicated clearly that the three sets of genes show different allelic variation. No variation was displayed by any of the *Est-2* loci. 'CS' and 'Synthetic' displayed different phenotypes for EST-5 encoded by the genes on 3B and 3D, while differences were observed for the EST-8 isozymes encoded by the genes on 3A and 3D. (Fig. 7). The relationship between *Est-D5* and *Est-D8* could be further explored by segregational analysis of the 'CS' × 'Synthetic' F_3 families. No recombination between genes controlling the EST-D5 and EST-D8 phenotypes was observed in the 50 F_3 lines. Thus, it was concluded that if the two sets of isozymes are under independent control, the genes are not widely dispersed on chromosome arm 3L.

Discussion

It has been demonstrated with the use of the single separation method, IEF, that there are at least eight groups of

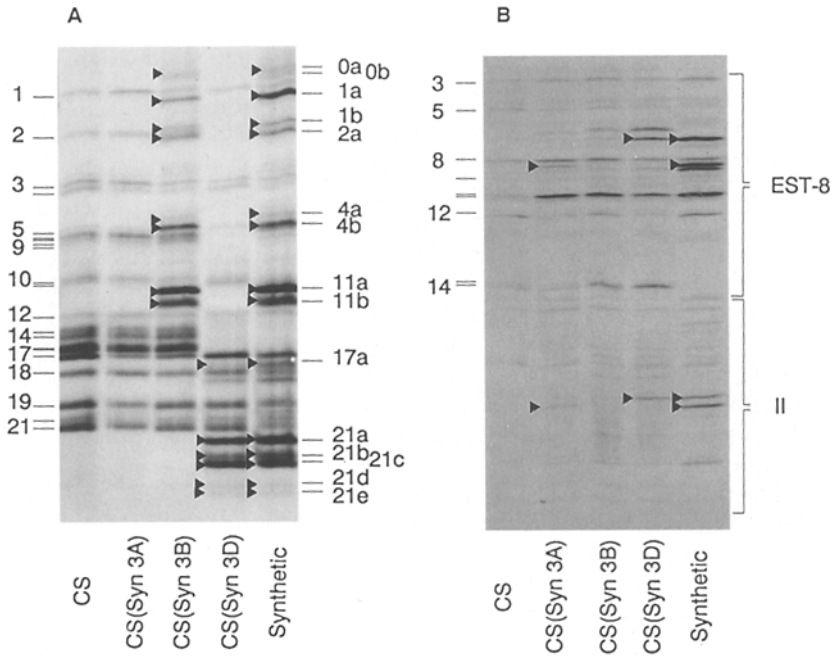

Fig. 7 The phenotypes of 'CS', 'Synthetic' and their homoeologous group 3 intervarietal substitution lines. (**A**) The EST-5 isozymes of endosperm extracts and (**B**) young (4-day-old) seedling isozymes (EST-8 and those in zone II). Isozymes not present in 'CS' but present in the intervarietal substitution lines are marked (▶)

esterase isozymes produced in different tissues of the wheat plant. These isozymes are encoded by genes on the long (*Est-7*) and short arms (*Est-6*) of group 2 chromosomes, on the long (*Est-2*, *Est-5* and *Est-8*) and short arms (*Est-1* and *Est-9*) of group 3 chromosomes and on the long arms of group 6 chromosomes (Est-4). Two of these, *Est-1* and *Est-6*, are dimeric isozymes.

It is unarguable that EST-4, EST-6 and EST-7 are encoded by three different sets of genes because they are located on three different chromosome arms, i.e. on the long arms of group 6, short arms of group 2 and long arms of group 2 chromosomes. On the other hand, the situation regarding the isozymes controlled by genes on the group 3 chromosomes is more ambiguous. More than one 'locus' controlling esterase production has been assigned to each of these chromosome arms. However, no recombinational evidence is available to prove that each is distinct. Thus, the difficulty of designation arises.

The three groups of isozymes encoded by genes on the long arms of homoeologous group 3 chromosome are expressed in different tissues and have different electrophoretic characteristics. Two of them, EST-5 and EST-8, showed independent varietal variation, while EST-2 was found to be monomorphic among the genotypes used here. However, no recombination was detected between the genes that encode EST-D5 and EST-D8 isozymes from a sample of 50 F_3 lines from the cross of 'CS'×'Synthetic', indicating that these two groups of isozymes are either encoded by genes in a compound locus or by closely linked genes. This close linkage relationship is supported by the fact that homoeoloci for genes controlling both groups of isozymes were detected on chromosome 6R in rye. A distal segment of 6RL is syntenous with a distal segment of the long arms of wheat group 3 chromosomes (Devos et al. 1993). Unfortunately, no intervarietal variation could be detected for the coleoptile-specific isozymes (EST-2), thus a similar segregational analysis involving *Est-2* with *Est-5* or *Est-8* was not possible.

According to the guidelines of gene nomenclature (Hart and Gale 1988) in wheat, a single locus should be assigned to genes on the same chromosome arms if no recombination evidence is available to prove that they are distinct. Although we have no evidence of spatial separation the other evidence relative to tissue specificity, electrophoretic characterization and allele variation provides adequate reasons to maintain the different gene symbols for these three sets of loci.

Two groups of esterase isozymes were also found to be encoded by genes on the short arms of the group 3 chromosomes. The group with lower pIs were shown to be dimeric and lack tissue-specificity, and thus are likely to be EST-1, as first reported by Barber et al. (1968). Isozymes in the second group are monomeric and embryo-specific, and thus different from EST-1 and those reported by Rebordinos and Perez de la Vega (1989). For this group, described for the first time here, we propose the symbol EST-9.

It would be desirable to find different substrates that discriminate, unequivocally, each group of wheat esterase isozymes. However, the protocols reported in this paper provide another relatively easy and reliable method to separate these different groups of isozymes, thus making these loci more useful as genetic markers.

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