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Identification and chromosomal locations of aconitase gene loci in Triticeae species*

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Summary. Two systems of monomeric aconitase (ACO) isozymes, designated ACO-1 and ACO-2, were identified in *Triticum aestivum* and in five diploid Triticeae species. The gene loci *Aco-A1*, *Aco-B1*, and *Aco-D1* were located in *T. aestivum* cv. 'Chinese Spring' chromosome arms 6Aq, 6Bq, and 6Dq, respectively, and the gene loci *Aco-A2*, *Aco-B2*, and *Aco-D2* in 5 Aq, 5 Bq, and 5Dq, respectively. *Aco-1* gene loci were also identified in 6Eβ of *Elytrigia elongata*, 6HL of *Hordeum vulgare* cv. 'Betzes', 6RL of *Secale cereale* 'PI 252003', $6S^l$ of *T. longissimum*, and CSU-31 of *T. umbellulatum*. Other *Aco-2* gene loci were identified in 5RL of *S. cereale* cv. 'King II' and 4EL of *E. elongata*. Conservation of synteny relationships is indicated among the species studied for the genes identified, with the exception of *Aco-E2*; the presence of this gene in 4EL suggests that *E. elongata* differs from 'Chinese Spring' and 'King II' by a translocation involving 4E and 5E.

Key words: Aconitase – Isozymes – Structural genes – Triticeae – Wheat

1 Introduction

Molecular markers have a number of inherent properties that make them more useful as genetic markers than genes that affect morphological characters (Tanksley 1983). Because of these properties, the availability of a large number of molecular markers makes feasible many basic and applied genetic investigations of crop species and their wild relatives that would otherwise be difficult or impossible.

For example, genes of agronomic importance that are not easily identified can be followed if they are tightly linked to a molecular marker or if they are flanked by two, more loosely linked molecular markers (Tanksley 1983). Also, studying selected markers whose chromosomal locations are known, selected chromosomes and chromosomal segments can be readily followed in complex cytogenetic procedures such as the development and verification of wheat-alien species chromosome addition and substitution lines (Hart et al. 1980; Hart and Tuleen 1983a, b) and wheat-intervarietal chromosome substitution lines. Furthermore, insights into evolutionary relationships within and among chromosomes are provided by knowledge of the chromosomal locations of homologous molecular markers in different genomes, chromosomes, and chromosomal segments (Hart 1979).

Approximately 115 gene loci that encode enzymes and endosperm storage proteins have been identified in *Triticum aestivum* cv. 'Chinese Spring' (Hart and Gale 1987). These comprise the vast majority of the known molecular markers, and in excess of 40% of the known gene loci, in hexaploid wheat (Cusick and McIntosh 1987). Fifteen or more enzyme and endosperm storage protein loci that are orthologous to known wheat loci have been localized in chromosomes in *Secale cereale*, *Hordeum vulgare*, *Elytrigia elongata*, and *Triticum longissimum* and a lesser number in six other Triticeae species (Hart and Gale 1987; Hart 1987).

This paper reports the results of studies designed to identify and to determine the chromosomal locations of the genes that encode aconitase (ACO, aconitase hydratase, E.C. 4.2.1.3) in *T. aestivum* cv. 'Chinese Spring' and in five other Triticeae species. Aconitase catalyzes the reversible interconversion of citrate and isocitrate via the intermediate *cis*-aconitate.

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2 Materials and methods

Strains

'Chinese Spring' (CS) aneuploids examined included all of the possible compensating nullisomic-tetrasomic types except nulli-2A tetra-2B. (Nulli-2A tetra-2D, nulli-4A tetra-4B, and nulli-4A tetra-4D plants were obtained as derivatives of monosomic-2A tetrasomic-2D, mono-4A tetra-4B and mono-4A tetra-4D plants, respectively.) Also examined were the three available homoeologous chromosome group 5 ditelosomic strains, namely, the ditelo-5Aq, ditelo-5Bq, and ditelo-5Dq strains, and four homoeologous group 6 ditelosomic strains, namely, the ditelo-6Ap, ditelo-6Bp, ditelo-6Dp, and ditelo-6Dq strains.

The available disomic chromosome addition lines of seven wheat-alien species chromosome addition series and a number of other wheat lines containing alien chromosomes or telosomes were analyzed. The lines studied and the recipient varieties, donor species, and original sources are given in Table 1. Also analyzed were the CS-*E. elongata*, 'CS-Imperial', CS-*T. longissimum*, and CS-*T. umbellulatum* amphiploids; cvs. 'Chinese Spring', 'Kharkov', 'Sturdy', 'Betzes', 'Imperial', 'King II', and 'Dakold'; and several accessions each of *T. longissimum* and *T. umbellulatum*.

Zymogram procedures

Zymogram analyses were conducted using extracts obtained from leaves of 5-day-old green plants. The leaves were macerated in a 12.5% sucrose solution with sand in a mortar with pestle at 4 °C, using a 1 : 1 weight : volume ratio of tissue: sucrose solution.

Electrophoresis was performed in horizontal Electrostarch gels (12%, W:V). The electrode buffer was composed of 0.135 M Tris and 0.043 M citric acid adjusted to pH 7.0 and the gel buffer consisted of a 1 : 14 dilution of electrode buffer (Siciliano and Shaw 1976). Filter paper wicks (Whatman No. 3) into which tissue extracts had been absorbed were inserted into the starch gels. Electrophoresis was conducted for about 5.5 h at a constant voltage of 150 volts. Gels were stained for aconitase by the method of Harris and Hopkinson (1976). Seventy-five milligrams of cis-aconitic acid were dissolved in 20 ml of 0.2 M Tris-HCl buffer, and the pH adjusted to 8.0 with 1 N NaOH. Five milliliters of this stock solution were combined with 45 ml of 0.2 M Tris-HCl buffer, pH 8.0, 15 mg NADP, 6 units isocitrate dehydrogenase, 1.5 ml of a 1.0 M $MgCl_2$ solution, 0.5 ml of a solution containing 5 mg/ml of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenol tetrazolium bromide (MTT), and 0.1 ml of a solution containing 5 mg/ml of phenazine methosulfate (PMS). Sliced gels were incubated in this solution at 37 °C for 20 min to 1 h until optimum intensity and resolution of bands were obtained, after which the gel slices were washed and fixed in a solution composed of 3% glycerol and 10% acetic acid.

3 Results

Genetic control of hexaploid wheat aconitase

The aconitase zymogram phenotype of green leaves of *T. aestivum* cv. 'Chinese Spring' consists of five bands (Fig. 1 A, H). Aneuploid analyses indicate that the three more anodal bands are produced by one group of ACO isozymes (designated ACO-1) and the other two bands by a genetically independent group of isozymes (designated ACO-2).

Table 1. Wheat-alien species chromosome addition lines analyzed, along with recipient varieties, donors, and sources

Recipient variety	Donor species	Lines examined	Original source
'Chinese Spring'	*Elytrigia elongata*	Disomic addns. 1E–7E and ditelosomic addns. 4EL, 6Eα, 6Eβ	Dvorak and Knott (1974) Dvorak (1980) Hart and Tuleen (1983a)
'Chinese Spring'	*Hordeum vulgare* cv. 'Betzes'	Disomic addns. 2H–7H and ditelosomic addns. 6HS, 6HL	Islam et al. (1981)
'Chinese Spring'	*Secale cereale* cv. 'Imperial'	Disomic addns. 1R–7R	Driscoll and Sears (1971)
'Chinese Spring'	*Secale cereale* cv. 'King II'	Disomic addns. 1R–6R	Chapman et al. (1974)
'Holdfast'	*Secale cereale* cv. 'King II'	Ditelosomic addns. 5RS, 5RL	Riley and Chapman (1958)
'Kharkov'	*Secale cereale* cv. 'Dakold'	Disomic addns. 1R, 3R, 4R, 5R, 6R	Evans and Jenkins (1960)
'Sturdy'	*Secale cereale* 'PI 252003'	Disomic addn. 6R and ditelosomic addn. 6RL	Tuleen NA (unpubl.)
'Chinese Spring'	*Triticum longissimum*	[a] Disomic addns. $1S^1$, $2S^1$, $3S^1$, $5S^1$, B, D, E and disomic substitution $6S^1$ (6B)	Feldman (1975) Hart and Tuleen (1983b)
'Chinese Spring'	*Triticum umbellulatum*	Disomic addns. 1U, 5U, 7U, A, D, E, G, CSU-31 and monosomic addn. F	Kimber (1967)

[a] Lines B and E of the *T. longissimum* series each contain 20 pairs of 'Chinese Spring' chromosomes and two pairs of *T. longissimum* chromosomes, including a pair of $6S^1$ chromosomes

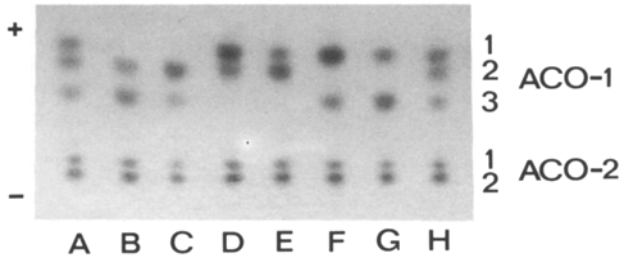

Fig. 1. Photograph of aconitase zymogram phenotypes produced by 'Chinese Spring' (CS) and by the CS homoeologous chromosome group 6 compensating nullisomic-tetrasomic strains. *A*, *H* CS; *B* nulli-6D tetra-6B; *C* nulli-6D tetra-6A; *D* nulli-6B tetra-6D; *E* nulli-6B tetra-6A; *F* nulli-6A tetra-6D; *G* nulli-6A tetra-6B. + = anode and – = cathode. Band numbers are shown on the right side of the photograph

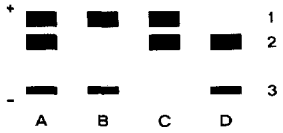

Fig. 2. Diagrams of the ACO-1 zymogram phenotypes produced by CS and by CS homoeologous chromosome group 6 ditelosomic strains. *A* CS and ditelo-6Dq; *B* ditelo-6Ap; *C* ditelo-6Bp; *D* ditelo-6Dp. + = anode and – = cathode. Band numbers are shown on the right side of the figure

Fig. 3. Diagrams of the ACO-2 zymogram phenotypes produced by CS and by aneuploid derivatives of CS. *A* CS, each of the nulli-tetra strains of homoeologous groups 1, 2, 3, 4, 6, and 7, nulli-5B tetra-5D, nulli-5D tetra-5B, ditelo-5Aq, ditelo-5Bq and ditelo-5Dq; *B* nulli-5A tetra-5B and nulli-5A tetra-5D; *C* nulli-5B tetra-5A and nulli-5D tetra-5A. + = anode and – = cathode. Band numbers are shown on the right side of the figure

Each of the compensating nulli-tetra strains of homoeologous chromosome groups 1, 2, 3, 4, 5, and 7 produces the same ACO-1 zymogram phenotype as does CS (Fig. 1 A, H). However, six different phenotypes are produced by the homoeologous group 6 nulli-tetra strains (Fig. 1 B–G). Bands 2, 3, and 1 are not present on the zymograms of strains nullisomic for chromosomes 6A, 6B, and 6D, respectively, and the staining intensities of bands 2, 3, and 1 are greater relative to the other bands present on zymograms of strains tetrasomic for chromosomes 6A, 6B, and 6D, respectively, than on CS zymograms. Diagrams of the ACO-1 zymogram phenotypes produced by the group 6 ditelosomic strains examined are shown in Fig. 2. Bands 2, 3, and 1 are not present on the zymograms of strains lacking the q arm of chromosomes 6A, 6B, and 6D, respectively. These results constitute strong evidence that the ACO-1 isozymes are monomers and that they are encoded by three gene loci located one each in the 6q chromosome arms. The aconitase structural gene loci located in 6Aq, 6Bq, and 6Dq are designated *Aco-A1, Aco-B1,* and *Aco-D1,* respectively, and the isozymes that they encode as ACO-1b, ACO-1c, and ACO-1a, respectively. These isozymes produce zymogram bands 2, 3, and 1, respectively.

The ACO-2 zymogram phenotype of CS consists of two bands, and the more anodal band stains much less intensely than the second band (Fig. 1 A, H). Among the nulli-tetra strains studied, only four produce ACO-2 phenotypes that differ from the ACO-2 phenotype of CS. The two nulli-tetra strains lacking chromosome 5A do not produce the anodal ACO-2 band and the two strains tetrasomic for 5A both produce a phenotype in which the relative staining intensities of the two ACO-2 bands are about equal. Diagrams of the ACO-2 phenotypes produced by the group 5 nulli-tetra strains and the ditelo-5Aq, -5Bq, and -5Dq strains are shown in Fig. 3.

The observed ACO-2 zymogram phenotypes support the hypothesis that the ACO-2 isozymes are encoded by three gene loci located one each in the 5q chromosome arms. The evidence that 5A, and specifically 5Aq, carries an *Aco-2* locus that encodes the anodal ACO-2 isozyme is very strong. This isozyme is not expressed by the two nulli-5A strains and is expressed by the ditelo-5Aq strain. Also, the relative intensity of the anodal band is greater on tetra-5A zymograms than on zymograms produced by strains disomic for 5A.

The evidence that the cathodal ACO-2 band is the site of two isozymes encoded by 5Bq and 5Dq genes is less direct than the aforementioned evidence for a 5Aq *Aco-2* gene locus. Observation of the staining intensities of the two ACO-2 bands relative to that of the ACO-1 bands indicates that the difference between the ACO-2 phenotype of CS and that of the strains tetrasomic for 5A and nullisomic for either 5B or 5D is due to differences in the staining intensities of both ACO-2 bands. Specifically, the nulli-5B tetra-5A and nulli-5D tetra-5A strains produce a zymogram on which the anodal ACO-2 band stains more intensely and the cathodal band less intensely than the same bands on CS zymograms. It is reasonable to postulate that the reduced staining intensity of the cathodal band is due to a reduction in *Aco-2* gene dosage and thus that the cathodal band is the site of ACO-2 isozymes produced by both 5B and 5D. The production by the nulli-5B tetra-5D and nulli-5D tetra-5B strains of the same ACO-2 phenotype as CS is consistent with this postulation. The ditelo-5Bq and ditelo-5Dq strains also produce the CS ACO-2 phenotype, indicating that the

Aco-2 loci are in the q arms of these chromosomes. The *Aco-2* loci located in 5Aq, 5Bq, and 5Dq are designated *Aco-A2*, *Aco-B2*, and *Aco-D2*, respectively, and the isozymes that they encode as ACO-2a, -2b, and -2c, respectively. The former isozyme is present at the site of band 1 and the latter two isozymes at the site of band 2. The results obtained indicate that the ACO-2 isozymes, like the ACO-1 isozymes, are active as monomers.

Subcellular fractionation of hexaploid wheat tissue extracts has disclosed that the ACO-1 isozymes are located in the cytosol and the ACO-2 isozymes in the mitochondria (Chenicek 1984). Since the two enzymes have different subcellular locations, it is to be expected that both of them will be present in all Triticeae species. As shown below, both of the enzymes were detected in each of the five 2n = 14 species analyzed in this study.

Chromosomal locations of Aco-1 and Aco-2 gene loci in diploid Triticeae species

Triticum umbellulatum. ACO-1 zymogram phenotypes differing from that of CS are expressed by *T. umbellulatum*, the CS-*T. umbellulatum* CSU-31 addition line, and the CS-*T. umbellulatum* amphiploid (Fig. 4 B, C). The expression of a novel ACO-1 isozyme by the latter two lines indicates that the alien chromosome present in the CSU-31 line contains an *Aco-1* gene locus; we designate this locus as *Aco-U1*. Apparently the *T. umbellulatum* strains studied contain a different *Aco-U1* allele than the amphiploid and the CSU-31 line since the electrophoretic mobilities of the products of the genes differ. *T. umbellulatum* ACO-2 activity was not detected in any of the chromosome addition lines (Fig 4 A, C), although it is clear that *T. umbellalatum*

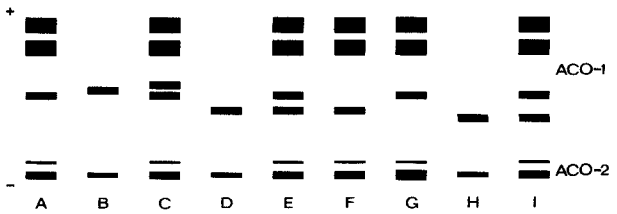

Fig. 4. Diagrams of ACO zymogram phenotypes observed in the study of the CS-*T. umbellulatum*, CS-*T. longissimum*, and CS-*H. vulgare* cv. 'Betzes' chromosome addition line series. *A* CS, CS-*T. umbellulatum* 1U, 5U, 7U, A, D, E, and G addition lines and addition line plants monosomic for *T. umbellulatum* chromosome F, CS-*T. longissimum* $1S^l$, $2S^l$, $3S^l$, and D addition lines, and CS-*H. vulgare* cv. 'Betzes' 2H, 3H, 4H, 5H, 7H, and 6HS addition lines; *B T. umbellulatum; C* CS-*T. umbellulatum* amphiploid and CSU-31 addition line; *D T. longissimum; E* CS-*T. longissimum* amphiploid; *F* CS-*T. longissimum* addition lines B and E and $6S^l$ (6B) substitution lines; *G* CS-*T. longissimum* $5S^l$ addition line; *H H. vulgare* cv. 'Betzes'; *I* CS-'Betzes' 6H and 6HL addition lines. + = anode and – = cathode

contains an *Aco-2* gene (Fig. 4 B). However, on some but not all amphiploid ACO-2 zymograms, the intensity of the cathodal ACO-2 band relative to that of the anodal band was greater than on the zymogram of CS. It is likely that the amphiploid carries an active *Aco-2* gene whose product co-electrophoreses with the cathodal CS ACO-2 isozyme.

Triticum longissimum. T. longissimum produces both an ACO-1 and an ACO-2 isozyme (Fig. 4 D). The CS-*T. longissimum* amphiploid expresses the three CS ACO-1 isozymes and the *T. longissimum* ACO-1 isozyme (Fig. 4 E). The CS-*T. longissimum* chromosome addition lines B and E and the $6S^l$ (6B) substitution line produce the *T. longissimum* ACO-1 isozyme but not the isozyme encoded by *Aco-B1* (Fig. 4 F). It is known that both line B and line E contain $6S^l$ disomically substituted for 6B and another pair of alien chromosomes (Hart and Tuleen 1983 b; Tuleen and Hart, unpubl. data). Consequently, it is clear that *Aco-*S^l*1* is located in $6S^l$.

It is likely that *Aco-*S^l*2* is located in $5S^l$ since, among the CS-*T. longissimum* lines studied, only the $5S^l$ line produced an ACO-2 zymogram that suggests the presence of an active *Aco-*S^l*2* gene in the line (Fig. 4 G). However, since the difference between the phenotypes of the CS-$5S^l$ addition line and CS consists only of a small difference in relative staining intensity among the ACO-2 bands, the assignment of *Aco-*S^l*2* to $5S^l$ must be regarded as tentative.

Hordeum vulgare cv. 'Betzes'. The zymogram phenotypes observed in the study of the 'CS-Betzes' addition lines are shown in Fig. 4 (A, H, I). Strong evidence was obtained that the 'Betzes' *Aco-1* structural gene is located in 6HL since among the 'CS-Betzes' addition lines studied, only the 6H and 6HL lines expressed the 'Betzes' ACO-1 isozyme (Fig. 4 I). The 'Betzes' ACO-2 isozyme is coincident in electrophoretic mobility with the products of *Aco-B2* and *Aco-D2*. None of the 'CS-Betzes' addition lines, including the 5H line, produced an ACO-2 phenotype that differs from that of CS, thus the chromosomal location of *Aco-H2* was not ascertained.

Elytrigia elongata. Strong evidence was obtained that the *Aco-1* and *Aco-2* genes of *E. elongata* are located in 6Eβ and 4EL, respectively. Novel ACO-1 and ACO-2 isozymes are produced by the CS-*E. elongata* amphiploid (Fig. 5 B). Among the CS-*E. elongata* addition lines, only the 6E and 6Eβ lines express the novel ACO-1 isozyme (Fig. 5 C). This indicates that *Aco-E1* is located in 6Eβ.

The ACO-2 phenotype produced by the ditelo-4EL addition line and by some disomic 4E addition line populations is shown in Fig. 5 (D). It is identical to that

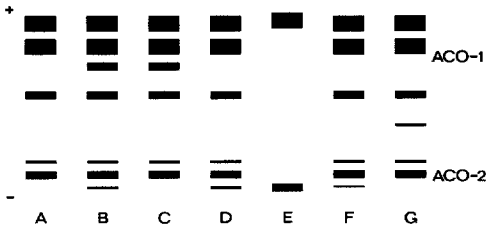

Fig. 5. Diagrams of ACO zymogram phenotypes observed in the study of the CS-*E. elongata*, CS-*S. cereale* cv. 'King II', 'Holdfast'-*S. cereale* cv. 'King II', and 'Sturdy'-'PI 252003' addition line series. *A* CS, CS-*E. elongata* 1E, 2E, 3E, 5E, 7E, and 6Eα addition lines, 'CS-King II' 1R, 2R, 3R, 4R, and 6R addition lines, and 'Holdfast'-'King II' 5RS addition line; *B* CS-*E. elongata* amphiploid; *C* CS-*E. elongata* 6E and 6Eβ addition lines; *D* CS-*E. elongata* 4E and 4EL addition lines; *E S. cereale* cv. 'King II'; *F* 'CS-King II' 5R and 'Holdfast'-'King II' 5RL addition lines; *G* 'Sturdy'-'PI 252003' 6R and 6RL addition lines. + = anode and – = cathode

Table 2. Chromosomal locations[a] of Aconitase-1 and Aconitase-2 genes in Triticeae species

	Aco-1	*Aco-2*
Triticum aestivum cv. 'Chinese Spring'	6Aq, 6Bq, 6Dq	5Aq, 5Bq, 5Dq
Triticum umbellulatum	CSU-31	nd
Triticum longissimum	$6S^1$	nd[b]
Elytrigia elongata	6Eβ	4EL
Hordeum vulgare cv. 'Betzes'	6HL	nd
Secale cereale cv. 'King II'	nd	5RL
Secale cereale 'PI 252003'	6RL	nd

[a] nd = not determined
[b] *Aco-*S^1*2* has been tentatively localized in $5S^1$

produced by the amphiploid. Since none of the other disomic addition lines produced a novel ACO-2 isozyme, these findings indicate that *Aco-E2* is located in 4EL. Two additional ACO-2 phenotypes were produced by some of the line 4E populations studied. These consisted of either the novel ACO-2 band shown in Fig. 5 plus another band of slower electrophoretic mobility, or of the band of slower mobility only. These findings indicate that two allelic forms of *Aco-E2* are present in these line 4E populations and that some plants are homozygous for one allele, some homozygous for the other allele, and some heterozygous.

Secale cereale cv. 'King II'. The ACO-1 isozyme produced by the 'King II' plants examined was approximately coincident in electrophoretic mobility with the anodal ACO-1 isozyme of CS (Fig. 5 E). None of the wheat-'King II' lines examined produced an ACO-1 phenotype that differs from that of CS, thus evidence regarding the chromosomal location of *Aco-R1* in 'King II' was not obtained.

Three ACO-2 phenotypes were observed among the 'King II' plants examined, including the one shown in Fig. 5 E. A phenotype composed of the band shown in Fig. 5 E, plus a more anodal band and a phenotype consisting of only the more anodal band were also observed. Presumably two *Aco-R2* alleles are present in the 'King II' population studied. The 'CS-King II' 5R addition line and the 'Holdfast'-'King II' 5RL addition line, but not the 5RS addition line, produce a novel ACO-2 isozyme not produced by CS (Fig. 5 F). This indicates that *Aco-R2* is in 5RL in 'King II'.

Secale cereale 'PI 252003'. Only two 'Sturdy'-'PI 252003' addition lines were available for study, namely, a line disomic for 6R and a line ditelosomic for 6RL. As expected, the ACO-2 phenotype of these lines did not differ from that of 'Sturdy'. However, both the 6R line and the 6RL line express a novel ACO-1 isozyme (Fig. 5 G). This indicates that *Aco-R1* is in 6RL in 'PI 252003'.

Secale cereale cvs. 'Imperial' and 'Dakold'. The anodal ACO-1 band and the cathodal ACO-2 on the zymograms produce by the 'CS-Imperial' 6R and 5R chromosome addition lines, respectively, stained more intensely relative to the other bands present than on the zymograms of CS and the other 'CS-Imperial' chromosome addition lines. This suggests that *Aco-R1* and *Aco-R2* are located in 'Imperial' chromosomes 6R and 5R, respectively. However, this assignment must be regarded as tentative, since the differences between the phenotypes are small.

No evidence was obtained regarding the chromosomal locations of *Aco-R1* and *Aco-R2* in 'Dakold.'

Table 2 lists the *Aco-1* and *Aco-2* gene locations determined in this study. In addition, as noted above, tentative localizations were made of *Aco-*S^l*2* to *T. longissimum* chromosome $5S^l$ and of *Aco-R1* and *Aco-R2* to 'Imperial' rye chromosomes 6R and 5R, respectively.

4 Discussion

Molecular markers for Triticeae homoeologous groups 5 and 6 chromosomes

The chromosomal locations of orthologous gene loci in different Triticeae species provide evidence of homoeology among the chromosomes and chromosomal segments in which the loci are located, just as the chromosomal locations of the members of paralogous

Table 3. Molecular markers identified in the 5p, 5q, 6p, and 6q chromosome arms of *T. aestivum* cv. 'Chinese Spring'[a]

5p	5q	6p	6q
Nor-3 set	*Aadh-1* set	*Amp-1* set	*Aadh-2* set
Skdh-1 set	*Aco-2* set	*Gli-2* set	*Aco-1* set
	β-Amy-A2	*Got-1* set	*α-Amy-1* set
	Lpx-2 set	*Nor-B2*	*α-Amy1*[b]
	Tpi-2 set		*Est-4* set
			Got-2 set

[a] For references, see Hart and Gale (1987)
[b] *α-Amy1* is located in 6Bq

sets of gene loci in different genomes within a polyploid Triticeae species provide evidence of homoeology among the chromosomes and chromosomal segments in which these loci are located (Hart 1979, 1987).

The molecular markers that have been identified to date in the chromosomes of homoeologous groups 5 and 6 of *T. aestivum* cv. 'Chinese Spring', including the aconitase gene loci identified in this study, are listed in Table 3. With the exception of *β-Amy-A2* and *α-Amy1*, each of these loci has been firmly identified as a member of a homologous set of Triticeae gene loci. Only a single *β-Amy* locus has been detected in six different diploid Triticeae species (Ainsworth et al. 1987), but in CS, *β-Amy* loci have been identified in two homoeologous chromosome groups; specifically, *β-Amy-A1* in 4A, *β-Amy-D1* in 4D, and *β-Amy-A2* in 5A (Joudrier and Cauderon 1976; Ainsworth et al. 1983; Dabrowska 1983). Also, Ainsworth et al. (1985) were unable to reconfirm that *α-Amy1* (referred to as *α-Amy2* by Hart (1983, 1984)) is a locus separate from *α-Amy-B2*, as reported by Nishikawa et al. (1981). Consequently, the status of *β-Amy-A2* and *α-Amy1* as members of homologous sets of Triticeae gene loci is uncertain.

Triticum umbellulatum

A *T. umbellulatum* chromosome that shows a high degree of homoeology with the CS group 6 chromosomes has not been identified. The long arm of the alien chromosome present in addition line A has been observed to pair with telosomes 6BL and 6DS (Athwal and Kimber 1972) and the chromosome carries a *Gli-2* locus (Shepherd 1973). However, *Aco-U1* is carried by the alien chromosome present in the CSU-31 addition line rather than by chromosome A and, while chromosome A has been substituted for 6A, 6B, and 6D (pers. commun. from E. R. Sears, cited in Kimber (1968)), the substitutions differ little phenotypically from the corresponding nullisomics. It appears that either an intact 6U is not present among the available CS-*T. umbellulatum* addition lines or that *T. umbellulatum* differs from CS by a translocation involving 6U.

Although we were unable to determine the chromosomal location of *Aco-U2* in this study, there is good evidence that the *T. umbellulatum* chromosome designated 5U is homoeologous to the CS group 5 chromosomes. Three molecular markers that are orthologous to CS group 5 markers have been identified in 5U, namely, *Nor-U3* (Martini et al. 1982), *Skdh-U1* (Koebner and Shepherd 1983) and *Tpi-U2* (Pietro and Hart 1985) [a *β-Amy* locus has also been identified in 5U (Ainsworth et al. 1987)] and 5U compensates for the chromosomes of wheat group 5 when substituted for them (Chapman and Riley 1970; see also Driscoll 1983).

Triticum longissimum

T. longissimum chromosome $6S^l$ compensates well for CS chromosome 6B when substituted for it (N. A. Tuleen, pers. commun.) and has been shown to contain four group 6 markers, namely, *Aco-S^{l}1* (this study) and *Amp-S^{l}1*, *Aadh-S^{l}2*, and *Got-S^{l}2* (Hart and Tuleen 1983 b). Consequently, there is good evidence that $6S^l$ is homoeologous to the CS group 6 chromosomes. The assignment of *Aco-S^{l}2* to $5S^l$ made in this study is only tentative but four other group 5 markers have been identified in $5S^l$, namely, *Aadh-S^{l}1* and *Lpx-S^{l}2* by Hart and Tuleen (1983 b), *Tpi-S^{l}2* by Pietro and Hart (1985), and *Skdh-S^{l}1* by Hart and Tuleen (1983 b) and Benedettelli and Hart (1986).

Hordeum vulgare cv. 'Betzes'

Brown and Munday (1982) identified an *Aco* gene locus in 'Betzes' chromosome 6H and we have shown in this study that the locus is a member of the Triticeae *Aco-1* set of gene loci. The identification of *Aco-H1* in 6HL provides additional evidence of the homoeology of 6H with the CS group 6 chromosomes. 6H has been successfully substituted for wheat chromosomes 6A, 6B, and 6D (Shepherd and Islam 1981) and carries, in addition to *Aco-H2*, four other group 6 markers, namely, *Amp-H1* and *Got-H2* (Hart et al. 1980), *Nor-H2* (Nicoloff et al. 1977; Appels et al. 1980; Saghai-Maroof et al. 1984), and *α-Amy-H1* (Brown and Jacobsen 1982; Muthukrishnan et al. 1984).

Elytrigia elongata

The results of tests of genetic compensation and chromosome pairing conducted with 6E (Dvorak 1979, 1980) as well as the molecular markers located in the chromosome attest to the homoeology of 6E to the CS group 6 chromosomes. *Amp-E1* was located in 6E*α* and *Aadh-E2* and *Got-E2* in 6E*β* by Hart and Tuleen (1983 a), *Nor-E2* in 6E*α* (=6Ep) by Dvorak et al. (1984), *α-Amy-E1* in 6E by Ainsworth et al. (1987), and *Aco-E1* in 6E*β* in this study. It should be noted that the arm locations of these loci are entirely consistent with the arm locations of the orthologous CS loci (see Table 3).

Aco-2 is a Triticeae group 5 q-arm marker, but *Aco-E2* was located in 4EL in this study. This is the first report of a difference in synteny relationships among orthologous *E. elongata* and CS gene loci. The presence of the CS *Aco-2* loci in the 5 q arms, of *Aco-R2* in 5RL in *S. cereale* cv. 'King II', and of *Aco-E2* in 4EL suggests that *E. elongata*, or at least the strain of *E. elongata* used to develop the CS-*E. elongata* chromosome addition series, differs from CS and 'King II' by a translocation involving 4E and 5E. The gametophytic and sporophytic compensation of 4E for 4A and 4D is excellent (Dvorak 1980) and of 4E for 4B (Dvorak 1980) and of 5E for 5A, 5B, and 5D is good (N. A. Tuleen, pers. commun.). *Aadh-E1* and *Lpx-E2*, two Triticeae group 5 q-arm markers, are located in 5E (Hart and Tuleen 1983 a). These findings suggest that *Aadh-1* and *Lpx-2* were located proximal to *Aco-2* in the q arm of chromosome 5 in the genome ancestral to genomes A, B, D, and E and are in this position today in CS. Furthermore, the findings indicate that the proposed translocation difference is non-centromeric and involves only a small part of 5EL. It is quite unlikely that the translocation difference arose during the development of the wheat-*E. elongata* chromosome addition lines but whether it occurs naturally throughout *E. elongata* is unknown.

Secale cereale cv. 'King II'

Aco-R2 was located in 5RL of 'King II' in this study. 5R appears to be in large part homoeologous with the group 5 chromosomes of CS but may also be partly homoeologous to the group 4 chromosomes (reviewed by Zeller and Hsam 1983). In addition to *Aco-R2*, group 5 molecular markers located in 5R include *Skdh-R1* in cvs. 'Imperial', 'King II', and 'Dakold' (Koebner and Shepherd 1983; Benedettelli and Hart 1986), *Tpi-R2* in 'Imperial' and 'Dakold' (Pietro and Hart 1985), and *Aadh-R1* in 'Imperial' and 'King II' (Hart and Tuleen, unpublished). There are no reports of known Triticeae group 4 molecular markers in 5R or of known group 5 molecular markers in 4R. However, a *β-Amy* locus has been identified in 5R, but not in 4R, in 'Imperial', 'King II', and 'Dakold' (Ainsworth et al. 1987) and, as noted above, *β-Amy* loci have been identified in 4A, 4D, and 5A in CS.

Secale cereale 'PI 252003'

Only two 'Sturdy'-'PI 252003' chromosome addition lines were available for study, namely, the 6R and 6RL lines. However, since both of these lines express a novel ACO-1 isozyme, i.e., an isozyme not expressed by 'Sturdy' (see Fig. 5), good evidence was obtained that *Aco-R1* is located in 6RL in 'PI 252003'.

Utility of Aco-1 and Aco-2 loci as chromosome markers

The results obtained in this study indicate that *Aco-1* is generally a good marker for alien group 6 chromosomes present in wheat aneuploids. *Aco-R1* is an exception; a *Secale cereale* ACO-1 isozyme that differs in electrophoretic mobility from the three wheat ACO-1 isozymes was found in only one of the four wheat-rye addition series studied. Also, the three wheat *Aco-1* loci should be useful varietal markers since extensive allelic variation exists at these loci (unpubl. results).

In contrast, *Aco-2* is generally a poor alien chromosome marker. An ACO-2 isozyme that differs in electrophoretic mobility from the wheat ACO-2 isozymes was found in only two of the seven wheat-alien addition series studied. Also, there are far fewer *Aco-2* variants than *Aco-1* variants in wheat (unpubl. results).

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References

Ainsworth CC, Doherty P, Edwards KGK, Martienssen RA, Gale MD (1985) Allelic variation at α-amylase loci in hexaploid wheat. Theor Appl Genet 70:400–406

Ainsworth CC, Gale MD, Baird S (1983) The genetics of β-amylase isozymes in wheat. 1. Allelic variation among hexaploid varieties and intrachromosomal gene locations. Theor Appl Genet 66:39–49

Ainsworth CC, Miller TE, Gale MD (1987) α-amylase and β-amylase homoeoloci in species related to wheat. Genet Res (in press)

Appels R, Gerlach WR, Dennis ES, Swift H, Peacock WJ (1980) Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma 78:293–311

Athwal RS, Kimber G (1972) The pairing of an alien chromosome with homoeologous chromosomes of wheat. Can J Genet Cytol 14:325–333

Benedettelli S, Hart GE (1986) Genetic analysis of Triticeae shikimate dehydrogenase. Agron Abstr, p 57

Brown AHD, Jacobsen JB (1982) Genetic basis and natural variations of α-amylase isozymes in barley. Genet Res 40: 315–324

Brown AHD, Munday J (1982) Population-genetic structure and optimal sampling of land races of barley from Iran. Genetica 58:85–96

Chapman V, Riley R (1970) Homoeologous meiotic chromosome pairing in *Triticum aestivum* in which chromosome 5B is replaced by an alien homoeologue. Nature 226:376–377

Chapman V, Riley R, Miller TE (1974) Annu Rep Plant Breeding Institute, Cambridge 1973, p 143

Chenicek KJ (1984) Evidence for the genetic control and subcellular locations of aconitase isozymes in Triticeae species. MSc Thesis, Texas A & M University, College Station

Cusick JE, McIntosh RA (1987) Linkage map of wheat (*Triticum aestivum:* 2n=42). In: O'Brien SJ (ed) Genetic maps, vol 4. Cold Spring Harbor Laboratory, New York (in press)

Dabrowska T (1983) Studies on chromosomal location of genes involved in beta-amylase isozymes in wheat kernels (*Triticum aestivum* L.) Genet Pol 24:9–19

Driscoll CJ (1983) Third compendium of wheat-alien chromosome lines. Suppl Proc 6th Int Wheat Genet Symp, Tokyo, Japan, 34 pp

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

Dvorak J (1979) Metaphase I pairing frequencies of individual *Agropyron elongatum* chromosome arms with *Triticum* chromosomes. Can J Genet Cytol 21:243–254

Dvorak J (1980) Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can J Genet Cytol 22:237–259

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

Dvorak J, Lassner MW, Kota RS, Chen KC (1984) The distribution of the ribosomal RNA genes in the *Triticum speltoides* and *Elytrigia elongata* genomes. Can J Genet Cytol 26:628–632

Evans LE, Jenkins BC (1960) Individual *Secale cereale* chromosome additions to *Triticum aestivum*. I. The addition of individual 'Dakold' fall rye chromosomes to 'Kharkov' winter wheat and their subsequent identification. Can J Genet Cytol 2:205–215

Feldman M (1975) Alien addition lines of common wheat containing Triticum longissimum chromosomes. Proc 12th Int Bot Congr 2:506

Harris H, Hopkinson DA (1976) Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam

Hart GE (1979) Genetical and chromosomal relationships among the wheats and their relatives. Stadler Genet Symp 11:9–29

Hart GE (1983) Hexaploid wheat. In: Tanksley S, Orton TJ (eds) Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam, pp 35–56

Hart GE (1984) Biochemical loci of hexaploid wheat (*Triticum aestivum*, $2n=42$, Genomes AABBDD). In: O'Brien SJ (ed) Genetic maps, vol 3. Cold Spring Harbor Laboratory, New York, pp 485–490

Hart GE (1987) Genetic and biochemical studies of enzymes. In: Heyne EG (ed) Wheat. American Society of Agronomy, Madison WI (in press)

Hart GE, Gale MD (1987) Biochemical/molecular loci of hexaploid wheat (*Triticum aestivum*, $2n=42$, Genomes AABBDD). In: O'Brien SJ (ed) Genetic maps, vol 4. Cold Spring Harbor Laboratory, New York (in press)

Hart GE, Islam AKMR, Shepherd KW (1980) Use of isozymes as chromosome markers in the isolation and characterization of wheat-barley chromosome addition lines. Heredity 36:311–325

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

Hart GE, Tuleen NA (1983b) Characterizing and selecting alien genetic material in derivatives of wheat-alien species hybrids by analyses of isozyme variation. In: Proc 6th Int Wheat Genet Symp. Tokyo, Japan, pp 377–385

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

Joudrier P, Cauderon Y (1976) Localisation chromosomique de gènes controlant la synthèse de certains constituants β amylasique du grain de Blé tendre. CR Acad Sci Paris, Ser D 282:115–118

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

Kimber G (1968) The relationships of single alien chromosomes to the homoeologous groups of *T. aestivum*. In: Finlay KW, Shephard KW (eds) Proc 3rd Int Wheat Genet Symp. Plenum Press, New York, pp 62–68

Koebner RMD, Shepherd KW (1983) Shikimate dehydrogenase – a biochemical marker for group 5 chromosomes in the Triticinae. Genet Res 41:209–213

Martini G, O'Dell M, Flavell RB (1982) Partial inactivation of wheat nucleolus organisers by the nucleolus organiser chromosomes from *Aegilops umbellulata*. Chromosoma 84: 687–700

Muthukrishnan S, Gill BS, Swegle M, Chandra GR (1984) Structural genes of α-amylases are located on barley chromosomes 1 and 6. J Biol Chem 259:13637–13639

Nicoloff H, Anastassova-Kristeva M, Kunzel G, Rieger R (1977) The behavior of nucleolus organisers in structurally changed karyotypes of barley. Chromosoma 62:103–109

Nishikawa K, Furuta Y, Hina Y, Yamada Y (1981) Genetic studies of α-amylase isozymes in wheat. IV. Genetic analyses in hexaploid wheat. Jpn J Genet 56:385–395

Pietro ME, Hart GE (1985) The genetic control of triosephosphate isomerase in hexaploid wheat and other Triticeae species. Genet Res 45:127–142

Riley R, Chapman V (1958) The production and phenotypes of wheat-rye chromosome addition lines. Heredity 12: 301–315

Saghai-Maroof MA, Soliman KM, Jorgenson RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian genetics, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Shepherd KW (1973) Homoeology of wheat and alien chromosomes controlling endosperm proteins. In: Proc 4th Int Wheat Genet Symp, pp 745–760

Shepherd KW, Islam AKMR (1981) Wheat: barley hybrids – the first eighty years. In: Evans LT, Peacock WJ (eds) Wheat science – today and tomorrow. Cambridge University Press, London, pp 107–128

Siciliano MJ, Shaw CR (1976) Separation and visualization of enzymes on gels. In: Smith I (ed) Chromatographic and electrophoretic techniques, vol 2, 4th edn. William Heineman, Medical Books Publication, London, pp 185–209

Tanksley SD (1983) Molecular markers in plant breeding. Plant Mol Biol Rep 1:3–8

Zeller FJ, Hsam SLK (1983) Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Proc 6th Int Wheat Genet Symp. Tokyo, Japan, pp 161–173