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SHORT COMMUNICATION

HOMEOLOGOUS PROTEINS SYNTHESIS CONTROLLED BY HOMEOLOGOUS CHROMOSOMES IN WHEAT

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Abstract—Two homeologous proteins have been isolated from the endosperm of common wheat (genomes ABD). Synthesis of these two proteins is controlled by the homeologous chromosomes 7B and 7D respectively. However, *Aegilops speltoides*, the more generally accepted B genome donor, does not synthesize the 7B protein.

WE HAVE previously described two sets of homeologous biochemical systems in wheat: the β-sitosterol esters systems[1] and the purothionins.[2] In both cases each homeologous system was regulated by a different genome, but we were unable to show which particular chromosomes were involved. We now report a pair of homeologous proteins from *Triticum aestivum* L. endosperm whose synthesis is controlled by homeologous chromosomes.

An electrophoretic component (Fig. 1, CM1) of the chloroform–methanol (2:1, v/v) extracted proteins from the endosperm of *T. aestivum* L. has been used to detect this wheat in mixtures with *T. durum* Desf.[3] Estimation of the amount of common wheat present was achieved by densitometric measurement of the ratio between CM1 and a second component (CM2) present in both wheat species. We have now purified these two components. A mol. wt. of 17,000 was obtained for CM1 and CM2 after reduction and denaturation by the method of Shapiro *et al.*[4] The unreduced proteins gave a mol. wt. of 22,000–23,000 both by the above method and by Sephadex G-100 chromatography. Further chemical evidence of the close relationship between CM1 and CM2 was obtained by finger-printing tryptic digests of CM2 from *T. durum*, CM1 and CM1 plus CM2 from *T. aestivum* following the thin-layer technique of Ballieux *et al.*[5]

In order to investigate which chromosomes control the synthesis of CM1 and CM2, half kernels of the monosomic and ditelocentric series of Chinese Spring wheat were analysed. Results are summarized in Fig. 1. About equal amounts of CM1 and CM2 are synthesized in Chinese Spring. Monosomics 2A and 7D showed a decreased proportion of CM1 and only mono 7B a depression of CM2. No synthesis of CM1 was observed in a ditelo 7D and of CM2 in a ditelo 7B. These results indicate that the synthesis of CM1 and CM2 is controlled by the homeologous chromosomes (ancestral homologues) 7D and 7B respectively.

No CM1 is present in the tetraploid Rescue variety (genomes AB) obtained by Kaltsikes *et al.*[6] by extraction of the D genome from hexaploid Rescue (genomes ABD) which does

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synthesize this protein. A synthetic *T. spelta* (ABD) shows the normal *T. aestivum* L. pattern and its parental species *T. carthlicum* (AB) and *Aegilops squarrosa* (D) show CM2 and CM1 respectively. However, CM2 was not found either in *Ae. speltoides* (B) or the synthetic AB alloploids *Ae. speltoides* × *T. monococcum*-TA396 and *Ae. speltoides* × *T. aegilopoides*-TA398 obtained by R. Riley. This fact, as well as other biochemical evidence,[7] seems to indicate that either the B genome donor of wheat was not exactly *Ae. speltoides* or that the genome has been extensively modified after the tetraploid was formed.

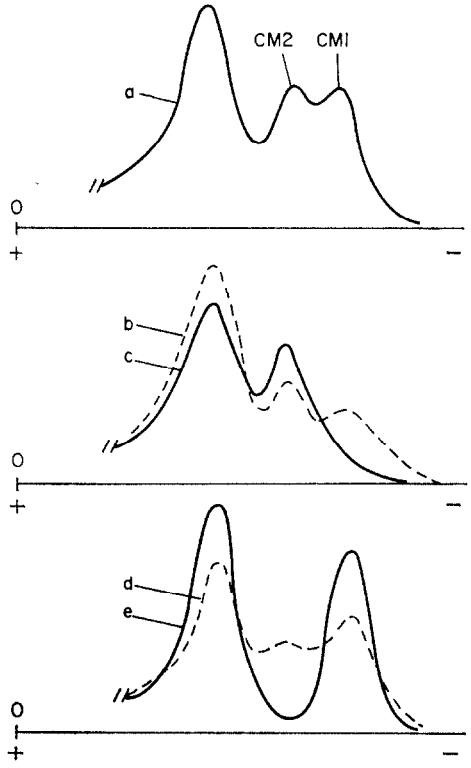

FIG. 1. DENSITOGRAMS OF PROTEINS CM1 AND CM2 SEPARATED BY UREA STARCH-GEL ELECTROPHORESIS[8] IN (a) EUPLOID CHINESE SPRING WHEAT, (b) MONO 7D, (c) DITELO 7D, (d) MONO 7B AND (e) DITELO 7B.

EXPERIMENTAL

Purification of Proteins CM1 *and* CM2

Flour was defatted with petroleum ether and the protein extracted with $CHCl_3$–MeOH (2:1, v/v) as previously described.[3] The extracted protein (10 g) was dissolved in 3 M urea (150 ml). Most of the protein was precipitated first by addition of 0·1% NaCl (50 ml) and then by dialysis against the same solution. The clear supernatant was fractionated by salting out with $(NH_4)_2SO_4$. Electrophoretically pure CM1 was obtained from *Triticum aestivum* between 35% and 55% saturation and a mixture of CM1 and CM2 between 25% and 35%. Electrophoretically pure CM2 was obtained from *T. durum* (which lacks CM1) between 25% and 35% saturation. Urea starch-gel electrophoresis of fractions was performed after each purification step by the method of Woychik *et al.*[8]

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[8] J. H. WOYCHIK, J. A. BOUNDY and R. J. DIMLER, *Arch. Biochem. Biophys.* **94**, 477 (1961).

Analysis of Proteins CM1 *and* CM2 *in Half Kernels*

The crushed half kernels were defatted with petroleum ether (b.p. 35–60°) and extracted with 0·15 ml of $CHCl_3$–MeOH (2:1, v/v). The extract was transferred with the aid of a capillary to a piece of filter paper (Whatman No. 3, 3 mm × 8 mm) and evaporated in the process. The absorbed protein was subjected to urea starch-gel electrophoresis.

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