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# Evidence that *Aco-B2* and *Aco-D2* of *Triticum aestivum* are located in chromosomes 4B and 4D*

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**Summary**. Studies designed to determine the chromosomal locations of variant *Aconitase-2* alleles of *Triticum aestivum* disclosed that *Aco-B2* and *Aco-D2* are not located in chromosomes 5B and 5D, as formerly reported. Reinvestigation of the chromosomal locations of the genes provided strong evidence they are instead located in chromosomes 4B and 4D. Also, four *Aco-B2* alleles were identified but no variant *Aco-D2* alleles were detected.

**Key words:** Aconitase – Isozyme – *Triticum* – Wheat

## Introduction

Chenicek and Hart (1987) reported that the genes that encode aconitase-2 (ACO-2) of *Triticum aestivum* cv 'Chinese Spring' (CS) are located in the long arms of the homoeologous group 5 chromosomes. So that we might genetically map the *Aconitase*-2 (*Aco-2*) genes, we sought to identify variant alleles of the genes and to assign each allele to a specific homoeologous group 5 chromosome. Our investigations ascertained, however, that *Aco-B2* and *Aco-D2* are not located in 5B and 5D, as previously reported. Subsequently, we obtained strong evidence that the genes are instead located in 4B and 4D. This paper reports the results of these investigations.

## Materials and methods

Aconitase zymogram analyses were performed using the procedures described in Chenicek and Hart (1987). The chromosome composition of plants was determined by study of Feulgen-stained root tip squashes and/or acetocarmine-stained microspore mother cells, as appropriate.

## Results and discussion

Thirteen of 340 hexaploid wheat accessions studied displayed an ACO-2 zymogram phenotype different than that of CS, and four variant ACO-2 phenotypes were found among the 13 accessions. Three of the phenotypes, each of which differs from that of CS by the presence of an additional cathodal band, are shown in Fig. 1 B–F. The fourth variant ACO-2 phenotype, which was not investigated genetically, is identical to that shown in channels C and D of Fig. 1, except for the absence of band 2.

If the cathodal ACO-2 band of CS is produced by isozymes encoded by genes located in 5BL and 5DL, as postulated by Chenicek and Hart (1987), then it is logical to assume that the additional cathodal band present in channels B–F of Fig. 1 is produced by a variant allele

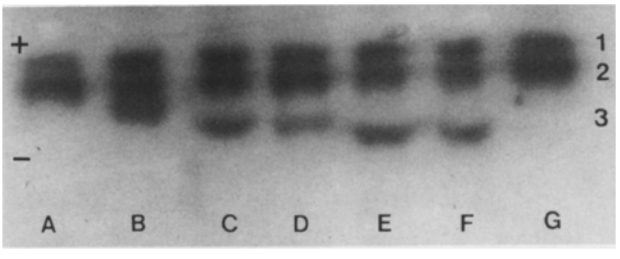


**Fig. 1.** Photograph of aconitase-2 zymogram phenotypes produced by 'Chinese Spring' (CS) and the five variant P.I. lines. *A* and *G* CS, *B* P.I. 278437, *C* P.I. 278543, *D* P.I. 182575, *E* P.I. 157589, *F* P.I. 340710. + anode and – cathode. *Band numbers* are shown on the *right side* of the photograph

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located in either 5BL or 5DL. To determine which one of these chromosomes carries the mutant allele in a given strain, the mutant strain was crossed as the male parent to a monotelosomic-5BL line and a monotelo-5DL line, and mono-5B and mono-5D plants derived from this cross were used as female parents in crosses with CS. If the mutant allele is in 5B, then among the progeny of the cross involving a mono-5B female parent, all of the 42-chromosome plants should produce an ACO-2 phenotype that includes the third cathodal band, and all of the 41-chromosome plants should produce an ACO-2 phenotype consisting only of the two anodal bands, while in the progeny of the cross involving 5D monosomy the two ACO-2 phenotypes should segregate independently of chromosome number (i.e., of monosomy or disomy for 5D). If the variant allele is instead in 5D, then independent assortment of ACO-2 phenotype and chromosome number should occur in the former group of progeny and association of ACO-2 phenotype with chromosome number in the latter group. The findings obtained in tests with P.I. 157589 and P.I. 182575 (see Table 1) were that chromosome number and ACO-2 phenotype segregated independently in *both* populations, indicating that the gene that encodes the cathodal isozyme is not located in either 5B or 5D.

**Table 1.** Segregation for chromosome number and ACO-2 zymogram phenotype among backcross progeny derived from pollinations by 'Chinese Spring' of monosomic $F_1$ plants produced in crosses of P.I. parent × monotelosomic-5BL, 5DL, and 4BS and P.I. parent × monosomic-4D

| Chromosome number | | 41 | | 42 | |
|---|---|---|---|---|---|
| Zymogram | Phenotype[a] | Bands 1 and 2 | Bands 1, 2, and 3 | Bands 1 and 2 | Bands 1, 2, and 3 |
| P.I. Parent | Chromosome involved | | | | |
| 157589 | 5B | 4 | 4 | 2 | 2 |
| | 5D | 5 | 3 | 3 | 1 |
| | 4B | 12 | 0 | 0 | 6 |
| | 4D | 2 | 1 | 2 | 3 |
| 182575 | 5B | 5 | 4 | 1 | 2 |
| | 5D | 5 | 5 | 0 | 2 |
| | 4B | 23 | 0 | 0 | 23 |
| | 4D | 3 | 7 | 4 | 1 |
| 278437 | 4B | 13 | 0 | 0 | 9 |
| | 4D | 2 | 3 | 2 | 1 |
| 278543 | 4B | 15 | 0 | 0 | 5 |
| | 4D | 5 | 6 | 1 | 1 |
| 340710 | 4B | 11 | 0 | 0 | 5 |
| | 4D | 5 | 4 | 5 | 2 |

[a] See Fig. 1

Naranjo et al. (1987) obtained evidence that a reciprocal interchange occurred between the long arms of chromosomes 4A and 5A prior to the origin to tetraploid wheat. Since *Aco-A2* is located in the long arm of chromosome 5A of CS, we hypothesized that the *Aco-2* gene set was located on the chromosomes of homeologous group 4 in the ancestral Triticeae genome and that *Aco-A2* was moved from 4AL to 5AL by the reciprocal 4AL-5AL interchange. According to this hypothesis, *Aco-B2* and *Aco-D2* are located in chromosomes 4B and 4D, respectively.

To test this hypothesis, we first determined if the isozyme that produces ACO-2 band 3, as shown in Fig. 1, is encoded by a gene located in either 4B or 4D. This was accomplished by using plants from the five P.I. lines listed in the Fig. 1 legend in tests the same as those described above but involving monosomy for 4B and 4D rather than 5B and 5D. The findings obtained with each of the five mutant lines indicate that the isozyme is encoded by a gene located in chromosome 4B. In the five populations derived from a mono-4B female parent, all mono-4B plants produced an ACO-2 phenotype composed of three bands, and all di-4B plants displayed the CS ACO-2 phenotype, while the two ACO-2 phenotypes segregated independently of 4D monosomy-disomy in the other five populations (see Table 1). We designate the *Aco-B2* allele present in 'Chinese Spring' as *Aco-B2a*, the allele in P.I. 278437 as *Aco-B2b*, the allele in P.I. 182575 and P.I. 278543 as *Aco-B2c*, and the allele in P.I. 157589 and P.I. 340710 as *Aco-B2d*.

Tests were next conducted to determine if 4D carries a gene that encodes an isozyme located at the site of band 2 of ACO-2. This was accomplished by analyzing progeny obtained by self-fertilization of plants nullisomic for the long arm of 4D and heterozygous for *Aco-B2a* and *Aco-B2c*. The plants were produced by crossing CS mono-4D plants as females with the P.I. 182575 line, crossing monosomic derivatives of this cross with CS ditelo-4DS, and selecting progeny that had 40 chromosomes plus a telosome (4DS) and displayed an ACO-2 phenotype consisting of three bands. If *Aco-D2* is in 4DL, then the intermediate and cathodal ACO-2 bands of these plants were produced by the products of *Aco-B2a* and *Aco-B2c*, respectively, and three different ACO-2 phenotypes should segregate in the progeny obtained by self-fertilization of the plants. Homozygous *Aco-B2a* plants should express band 2 but not band 3, homozygous *Aco-B2c* plants should express band 3 but not band 2, and heterozygotes should express both bands. Of 12 progeny tested, 8 produced all three ACO-2 bands, 1 produced bands 1 and 2 only, and 3 produced bands 1 and 3 only (see Fig. 2). These data strongly indicate that *Aco-D2* is located in 4D.

Based on the localization of *Aco-E2* in the long arm of chromosome 4E of *Lophopyrum elongatum* (formerly

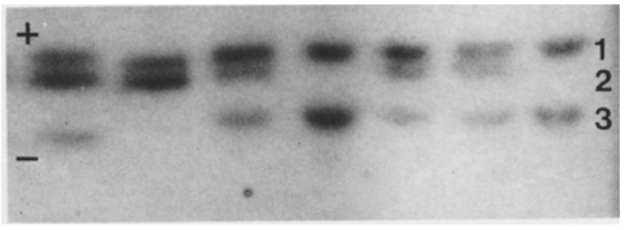


**Fig. 2.** Photograph of aconitase-2 zymogram phenotypes of segregating $F_2$ progeny obtained by self-fertilizing a plant of genotype *Aco-B2a/Aco-B2c* that was nullisomic for the long arm of chromosome 4D and displayed three ACO-2 zymogram bands (see text). + anode and − cathode. *Band numbers* are shown on the *right side* of the photograph

designated *Elytrigia elongatum*) and the apparent localization of the *Aco-2* genes of CS in the homoeologous group 5 chromosomes, Chenicek and Hart (1987) suggested that *L. elongatum* may differ from CS by a reciprocal translocation involving chromosomes 4 and 5. Pietro et al. (1988) made a similar suggestion regarding *T. searsii* and CS, based on the localization of *Aco-S*$^s$*2* in 4S$^s$. The findings reported in this paper remove the basis for these suggested translocations.

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