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Chromosomal location of peptidase, *PEPT-1*, genes in *Triticum aestivum* var. Chinese Spring

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Summary

Ditelocentric accessions of the Chinese Spring cultivar of *Triticum aestivum* were analysed electrophoretically for peptidase (PEPT) and amino peptidase (AMP = LAP) activity. Isozymic activity was missing in the accessions CSDT6AS and CSDT6BS when stained for PEPT. This was taken as evidence that the structural genes encoding these isozymes are located on the long arms of chromosomes 6A and 6B. These genes have been named *Pept-A1* and *Pept-B1*, respectively. Isozymic activity was missing in the CSDT6BL accession when stained for AMP. This result reconfirms the previously published location of the gene *Amp-B1* on the short arm of chromosome 6B and demonstrates that the two sets of loci are clearly different.

1. Introduction

The chromosomal mapping of isozyme encoding genes in wheat is of supreme importance (Ainsworth, 1983). The incorporation of desirable traits into new cultivars, especially of phenological or adult morphological characters, by crossing programmes is a tedious and long-term process. If chromosomal relationships can be determined with electrophoretic enzyme loci, relatively fast and simple analyses may be used to determine the presence or absence of desired chromosomes or chromosome segments both in early life stages and generations. Secondly, genetic analysis of polygenic characters may be facilitated by associations with gene markers of known locations.

Aneuploid and ditelocentric accessions of cultivated wheats have been used for analysis of locations of isozyme encoding genes for the past 15 years (e.g. Barber *et al.* 1968; Hart, 1973; Ainsworth, 1983; McIntosh, 1983). Specifically, two general peptidase isozyme systems have been described and mapped to wheat chromosomes. Hart (1979) described a homologous set of amino peptidase loci, *Amp-1*, and has located them on the short arms of chromosomes 6A, 6B and 6D. A second set of loci *Ep-1* are located on the group 7 chromosomes (Hart & Langston, 1977). This paper presents evidence of the peptidase loci, *Pept-1*, located on the long arms of chromosomes 6A and 6B in *Triticum aestivum* variety Chinese Spring.

2. Materials and Methods

Seeds of *T. aestivum* var. Chinese Spring, of the ditelocentric accessions CSDT6AS, CSDT6AL, CSDT6BS, CSDT6BL, CSDT6DS, CSDT6DL, CSDT7AS, CSDT7AL, CSDT7BS, CSDT7BL and CSDT7DS were germinated on moist filter paper. After germination, the seedlings were transferred to small containers filled with washed sand. After 8–10 days, leaf tissue was sampled from each plant and crushed in a 0·2 M phosphate buffer, pH 7·0, with mercaptoethanol. The crude extract was absorbed on filter paper wicks and the wicks were inserted into horizontal starch gels (12% w/v). The buffer system for the isozyme assays was Trisma base 0·135 M and 0·043 M citric acid, pH 7·0. The gel buffer was diluted 1:15. The staining procedures for PEPT (E.C. 3.4.13.11) and for AMP as listed for LAP (E.C. 3.4.11.1) are listed in Brown *et al.* (1978). Peptidase may also be stained using phenylalanyl-alanine as a substrate, in which case an additional isozyme appears anodal to PEPT-1.

3. Results and Discussion

Photographs of the electrophoretic assays are shown in Figs. 1 and 2. The common PEPT-1 phenotype of Chinese Spring consists of two overlapping bands each representing one isozyme. Deviations from the common PEPT-1 phenotype of Chinese Spring occur in CSDT6AS and CSDT6BS. In CSDT6AS the upper band of the system does not appear, and only a single band of slow mobility is apparent. The complimentary band of the PEPT-1 phenotype is similarly missing in CSDT6BS. The more mobile isozyme is clearly apparent, whereas the slower is missing. These results were obtained repeatably in several different trials. The absence of the slower isozyme in the acces-

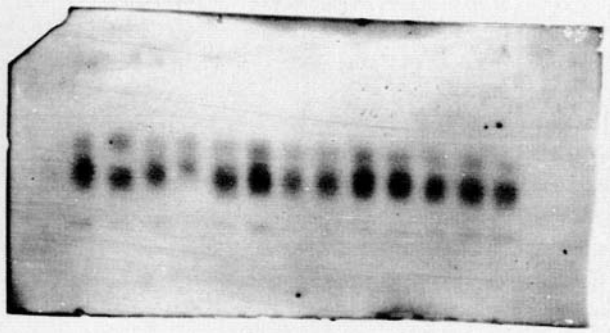

Fig. 1. Photograph of a gel stained for PEPT. The samples are in the following order: (1) Chinese Spring; (2) CSDT6AS; (3) CSDT6AL; (4) CSDT6BS; (5) CSDT6BL; (6) CSDT6DS; (7) CSDT6DL; (8) CSDT7AS; (9) CSDT7AL; (10) CSDT7BS; (11) CSDT7BL; (12) CSDT7DS; (13) Chinese Spring. Samples 2 and 4 show deviant patterns with the faster and slower isozyme bands missing, respectively.

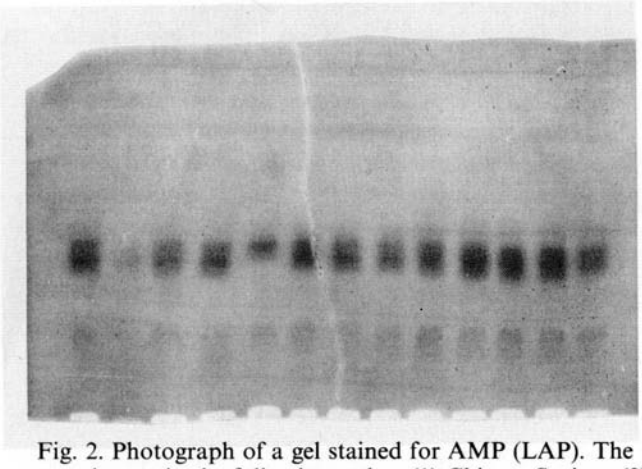

Fig. 2. Photograph of a gel stained for AMP (LAP). The samples are in the following order: (1) Chinese Spring; (2) (6) CSDT6DS; (7) CSDT6DL; (8) CSDT7AS; (9) CSDT7AL; (10) CSDT7BS; (11) CSDT7BL; (12) CSDT7DS; (13) Chinese Spring. Sample 5 displays a deviant pattern with the less mobile isozyme band missing.

sion missing the chromosomal arm 6AL and its compliment missing in the accession without the arm 6BL serve as evidence that the homologous structural genes for PEPT-1 are located on the long arms of chromosome 6A and 6B. These two loci will be designated *Pept-A1* and *Pept-B1*. No deviation from the common phenotype was noticeable in the 6D ditelocentric accessions. In addition, the phenotype of Chinese Spring was similar to that of the tetraploid *T. dicoccoides* (genome AABB) (Golenberg, in press). Thus, if present, a homologous structural gene for PEPT-1 could not be distinguished by the present techniques.

The electrophoretic pattern obtained for AMP similarly has two distinct zones of isozyme activity in Chinese Spring. A third isozyme such as described by Hart (1979) was not apparent, or overlapped one other isozyme zone in the buffer system used herein. The accession CSDT6BL displayed the only deviation from this pattern, having only one active isozyme band. The missing enzyme is encoded by a gene located on the short arm of chromosome 6B. This gene must be synonymous with *Amp-B1* reported by Hart (1979).

The genes encoding the two peptidase systems analysed are located on different chromosome arms in the *T. aestivum* genome and are thus clearly independent loci. The location of these genes in the *T. dicoccoides* genome has not been directly determined. Linkage of a *Pept-1* gene with a coleoptile pigment locus *Rc* has been reported (Golenberg, in press). Double heterozygous individuals were produced by crossing inbred lines of *T. dicoccoides*. The F1 plants were allowed to self and the F2 offspring were analysed for the recombination fraction between the *Pept-1* and *Rc* loci. The recombination fraction was estimated to be 0·2758 (S.E. = 0·0677). An *Rc* locus has similarly been described in *T. aestivum* and located on the 6B chromosome (Sutko, 1977). It was suggested that this pigment locus is homologous to the *T. dicoccoides* locus and that the chromosomal segment containing the two loci has been conserved from *T. dicoccoides* to *T. aestivum* (Golenberg, in press).

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