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The genetic control of grain esterases in hexaploid wheat

1. Allelic variation

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Summary. Analysis of grain esterase isozymes in Chinese Spring aneuploid genotypes by IEF confirmed that genes on the long arms of chromosomes 3A, 3B and 3D (*Est-5*) control the production of 19 isozymes. Allelic variants have been found for the isozyme pattern controlled by each chromosome. Segregational data involving null alleles and complex phenotypic differences indicate that the wheat grain esterases are encoded by three compound and probably homoeoallelic loci, each capable of producing at least six different isozymes. In a sample of 138 hexaploid genotypes, seven alleles were distinguished.

Key words: Hexaploid genotypes – Allelic variation – Esterase – Isozymes – Isoelectric focusing – Wheat

1 Introduction

A large number of different carboxylic ester hydrolases (EC 3.1.1.1), known simply as 'esterases' have been detected in plant tissues. Using electrophoretic techniques and azo-dye coupled stains to locate esterases, multiple isozymes have been shown to occur in a wide variety of plant species (Van der Jooste and Moreland 1963; Nakai 1977).

The role of the different plant esterases is poorly understood. In animals, most esterase isozymes are apparently non-specific and exhibit overlapping substrate specificities (Coates et al. 1975). In certain cases the isozymes are relatively specific, such as the cholinesterases and carbonic anhydrases.

In plants, the esterases are possibly the best studied group of enzymes, and in wheat have been the subject of numerous studies with various objectives. Much of the available literature is concerned with the genetic control of esterase (Nakai 1973, 1976; May et al. 1973; Bozzini et al. 1973; Cubadda et al. 1975; Jaaska 1980) or the evolution of wheat (Nakai 1973, 1978, 1979; Belea and Fejer 1980; Jaaska 1980) but there have also been studies concerned with defining alien-wheat chromosome homoeologies (Artemova 1982; Barber et al. 1968), the identification of alien-wheat hybrids (Fedak 1977; Fejer and Belea 1978) and the extent of esterase variation in hexaploid genotypes (Alexandrescu and Saulescu 1982).

This paper sets out to clarify the situation with respect to the genetic control of grain esterases in hexaploid wheat, *Triticum aestivum* (2n = 6x = 42), and to document the extent of allelic variation in a sample of hexaploid varieties.

2 Materials and methods

2.1 Genotypes

2.1.1 Aneuploid lines. The nullisomic-tetrasomic (NT) and ditelosomic (DT) genotypes of Chinese Spring (CS) developed by E. R. Sears (Sears 1954, 1966a, 1966b) were employed to examine the effect of nullisomy for particular chromosomes on grain esterase zymograms. All available nullisomic-tetrasomic genotypes for the seven chromosome groups were screened together with the ditelocentrics for group 3.

2.1.2 Hexaploid genotypes. The 138 hexaploid wheats examined are listed in Table 1. These were obtained from the Plant Breeding Institute collection, the Small Grain Collection, Germplasm Resources Laboratory, Beltsville, USA, and Mr. Xin Zhi-Yong, Institute of Crop Breeding and Cultivation, Peking, China.

2.1.3 Intervarietal chromosome substitution lines. Two substitution series were used to identify the chromosomal control of novel esterase isozymes and to verify chromosomal locations established by nullisomic analysis: CS (Timstein) developed by E. R. Sears and CS (Synthetic) developed by C. N. Law and A. J. Worland. Synthetic is a synthetic hexaploid; an amphidiploid of *T. dicoccum* × *Aegilops squarrosa* (McFadden and Sears 1946).

2.1.4 Random lines. F_2 progenies from two crosses were analysed for segregation of esterase isozymes: CS × Synthetic and CS × Kalyansona.

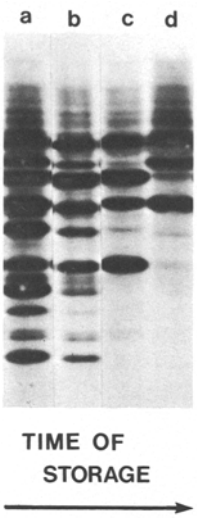

Fig. 1. Esterase zymograms of Chinese Spring grain obtained from extracts (*a*) run immediately and stored at 4 °C for (*b*) 2 days, (*c*) 3 days, (*d*) 4 days. The activity of the cathodal isozymes decreases markedly with extract storage time

2.1.5 Enzyme extraction. Single mature grains were milled in a microhammer mill (Paulis and Wall 1979) and the flour mixed with extraction buffer (1 : 5 w/v 0.05 M phosphate pH 7.5). The extracts were allowed to stand for 1 h and then centrifuged at 12,000×g for 10 min. The supernatants were used immediately for isoelectric focusing. Storage of extracts, even at 4 °C, was observed to result in loss of enzyme activity in specific isozymes (Fig. 1).

2.1.6 Isoelectric focusing. Esterase isozymes were separated by flat-bed isoelectric focusing (IEF) using Ampholine PAG plates pH 3.5–9.5 (LKB). Gels were prefocused for 30 min before applying 20 µl sample extracts absorbed in 5×10 mm filter-paper wicks (Whatman 3MM) and placed 20 mm from the cathode, directly onto the gel surface. The sample application pieces were removed after 30 min focusing at a constant power of 1.2 W cm^{-1} width of gel. Electrofocusing was terminated after a further 2 h. pH gradients were obtained, prior to staining, by taking pH readings at 5 mm intervals across the surfaces of the gels.

2.1.7 Enzyme visualisation. Gels were stained for esterase activity with a mixture of α-naphthyl acetate (50 mg) and Fast Blue RR salt (100 mg) dissolved in 5 ml acetone and made up to 100 ml with 0.05 M phosphate buffer pH 7.5. Gels were destained, to remove background stain, with 7% acetic acid solution.

3 Results

3.1 Esterase isozymes in Chinese Spring

The Chinese Spring esterase zymogram, obtained from extracts of mature grains, comprised at least 30 isozymes, with isoelectric points (pIs) between pH 5.5 and 7.2 (Fig. 2). However, the most anodal isozymes were poorly resolved and only the 21 most cathodal isozymes, with pIs greater than 6.2, were considered in the analysis.

The group 3 chromosomes have been implicated in the control of the production of grain esterases in CS by a number of workers. Analysis of the group 3 nullisomic-tetrasomics of CS confirmed this result (Fig. 3). Chromosomes 3A, 3B and 3D controlled the production of six, seven and six isozymes respectively (3A: isozymes 11, 12, 18–21; 3B: isozymes 1, 5, 6, 9, 10, 16, 17; 3D: isozymes 3, 7, 8, 13, 14, 15). Two of the 21 isozymes proved too faint to be assigned to a chromosome (bands 2 and 4).

In each case the long-arm was responsible for the isozymes since the zymograms of CSDT3AL, CSDT3BL and CSDT3DL were the same as the euploid zymogram, while those of CSDT3AS and CSDT3DS lacked the respective 3A and 3D isozymes.

Isozyme 7, controlled by 3D, was not completely removed (together with a number of other isozymes) in the relevant nullisomic genotypes although it was reduced in intensity. It is likely that another isozyme with similar pI but controlled by a different homoeologous chromosome remained after the removal of chromosome 3D.

3.2 Allelic variation

3.2.1 Number of loci. At least six esterase isozymes are controlled by each of the chromosomes 3A, 3B and 3D. Such multiple band patterns could be produced (a) by several loci on each chromosome or (b) by single loci which either are compound and have several closely-linked subunits or encode products which are subject to post-translational modification.

This question was investigated by examining segregation of the multiple bands encoded by chromosome

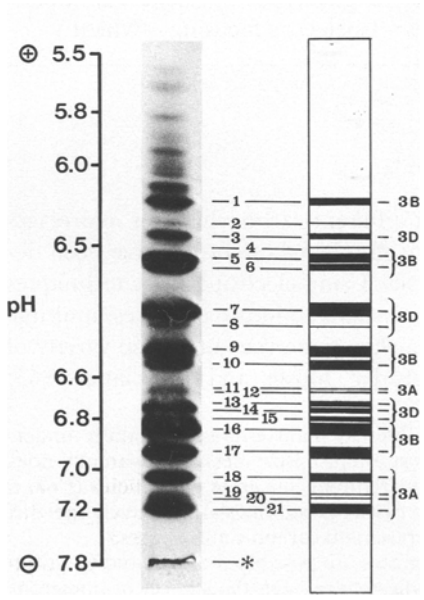

Fig. 2. Chinese Spring euploid grain esterase zymogram, and diagrammatic representation showing the isozymes under consideration and their chromosomal control, *indicates the position of the anodal end of the sample application wick

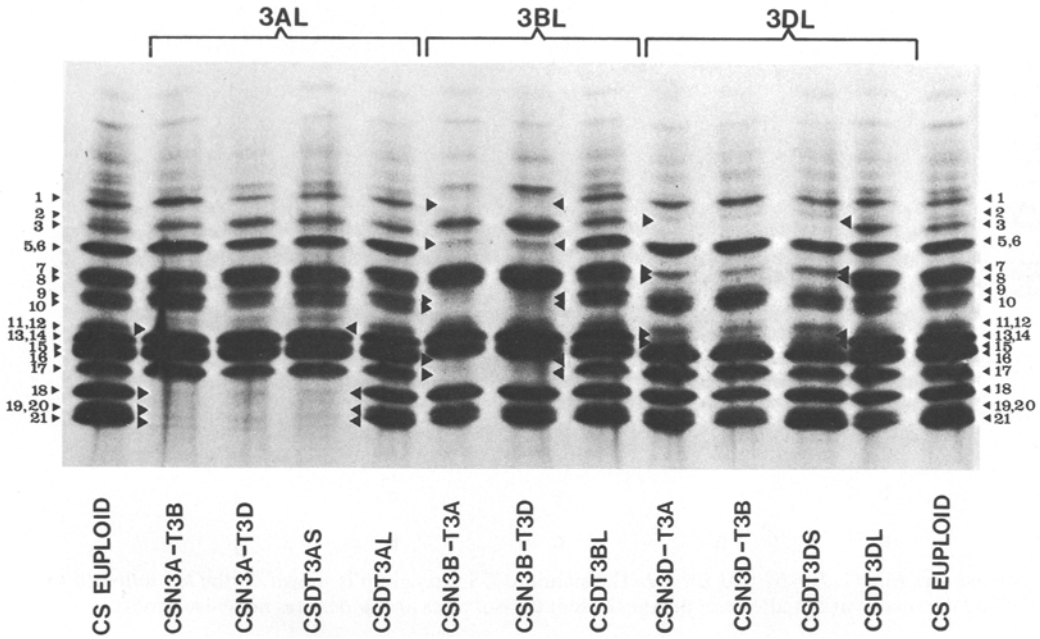

Fig. 3. Esterase zymograms of chromosome group 3 nullisomic-tetrasomic and ditelosomic aneuploid genotypes of Chinese Spring. *Arrows* indicate the absence of CS bands

3A in F_2 progeny derived from a cross between Chinese Spring and Kalyansona, which has a null phenotype for all six 3A bands (Fig. 4). In the 69 F_2 grains examined, parental esterase phenotypes only were observed; bands 11, 12 and 18–21 always segregated together, indicating the presence of, for mapping purposes, a single locus on 3AL. Segregation of 3B and 3D multiple band differences suggested that single, although possibly compound, loci are also involved on these chromosomes (see 3.2.5 below and Fig. 8).

The three esterase loci on 3AL, 3BL and 3DL which comprise the *Est-5* homoeoallelic series are designated *Est-A5*, *Est-B5* and *Est-D5*, respectively. For details of *Est-1* to *Est-4* see 'Discussion' and Hart (1984). The dif-

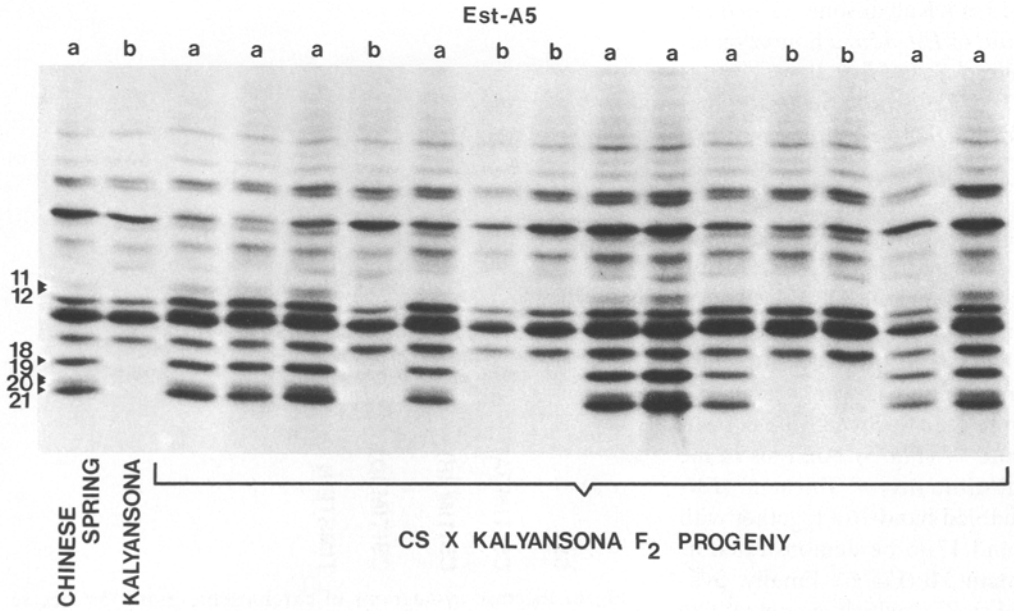

Fig. 4. Esterase zymograms of a sample of Chinese Spring (*Est-A5a*) × Kalyansona (*Est-A5b*) F_2 progeny showing segregation of isozyme bands controlled by chromosome 3A. Two classes only are distinguished: *a: Est-A5a/a* homozygotes plus *Est-A5a/b* heterozygotes, the two being indistinguishable and *b: Est-A5b/b* homozygotes

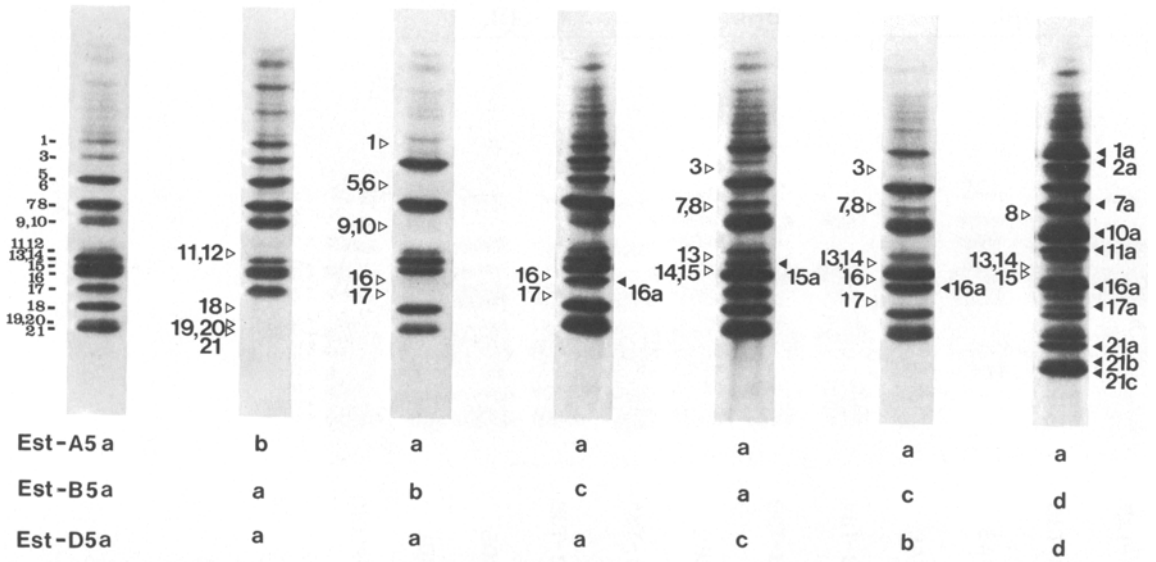

Fig. 5. Allelic variation at esterase loci *Est-A1, Est-B1* and *Est-D1.* The standard CS zymogram is shown on the *left* followed by six variant zymograms which include the seven variant alleles. ▷ denotes absent CS isozymes and ▶ denotes novel isozymes

ferent segregating combinations are assigned allele symbols by suffixing the locus symbols with letters. Chinese Spring is always assigned the '*a*' allele and variants are identified by '*b*', '*c*' etc.

3.2.2 Est-A5 alleles. Two alleles were recognised. *Est-A5a,* the allele carried by CS and 123 varieties and encoding the six isozymes already mentioned. A null allele, *Est-A5b,* carried by 15 varieties, produced a null phenotype for these isozymes (Table 1; Fig. 5). Transmission of *Est-A5b* was normal when segregating in F_2 progeny from CS (*Est-A5a*) × Kalyansona (*Est-A5b*) and fitted closely a 3 : 1 ratio of *Est-A5a/a* homozygotes + *Est-A5a/b* heterozygotes : *Est-A5b/b* homozygotes (51:18 $\chi^2_{(1)}=0.022$, $P>0.05$). *Est-A5a/b* heterozygotes were not distinguishable from *Est-A5a/a* homozygotes.

3.2.3 Est-B5 alleles. Four alleles were identified at *Est-B5.* The CS allele *Est-A5a,* encoding seven isozymes, was present in 128 of the 138 genotypes (Fig. 5). A null allele, *Est-B5b,* giving a completely null 3B esterase phenotype was present in Big Club. *Est-B5c* was present in seven varieties (Table 1) and produced a phenotype in which isozyme bands 16 and 17 were absent and replaced by a novel isozyme (16a) between the expected positions of bands 16 and 17 (Fig. 5). Analysis of the group 3 chromosome substitutions of Timstein (*Est-B5c*) into CS (*Est-B5a*) enabled band 16a together with the absence of bands 16 and 17 to be demonstrated as under the control of Timstein 3B (Fig. 6). Finally, Synthetic carried the allele *Est-B5d* which encoded five novel isozymes (1a, 2a, 7a, 10a and 11a) in addition to the seven isozymes encoded by *Est-B5a.* These five novel bands were present in the zymogram of the 3B substitution of Synthetic into CS (Fig. 7).

3.2.4 Est-D5 alleles. Three allelic variants were identified at *Est-D5* in addition to the '*a*' allele carried by CS and 134 genotypes, and encoding the six 3D isozymes identified in the nullisomic analysis.

Est-D5b carried by *T. macha* produced a null 3D phenotype in which bands 3, 8, 13 and 14 were absent and band 7 was reduced in intensity (Fig. 5). (Band 7 was not completely removed by nullisomy for 3D.)

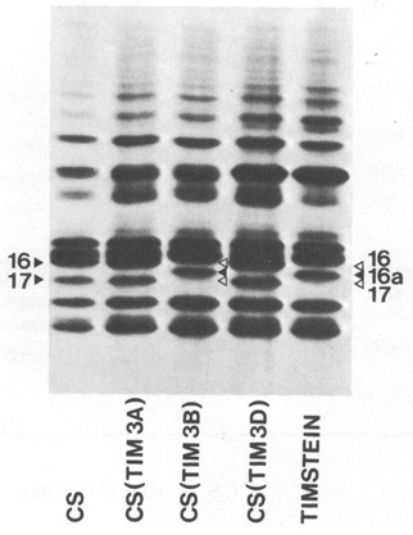

Fig. 6. Esterase zymograms of chromosome group 3 Chinese Spring (Timstein) chromosome substitution lines. The two CS isozymes (*16* and *17*) absent from Timstein are *arrowed,* together with the novel band *16a* present in Timstein

Table 1. The hexaploid genotypes surveyed for esterase isozyme variation and the alleles present at the *Est-A5, Est-B5* and *Est-D5* loci. The origins of the genotypes are given in parenthesis. Arab = Aarabia, Arg = Argentina, Aust = Australia, Belg = Belgium, Can = Canada, Ch = China, Fr = France, Ger = Germany, Ind = India, Ir = Iran, It = Italy, Ken = Kenya, Mex = Mexico, Mor = Morocco, Neth = Netherlands, NZ = New Zealand, Nor = Norway, Port = Portugal, Swe = Sweden, UK = United Kingdom, USA = United States of America, USSR = Russia

Est-A5a, Est-B5a, Est-D5a

Arawa (NZ), Arjun (Ind), Azteca (Mex), Atlas 66 (USA), Bei Jing 8 (Ch), Bei Jing 10 (Ch), Bersee (Fr), Besostaya 1 (USSR), Bounty (UK), Brigand (UK), Capitole (Fr), Cappelle-Desprez (Fr), Champlein (Fr), Cheyenne (USA), CHINESE SPRING (Ch), Ciano 67 (Mex), C306 (Ind), Hybride de Joncquois (Fr), Druchamp (Fr), Feng Kang 2 (Ch), Feng Kang 4 (Ch), Feng Kang 13 (Ch), Feng Kang 15 (Ch), Fenman (UK), Gan Mai 8 (Ch), Glennson (Mex), Graecum 787A (USSR), Highbury (UK), Hobbit (UK), Holdfast (UK), Hope (USA), Hsiang Yang 2 (Ch), H14471 (Ger), HD 2177 (Ind), Inia 66 (Mex), IWP 72 (Ind), Janak 19 (Ind), Jin 2148 (Ch), Jing 771 (Ch), Jing Hong 4 (Ch), Jing Hong 5 (Ch), Ke Chun 14 (Ch), Ke Chun 17 (Ch), Kenya 321 (Ken), Kenya 341 (Ken), Kenya Selection (Ken), Kharkov (USSR), Kharkov 22MC (Can), Koga II (Ger), Liao Chun 5 (Ch), Liao Chun 8 (Ch), Little Joss (UK), Lutescens 62 (USSR), Mara (It), Maris Huntsman (UK), Maris Ranger (UK), Maris Settler (UK), Maris Sportsman (UK), Manella (Neth), Minister (Belg), Minister Dwarf (Belg), Nong Da 311 (Ch), Novosibirskaja 67 (USSR), NP4 (Ind), Penjamo 62 (Mex), Pinyte (Mor), Pratap 16 (Ind), Poros (Ger), Red Chief 3392 (USA), Red Chief 12104 (USA), Restauracao (Port), RL 4137 (Can), Sandomirka (Port), Sappo (Swe), Sava (Yug), Sefidak (Ir), Sharbati Sonora (Ind), Sicco (Neth), Soissonais (Fr), Spica (Aust), Stewart (USA), SD1 (USA), SD2 (USA), Tai Shang 1 (Ch), Timmo (Swe), Tom Thumb (USA), *Triticum spelta, T. sphaerococcum* 1–12, Trym (Nor), Var 9 11 (Arab), Vilmorin 27 (Fr), VPM (Fr), Wembley (UK), White Fife (Can), Yang 1 (Ch), Zhong 7602 (Ch), Zhong 7605 (Ch), Zhong 7606 W (Ch), Zhong 7725 (Ch), Zhong 8604 (Ch)

Est-A5b, Est-B5a, Est-D5a

Chota Lerma (Ind), Jiang Dong Men (Ch), Juliana (Neth), Jumbo (UK), Kalyansona (Ind), Mexipak (Mex), Piamontes (Arg), Safed Lerma (Ind), Siete Cerros (Mex), Sonalika (Ind), Sona 227 (Ind), Super X (Mex), Welcome (UK), Wilhelmina (Neth), WL 410 (Ind)

Est-A5a, Est-B5b, Est-D5a

Big Club (USA)

Est-A5a, Est-B5c, Est-D5a

Flamingo (Ger), Gabo, (Aust), Hua Dong 6 (Ch), H 8810/47 (Ger), Maris Dove (UK), NP 839 (Ind), Timstein (Aust)

Est-A5a, Est-B5a, Est-D5c

Hobbit 'S' (UK)

Est-A5a, Est-B5c, Est-D5b

T. macha

Est-A5a, Est-B5d, Est-D5d

Synthetic hexaploid

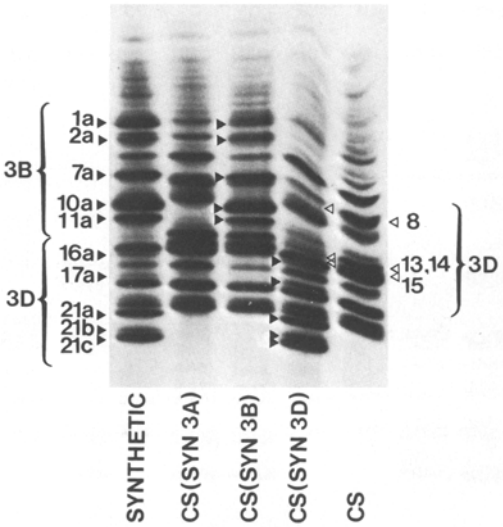

Fig. 7. Esterase zymograms of chromosome group 3 Chinese Spring (Synthetic) chromosome substitution lines. Novel bands present in the zymogram of Synthetic, controlled by chromosome 3B and 3D (▶), and CS bands absent from Synthetic (▷), are *arrowed*

A partially null phenotype was produced by the variant allele *Est-D5c*. This allele, carried by Hobbit 'S', encoded a novel band, 15a, whilst bands 8, 13, 14 and 15 were absent, and bands 3 and 7 were reduced in intensity (Fig. 5).

The third allele, *Est-D5d*, was carried by Synthetic and encoded five novel bands (16a, 17a, 21a, 21b and 21c) in addition to being null for bands 8, 13, 14 and 15 (Fig. 5). In the substitution of Synthetic 3D into CS, bands 8, 13, 14 and 15 were removed and the five novel bands were added to the zymogram (Fig. 7).

3.2.5 Segregation of Est-B5 and Est-D5 alleles. Despite the complex nature of the isozyme patterns encoded by the variant 3B and 3D alleles of Synthetic (*Est-B5d* and *Est-D5d*), studies of segregation of these alleles show that a single compound locus is involved on each chromosome. 50 F_2 progeny from a cross between CS (*Est-B5a, Est-D5a*) and Synthetic (*Est-B5d, Est-D5d*) were screened for segregation of 3B and 3D isozymes and at both loci parental band patterns only (together with heterozygotes) were observed (Fig. 8).

Transmission at *Est-B5* was normal and fitted the expected 3 : 1 ratio (8 *Est-B5a/a* : 30 *Est-B5a/d* + *EstB5d/d*; $\chi^2_{(1)} = 0.11$, $P > 0.5$). Segregation at *Est-D5* did not, however, fit the expected 1 : 2 : 1 ratio (9 *Est-D5a/a* : 35 *Est-D5a/d* : 6 *Est-D5d/d*; $\chi^2_{(2)} = 7.24$, $P < 0.05$).

3.2.6 Varietal variation. In the sample of 138 hexaploid genotypes examined, seven different esterase phenotypes can be distinguished (Fig. 5) which can be classi-

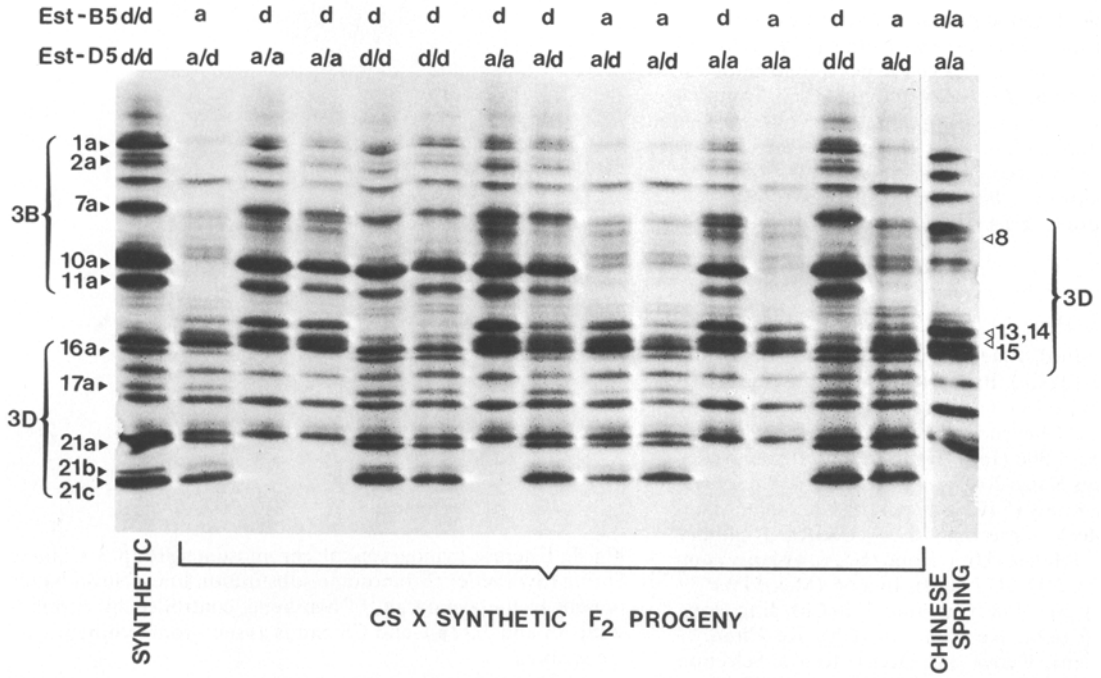

Fig. 8. Esterase zymograms of a sample of Chinese Spring (*Est-B5a, Est-D5a*) × Synthetic (*Est-B5d, Est-D5d*) showing segregation of isozyme bands controlled by chromosomes 3B and 3D. At the *Est-B5* locus, two classes are distinguished: *a: Est-B5a/a* homozygotes and *d: Est-B5d/d* homozygotes plus *Est-B5a/d* heterozygotes, the two being indistinguishable. Both homozygotes and the heterozygote are distinguishable in the segregation at *Est-D5*. Novel bands present in the zymogram of Synthetic, controlled by chromosomes 3B and 3D (▶), and CS bands absent from Synthetic (▷) are *arrowed*

fied according to the alleles carried at the *Est-A5, Est-B5* and *Est-D5* loci (Table 1). By far the majority of genotypes carry the three '*a*' alleles of CS. Of the six variants, four carried a single variant allele at one of the 3A, 3B or 3D loci and two carried two variant alleles; *T. macha* and Synthetic carried variant 3A and 3D alleles.

4 Discussion

Previous studies on the esterases of hexaploid wheat have identified four sets of homoeologous loci, which have been assigned gene symbols by Hart (1984). The *Est-1* series of loci have been located on chromosome arms 3AS, 3BS and 3DS in immature grains, roots, leaf sheaths and leaves of green plants (Barber et al. 1968, 1969; Bergman 1972). The *Est-2* loci have been located on 3AL, 3BL and 3DL in coleoptiles (Jaaska 1980). *Est-3* loci have been identified on 7BS and 7DS in coleoptiles by Jaaska (1980). A locus on 7AS encoding three isozymes in immature grains (28, 25 days post anthesis) has recently been identified (Ainsworth, unpublished). This locus may also be part of the *Est-3* series. The triplicate series of *Est-4* loci has been identified on chromosome arms 6AL, 6BL and 6DL in leaves and etiolated leaves (May et al. 1973; Nakai 1976; Jaaska 1980).

Esterases in mature wheat grains have previously been described by Bozzini et al. (1983); Nakai (1973, 1976) and Cubadda et al. (1975) and the loci responsible identified on the long arms of chromosomes 3A, 3B and 3D. However, no gene symbols have previously been assigned. We propose that this triplicate set of homoeologous loci be designated *Est-5*, as distinct from the *Est-2* series of loci which are specific to coleoptiles (Jaaska 1980). The same *Est-5* isozymes have also been shown to be expressed in immature grains (Ainsworth, unpublished).

The three homoeoallelic esterase loci, *Est-A5, Est-B5* and *Est-D5* each encode at least six isozymes. Analysis of F_2 progenies from crosses between varieties carrying different *Est-A5, Est-B5* and *Est-D5* alleles, segregating for multiple band differences, showed that only parental EST phenotypes were recovered, indicating that single loci are involved on each chromosome. It is likely that the loci are compound and composed of tightly-linked subunits, each subunit encoding a single product. Alternatively, a single product from each locus could be modified post-translationally to produce the different isozymes. Evidence against post-translational modification comes from consideration of the allele *Est-*

B5c, which produces a variant phenotype in which two of the seven isozymes controlled by chromosome 3B in CS remain unaltered, despite the fact that two 3B isozymes are replaced by a novel isozyme. Post-translational modification of a single, but mutant gene product would be expected to result in alteration of all the isozymes.

Similar complex gene loci have been demonstrated for wheat for α-amylase (*α-Amy-B2*; Gale et al. 1983), β-amylase (*β-Amy-D1* and *β-Amy-A2*; Ainsworth et al. 1983) and endosperm proteins (Law and Payne 1983). Complex esterase loci encoding multiple bands have also been described in rye (Schmidt-Stohn and Wehling 1983) and maize (MacDonald and Brewbaker 1974). Complex loci encoding leaf esterases in wheat have been described by Jaaska (1980).

Little evidence concerning the subunit number of plant esterases is available although esterase isozymes are known to exist both as monomers and dimers in animals (Harris and Hopkinson 1976). The EST-1 isozymes have been shown to be dimeric by Barber (1968, 1969), and Jaaska (1980). No good evidence for the subunit structure of the EST-2, EST-3 and EST-4 isozymes is available.

In the present study there is no evidence for dimerisation of EST-5 isozymes: no hybrid bands were seen in the zymograms of substitution lines or nullisomic genotypes, where in a dimeric situation, a single hybrid band could be removed by removal of either of two different group 3 homoeologues. In an analysis of alien-wheat chromosome addition lines and mixtures of extracts, no hybrid enzymes have been noted (Ainsworth et al., in preparation). It is therefore likely that the EST-5 esterases are monomeric.

Allelic variation occurs at all three esterase loci: two alleles were found at *Est-A5* and four each at *Est-B5* and *Est-D5*. Alleles were found at *Est-A5*, *Est-B5* and *Est-D5* which were null for all CS bands previously established as being under the control of chromosome 3A, 3B or 3D. It is unlikely that such null phenotypes result from large chromosomal deletions since there seem to be no correlated deleterious effects on either transmission or agronomic performance – *Est-A5b*, the null allele for the 3A locus, for instance, appears in a number of commercial varieties. In addition, the partial null allele at the locus on 3D, *Est-D5b*, would be difficult to explain in terms of a deletion of any size.

Allelic variation at esterase loci is not well documented in wheat. Alexandrescu and Saulescu (1982) distinguished five different esterase phenotypes in a study of grain esterases in hexaploid varieties. The phenotypes were distinguished mainly on the basis of band intensity differences using conventional disc polyacrylamide gel electrophoresis and it was concluded by these authors that such differences were caused by the action of regulator genes. Five varieties in the present study were also examined by Alexandrescu and Saulescu. These varieties, Atlas 66, Besostaya 1, Mara, Maris Huntsman and Sava, were representative of three of the phenotypes identified by Alexandrescu and Saulescu but were all found to carry the CS alleles at all three loci in this study. Here, allelic variants were distinguished solely on the basis of the absence of CS bands and the presence of novel bands. This presumably is indicative of the superior resolving power of isoelectric focusing over disc electrophoresis and has been previously demonstrated for β-amylase (Ainsworth et al. 1983). Nakai (1973) who also used IEF, reports a null phenotype at the 3A locus, which was seen in about 10% of hexaploids tested. Most of the genotypes carrying the null allele were *T. compactum* wheats.

The two most common allelic variants, *Est-A5b* and *Est-B5c* are carried by varieties which originate from a number of different countries. *Est-A5b* is carried by varieties from India, China, the Netherlands, Britain and Mexico. *Est-B5c* is carried by varieties from Germany, Australia, China, Britain and India. It is tempting to postulate that the two variant alleles have each arisen several times independently. However, inspection of pedigrees reveals that this is likely not to be the case. Eight varieties carrying the null allele *Est-A5b* (Juliana, Sonalika, Kalyansona, Welcome, Sona 227, Siete Cerros, Mexipak and Super X) were all found to have Wilhelmina (*Est-A5b*), a wheat from the Netherlands, in their ancestry. It is therefore possible that the mutation giving rise to *Est-A5b* has only occurred once and has been bred into varieties from other countries. The same may well be true for the *Est-B5c* allele found in seven varieties. Timstein and Gabo are closely related; NP839 has Gabo in its pedigree, and Maris Dove has H8810/47 in its pedigree. No common variety could be found in the pedigree of Flamingo, and pedigrees were not available for the breeders' lines H14471 and H8810/47.

The widespread occurrence of these null mutants might indicate that there is no associated selective disadvantage. However, a crossing programme has been initiated to enable the effects of different combinations of the null alleles on plant phenotype to be investigated.

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