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Extent of genetic variability of endosperm esterases in *Triticum aestivum* L. 2n=6x=42*

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Summary. Genetic variability of endosperm esterase has been studied in 42 cultivars of *Triticum aestivum* L. 2n=6x=42. Different techniques, including sequential electrophoresis and electrofocusing, have been used with various substrates and esterase inhibitors. The electrophoretic patterns in each cultivar are described. Chromosomal location using the nullitetrasomic and ditelosomic lines of Chinese Spring was carried out in order to relate and/or locate the esterase genes to specific chromosomes. Most of the esterase isozymes located were in the long arm of the chromosomes of the homoeology group 3; but we have found six located in the short arms, five of them in the chromosome 3AS and one in the 3DS. This location increases the number of esterase genes described, because no esterase genes had been described so far in short arms of chromosomes of the homoeology group 3. The genetic control is discussed and, according to our results, between 12 and 15 loci, organized in five "compound loci", control the endosperm esterases in wheat. Also one "modifier" gene modifying the mobility of two esterase bands and present in all the cultivars studied is postulated.

Key words: Esterases – Genetic variability – *Triticum aestivum*

Introduction

A knowledge of the total extent of variation in an enzymatic system is basic to its use in population and genetic studies. Generally, a single electrophoretic method is not able to show all the variability of a given isozymatic system (Coyne 1982), and the importance of such undetected or cryptic variability in population genetic studies has been repeatedly pointed out (Chakraborty and Nei 1976; Ayala 1982; Damerval et al. 1986). Several electrophoretic and biochemical methods have been described to detect cryptic variability, and they have been applied to animal population studies (Chambers et al. 1981; Heinstra et al. 1986). In plants, cryptic variability only has been studied in some enzymatic systems in *Avena barbata, Hordeum vulgare* and *Zea mays* (Shumaker et al. 1982) and in *Oryza* ssp. (Second 1982).

Esterases (EC 3.1.1.-) hydrolize esters and generally they have a very low substrate specificity. Esterase systems, as revealed by electrophoretic techniques, are characterized by the presence of a high number of isozymes and by a great variability among individuals and populations.

Hexaploid wheat (*Triticum aestivum* L.) is one of the plant species in which esterases have been genetically studied, but still their genetic control is poorly understood. Four sets of homoeologous loci have been described controlling wheat esterases in different organs and tissues. Genes for esterases of immature seeds, roots, leaf sheets and leaves of green plants have been located in chromosome arms 3AS, 3BS, and 3DS (Barber et al. 1968, 1969; Bergman 1972); loci for esterases of etiolated coleoptile have been located in chromosome arms 3AL, 3BL, 3DL, 7BS, and 7DS (Jaaska 1980); those of etiolated leaves in 6AL, 6BL, and 6DL (May et al. 1973; Nakai 1976; Jaaska 1980). Hart (1984) assigned symbols *Est-1, Est-2, Est-3,* and *Est-4* to each one of the four sets of homoeologous loci. Esterases of mature grains have been located in 3AL, 3BL, and 3DL (Nakai 1976; Cubadda et al. 1975; Ainsworth et al. 1984). In regard to the sub-

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unit composition of wheat esterases, these have been described as monomers or dimers (Barber et al. 1968; May et al. 1973; Jaaska 1980).

Our aims in this work were to study the extent of variability of endosperm esterases of hexaploid wheat mature seeds and to identify the chromosome arms in which the genes for endosperm esterases are located.

Materials and methods

Wheat cultivars

Hexaploid wheat, *Triticum aestivum* L. (2n = 6x = 42), cultivars used are listed in Table 1. Furthermore, the F_2 progeny from the cross Banks × Capitole was analyzed to score esterase segregation.

Aneuploid lines

The nullisomic-tetrasomic (NT) and ditelosomic (DT) lines of Chinese Spring wheat, kindly supplied by Prof. E. R. Sears, were used to locate the chromosomes which control seed esterases. All the available nulli-tetrasomic (lacking the NT 2A–2B, 2A–2D, 4A–4D, and 4D–4B) for the seven homoeology groups and the ditelosomic for group 3 were screened.

Enzyme extraction

Endosperms from mature dry seeds were crushed and the enzymes were extracted by adding 50 µl of phosphate buffer 0.02 *M*, pH 7.0, for 1.5 h at 4 °C.

Standard electrophoresis procedure

After extraction, paper wicks (Whatman 3M), 7 × 2 mm, were soaked with the crude extract and then inserted 4 cm from the cathode in 180 × 260 × 2 mm 10% w/v horizontal polyacrylamide gels (95:5 acrylamide:bisacrylamide). The buffers used were: 0.1 *M* NaOH, 0.3 *M* boric acid (pH 8.1) as electrode buffer, and 15 m*M* TRIS, 3.5 m*M* citric acid (pH 8.6) as gel buffer. Electrophoresis was carried out at 4 °C and 21 V cm^{-1} until the bromophenol blue migrated 12 cm. i.e. approximately 4 hours.

Sequential electrophoresis

Two different polyacrylamide concentrations were used in gels, 7.5% and 5.0%. All the other conditions were kept as above.

Electrofocusing

Polyacrylamide gels of 110 × 245 × 0.5 mm with a concentration of 7.5% w/v were used. Gradients of pH ranged from 4 to 9. In the anode and cathode, 0.5 *M* phosphoric acid and 1.0 *M* NaOH were used, respectively. Gels were prefocused for 2 h before applying sample extracts, absorbed in 4 × 8 mm paper wicks, directly onto the gel surface at 1.5 cm from the cathode. Electrofocusings were carried out at a constant voltage of 180 V cm^{-1} for 2 h. The gradients of pH were checked at 5 mm intervals across the surface of the gels.

Staining and inhibition

Gels were stained for esterase activity using a method adapted from Shaw and Prasad (1970). One hundred milligrams of Fast Blue RR salt was dissolved in 5 ml of α-naphthyl acetate (1% w/v in acetone) and then mixed with 5 ml of phosphate buffer 1 *M* (pH 6.0) and 90 ml of distilled water. Gels were incubated at 30 °C until dark bands appeared, then washed, and fixed in a solution of 7% v/v acetic acid.

In addition to α-naphthyl acetate, two other substrates were used: α-naphthyl propionate and α-napthyl butirate. All of them were utilized at concentrations of 1% w/v in acetone.

Table 1. Endosperm esterase patterns of the hexaploid wheat cultivars

Cultivars	Pattern		
	Origin	Electrophoresis[a]	Electrofocusing[b]
Anza	Mexico	A (6, 8)[c]	D (23, 25, 30, 32)
Ariana	Spain	A	E (24, 30, 32)
Avocet	Australia	A	E
Banks	Australia	C (7, 9)	I (24, 31, 33)
Barbilla	Spain	A	E
Bastion	Mexico	A	E
Bindawarra	Australia	A	D
Boulmiche	France	A	E
Cajeme	Mexico	B (6, 8, 15)	F (23, 25, 30, 32, 34,35)
Canaleja	Spain	A	E
Capitole	France	A	E
Castan	France	A	D
Chinese Spring	U.S.A.	A	E
Condor	Australia	C	J (19, 23, 25, 31, 33)
Cook	Australia	C	J
Gamenya	Australia	C	G (23, 25, 31, 33)
Halberd	Australia	A	E
Isis	Australia	A	D
Jabiru	Australia	A	D
Jacup	Australia	A	D
Jupateco	Mexico	A	D
Kite	Australia	A	D
Lance	Australia	B	F
Lutescens spring	U.S.S.R.	A	E
Lutescens winter	U.S.S.R.	A	E
Magdalena	Spain	A	D
Millewa	Australia	B	F
Nava		A	D
Pane 247	Spain	A	D
Rojo Basto	Spain	A	D
Shortim	Australia	C	G
Songlen	Australia	B	F
Spelta Spring	U.S.S.R.	A	D
Spelta winter	U.S.S.R.	C	G
Splendeur	France	A	E
Telar		A	E
Tincurrim	Australia	A	D
Winglen	Australia	A	D
Yecona		B	F
Yecora	Mexico	A	D
030		A	E
062		A	E

[a] The isozymes observed by electrophoresis were numbered 1–16
[b] The isozymes observed by electrofocusing were numbered 1–35
[c] Number in parenthesis indicates the isozymes lacking in each position

Table 2. Chromosomal location of wheat endosperm esterases and response to substrates and inhibitors, standard polyacrilamide technique

Aneuploid lines of Chinese Spring	Isozymes													
	EST 1					EST 2					EST 3			
	1	2	3	4	5	7	9	10	11	12	13	14	15	16
3A–3B	+	+	−	+−	−	+	+	++	+	+	+	−	−	+
3A–3D	+	+	−	+−	−	+	+	++	+	+	+	−	−	+
3AL	+	+	−	+	−	+	+	+	+	+	+	+	+	+
3AS	+	+	+	+	+	+	+	+	+	+	+	−	−	+
3B–3A	+	++	+	++	+	−	−	+	++	+	+	+	+	+
3B–3D	+	++	+	++	+	−	−	+	++	+	+	+	+	+
3BL	+	++	+	++	+	+	+	+	+	+	+	+	+	+
3BS	+	+	+	+	+	−	−	+	+	+	+	+	+	+
3D–3A	−	++	+	++	+	+	+	+	−	−	+	+	+	+
3D–3B	−	++	+	++	+	+	+	+	−	−	+	+	+	+
3DL	−	+	+	+	+	+	+	+	+	+	+	+	+	+
3DS	+	+	+	+	+	+	+	+	−	−	+	+	+	+
All other lines and euploid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Substrates/inhibitors														
β-naphtyl acetate	+	+	+	+	+	−	−	−	−	−	+	+	+	+
α-naphtyl propionate	−	+	−	+	−	+	+	+	+	+	+	+	+	+
pHMB	−	+	−	+	−	+	+	+	+	+	+	+	+	+

+ = isozyme present; − = isozyme absent; ++ = isozyme present with higher intensity; +− = isozyme present with lower intensity

Three reported inhibitors of esterases were employed: parahydroximercuribenzoate (pHMB) 10 m*M*, iodoacetamide 1 m*M*, and EDTA 3 m*M*. Iodoacetamide and EDTA were present both during preincubation (30 min at 30 °C) and staining of gels, while pHMB was added only during preincubation, and gels were washed before the staining solution was poured on. The substrate used with inhibitors was α-napthyl acetate.

Nomenclature

Each isozymatic activity zone was named with the abbreviation of the enzymatic system in capital letters (EST) followed by a number, 1–3, from faster to slower migration. Isozymes were also numbered from faster to slower migration in gels.

Results

Standard electrophoresis

Isozymatic patterns. Three isozymatic patterns, named A, B, and C, were observed for endosperm esterases (Fig. 1). In all the patterns, three zones of activity were distinguished: EST1, EST2, and EST3. The total of intensely stained isozymes observed was 16: EST1 included isozymes 1–5, EST2 isozymes 6–12, and EST3 isozymes 13–16. All the patterns showed a set of five weakly stained isozymes (Fig. 1); however, as these were usually difficult to detect, they were considered unsuitable for the purposes of distinguishing between the different patterns. The esterase patterns observered in each wheat cultivar are indicated in Table 1.

Chromosome location. The results of chromosome location of Chinese Spring wheat esterase loci are shown in Table 2. All the nulli-tetrasomic (NT) lines scored showed isozyme patterns identical to euploid Chinese Spring, except NT lines for homoeology group 3. Genes for isozymes 3 and 5 were located in the short arm of chromosome 3A, that for isozyme 1 in the short arm of 3 D. Genes for isozymes of the EST2 zone were located in the long arm of 3B (isozymes 7 and 9) and in the long arm of 3D (isozymes 11 and 12). Finally, genes for isozymes 14 and 15 (EST3) were located in the long arm of 3A.

Substrates and inhibitors. The differential staining when β-napthyl acetate was used as substrate corroborated the existence of three esterase zones. While esterases of EST1 and EST3 were shown in gels when this substrate was used, those of EST2 were not (Table 2). α-Napthyl butirate failed to reveal any esterase isozyme. Iodoacetamide and EDTA partially inhibited the appearance of all esterase bands, but none especifically (Table 2). These results were observed in all the cultivars.

Sequential electrophoresis. In addition to the 10% polyacrylamide concentration, another two concentrations were studied, 7.5% and 5%. Isozyme migration in gels was more distant from the origin the lower the polyacrylamide concentration, but no significant difference in the relative migration (compared to those in standard elec-

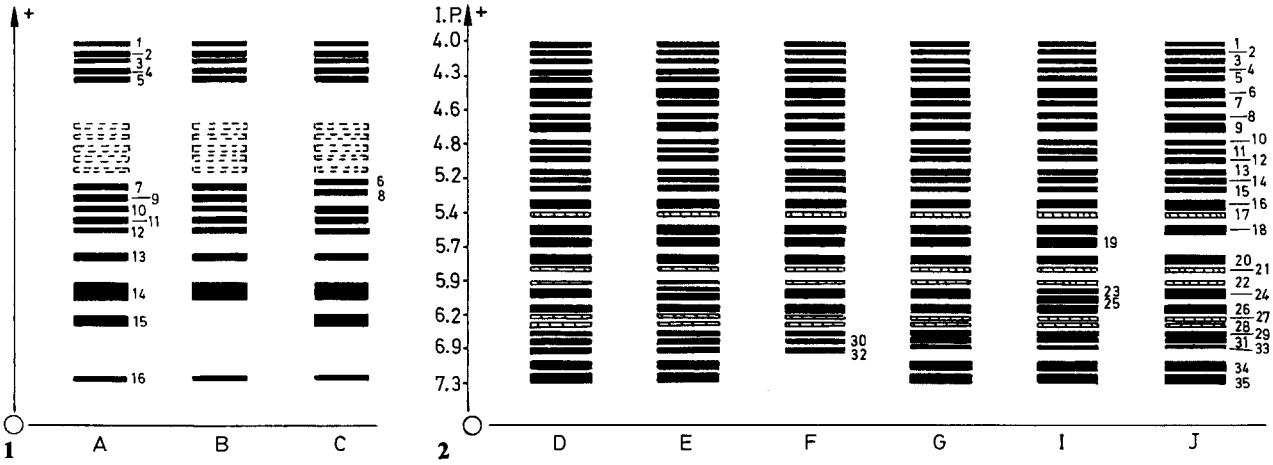

Fig. 1. Endosperm esterase patterns obtained using polyacrylamide gel electrophoresis

Fig. 2. Endosperm esterases patterns obtained using electrofocusing. The isoelectric points (I.P.) obtained at 5 mm interval across the gel surface are shown on the left side of the graphic

trophoresis) were observed in any band of any wheat cultivar. Thus, no cryptic variability, if it exists, was revealed by this technique.

Electrofocusing

Isozymatic patterns. Seven patterns with a total of 35 esterase bands were observed in electrofocusing gels. Isozymes were distributed in a pH range of 4.0–7.3 (Fig. 2). Isozymes 1–18, 20–22, and 26–29 were common to all the patterns. The pattern observed in each cultivar is shown in Table 1.

Chromosome location. The results of chromosome location are summarized in Table 3. Genes determining the presence of isozyme bands 1, 3, 5, 7, and 8 were located in chromosome arm 3AS; those determining isozymes 16, 17, 20, 25, 26, and 33 in chromosome arm 3BL; and those determining isozymes 14, 15, 18, 19, 21, 22, 23, and 29 in 3DL.

Substrates and inhibitors. The results obtained with substrates and inhibitors are summarized in Table 3. The EDTA partially inhibited all the isozymes but none specifically. As for standard electrophoresis, these results were observed in all the cultivars.

Genetic control. The F_2 progeny from the cross Banks × Capitole permitted the study of segregation of esterase pairs 30, 32 (Pattern I) and 31, 33 (Pattern E). Among the F_2 individuals, 76 simultaneously showed isozymes 31 and 33, while 30 simultaneously showed isozymes 30 and 32. This segregation fit to 3:1 single locus segregation with a χ^2 value of 0.62, $0.50 > P > 0.40$. No endosperm showed any other possible combination of these isozymes. Likewise, no cultivar showed isozyme combinations different from 30/32 or 31/33.

Discussion

Standard electrophoresis

We were able to distinguish 16 sharp esterase isozyme bands in polyacrylamide gels. These isozymes were distributed in three activity zones in gels and in three different patterns among cultivars. The use of substrates and inhibitors corroborated the distribution of endosperm esterases in three zones of activity. The assumption is that major functional differences between esterases, such as substrate and inhibitor specificities and electrophoretic mobility differences, reflect the existence of esterases encoded by different genetic systems (Matteo 1975). Thus, the fastest and slowest migrating isozymes (EST1 and EST3) were stained with β-naphthyl acetate, while the medium ones (EST2) were not. The pHMB inhibited specifically the same EST1 isozymes (1, 3, and 5) which were not stained by α-naphthyl propionate; thus, EST1 zone should be formed by at least two different groups of esterases.

EST1. At least some of the isozymes of the EST1 zone could have a dimeric structure. According to the location data (Table 2) and the response to α-naphthyl propionate and pHMB, we propose that isozymes 1 and 5 are, respectively, the homodimers of the "fast" (F) and "slow" (S) subunits of a homoeologous system of isozymes. The F and S subunits would be coded, respectively, by genes on arms 3DS and 3AS. Isozyme 3 would be the corre-

Table 3. Chromosomal location of wheat endosperm esterases as revealed by electrofocusing, and response to substrates and inhibitors

Lines	Isozymes																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29	31	33	34	35
3A–3B	−	+	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
3A–3D	−	+	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
3AL	−	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3AS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3B–3A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3B–3D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3BL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3BS	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3D–3A	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3D–3B	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3DL	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3DS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Substrates inhibitors																																
β-naphtyl-acetate	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
α-naphtyl-propionate	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pHMB	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iodoacetamide	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4. Proposal of correspondence between esterase isozymes by electrofocusing patterns

Ainsworth et al. (1984)		This work	
Isozyme	Chromosome	Isozyme	Chromosome arm
1	3B	16	3BL
2[a]	?	17[a]	3BL
3	3D	18	3DL
4[a]	?	19	3DL
5	3B	20	3BL
6	3B		
7	3D	21[a]	3DL
8[a]	3D	22[a]	3DL
		23	3DL
9	3B	25	3BL
10	3B	26	3BL
11[a]	3A	27[a]	3AL
12[a]	3A	28[a]	3AL
13	3D	29	3DL
14	3D		
15	3D		
16	3B	31[b]	?
17	3B	33	3BL
18	3A	34	3AL
19[a]	3A		
20[a]	3A		
21	3A	35	3AL

[a] Relatively thin isozymes
[b] This band should be the summation of at least two isozymes, since it never disappears in aneuploid lines

sponding heterodimer plus another isozyme also coded by 3AS. The dimeric structure for the most anodic esterase bands and located in the short arms of chromosomes of the homoeology group 3 have been described before (Barber et al. 1968; Jaaska 1980). Isozymes 2 and 4 must be the summation of products encoded by genes located on different chromosomes of group 3, in accordance with the intensities observed in nulli-tetrasomic lines (Table 2). From our data we cannot determine if isozymes in bands 2 and 4 are monomeric or dimeric. This genetic control means that at least five different genes are needed to produce the isozymes of EST1 zone.

EST2. Unfortunately, we cannot determine if esterase isozymes included in EST2 zone have a monomeric or dimeric structure. Bergman and Maan (1973) suggested a monomeric structure for esterases without affinity for β-napthyl acetate, which is the case of EST2 esterases. But monomeric or dimeric, the minimum number of loci controlling EST2 zone would be four. If they are monomeric, isozyme 10 has to be the result of the co-migrating of at least two different isozymes coded by different chromosomes, because they never disappear in aneuploid lines (Table 2). Isozyme pairs 7–9 and 11–12 are pairs of main-satellite bands, since in both cases the two

isozymes showed simultaneous appearance or absence in both aneuploid lines and cultivars (Tables 1 and 2). If they are dimeric enzymes, the minimum number of loci would also be four, chromosome arm 3BL would carry two non-allelic genes determining the "fast" (F) and "intermediate" (I) subunits, whilst 3DL would carry the "intermediate" and "slow" (S) subunits. Thus, isozymes 7, 9, 10, 11, and 12 of Chinese Spring wheat would be the dimers FF, FI, FS + II, IS and SS, respectively.

EST3. From three to six genes would be needed to determine this zone, depending upon different predictions about the monomeric or dimeric nature of cereal esterases. Therefore, it can be concluded that the number of genes controlling the esterases of wheat endosperm would be between 12 and 15.

Electrofocusing

This technique revealed 35 isozyme bands, distributed between pH 4.5 and 7.3, in comparison with the 16 isozymes observed by polyacrylamide gels.

Cubadda et al. (1975) studied esterases of wheat seeds by electrofocusing and located 17 isozymes of Chinese Spring in the homoeology group 3. Ainsworth et al. (1984) also analyzed by means of electrofocusing the seed esterases of different cultivars of hexaploid wheat. They observed seven patterns with a total of 21 isozymes ranging from pH 6.2 to 7.2. In spite of different technical procedures between our work and that of Ainsworth et al. (1984), the patterns correspond well; the isozymes described by these authors coincided with the isozymes 13–16 that we observed from pH 5.5 to 7.2 (Table 4). This coincidence was supported by the data of chromosomal location (Table 4).

We tried to relate band-to-band within the isozymes showed in standard electrophoresis with those appeared by electrofocusing, in order to know which ones were new esterase isozymes. On the basis of the response to β-napthyl acetate (Table 3), it is possible to relate isozymes 1–9, 10–28 and 29–35, respectively, with EST1, EST2 and EST3 zones. The response to α-napthyl propionate and the data of chromosomal location suggest that isozymes 3 and 5 of EST1 correspond to isozymes 1, 3, 5, 7 and 8 of electrofocusing. On the other hand, all the cultivars which showed in the first method the pair of isozymes 6/8, showed by electrofocusing the pair 30/32, and those which showed the pair 7/9 showed the pair 31/33. But since their response to β-naphthyl acetate is different (Tables 2 and 3), it would be necessary to ascertain if such differences are of a technical nature occurring during the staining procedure, before it is possible to determine the relationship of these isozymes. Isozyme 15 of EST3 by standard electrophoresis would correspond to 34 and 35 of electrofocusing, since the six cultivars lacking it (pattern B) also lack isozyme 34 and 35 of electrofocusing (pattern F) and in both cases they were located in 3AL. The isozyme 1 located in 3DS could not be related by anyone to electrofocusing, probably due to co-migrating bands.

Ainsworth et al. (1984) observed that all the isozymes of each group of homoeology co-segregated; they proposed the existence, for mapping purposes, of three "compound loci" (*Est-A5, Est-B5* and *Est-D5*) composed of tightly linked subunits, each subunit encoding a single product. According to our data of chromosomal location, at least five of these "compound loci" are required to encode wheat endosperm esterases. Complex esterase loci encoding multiple bands have also been previously described in leaf in wheat (Jaaska 1980) and in other cereals, barley (Kahler and Allard 1970; Kahler 19891) and rye (Schmidt-Stohn and Wehling 1983). If each "compound loci" located in different wheat chromosome arms has several true loci, then our previous estimation of 12–16 loci controlling endosperm esterases is clearly possible.

The 3:1 F_2 segregation obtained in the cross Banks × Capitole supports the hypothesis that the difference between isozyme pairs 30/32 and 31/33 is due to a change in mobility originated by a post-translational modification determined by a "modifier" gene specifically acting on these two isozymes. The hypothesis would be: (i) Isozymes 30/32 and 31/33 are encoded by the same gene(s). (ii) The gene(s) are present in all the cultivars analyzed, since all of them had isozymes 30/32 or 31/33. (iii) The dominant allele *M* of a "modifier" gene determines the slower migration of these isozymes (31/33), while the recessive allele *m* determines the faster couple (30/32).

Genes controlling the electrophoretic mobility of isozymes have been described for MDH of maize (Goodman and Stuber 1980) and peroxidases of petunia (Wijsman 1983).

Our data confirm that the esterases of hexaploid mature seed, and endosperm esterases in particular, are controlled by a numerous set of genes located in the homoeology group 3. The use of complementary electrophoretic and electrofocusing techniques has revealed that there are also esterase genes in the short arms of group 8 chromosomes, thus increasing the number of previously described genes related to wheat seed esterases. Segregational studies have revealed that some differences in esterase patterns between different cultivars are due to the change in mobility of isozymes controlled by "modifier" genes.

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