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CHROMOSOMAL LOCATION OF GENES CONTROLLING ENDOSPERM PROTEIN PRODUCTION IN TRITICUM AESTIVUM CV. CHINESE SPRING

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SUMMARY

Analysis of compensating nullisomic-tetrasomic lines of Triticum aestivum cv. Chinese Spring indicates that bands in the electrophoretic pattern of water-soluble, ethanol-extracted, seed proteins are controlled by genes on chromosomes 2A, 4A, 6A, 2B, 3B, 4B, 2D, 3D, 4D, 6D and 7D. Not all bands have yet been assigned to genes on particular chromosomes. Some bands appear to be formed of dimeric or multimeric protein molecules, for they are under the control of more than one chromosome. The characteristic electrophoretic pattern of T. turgidum is inferred to be controlled by genes on both A- and B-genome chromosomes.

INTRODUCTION

Water-soluble, ethanol-extracted seed proteins have several times been used to investigate evolutionary affinities in the wheat group (JOHNSON and HALL, 1965; WAINES, 1969; WILLIAMS, 1971; JOHNSON, 1972a; JOHNSON, 1972b). The impetus for these studies was the importance of bread wheat, *Triticum aestivum*, and its relatives as crop plants and their ease of handling as organisms for a chemosystematic investigation of evolution in a polyploid series. Bread wheat is a hexaploid with three sets of seven chromosomes in its haploid complement, the A, B and D genomes. The A genome is thought to have been contributed by *T. monococcum* and the D genome by *Aegilops squarrosa*, for both of these genomes exhibit respectively complete meiotic-chromosome-pairing homology with these two taxa. The source of the B genome is not clear, for it does not show complete chromosome-pairing homology with any known diploid taxon. Tetraploid wheat, *T. turgidum*, has two sets of seven chromosomes in its haploid complement, the A and B genomes, while another group of tetraploids, the *T. timopheevi* group, contain the A and G genomes. The source of the G genome is also unknown.

The water-soluble, ethanol-extracted seed proteins of *T. aestivum* are fractionated into a pattern of more than 20 bands by disc electrophoresis (JOHNSON *et al.*, 1967). The existence of more or less complete series of aneuploid lines in certain cultivars of bread wheat provides a potential means of assigning to chromosomes some of these protein bands along with the genes that govern their production. Such a procedure would indicate which bands are controlled by genes in the three different genomes and also indicate how many of the 21 possible haploid chromosomes are being sampled by this extraction technique.

The genetic control of some wheat-endosperm proteins has already been investigated (BOYD and LEE, 1967; SHEPHERD, 1968; BOYD *et al.*, 1969).

MATERIALS AND METHODS

The plant materials used in this study were the nullisomic-tetrasomic compensating lines of *Triticum aestivum* L. em. Thell. cv. Chinese Spring, seed of which was kindly supplied by Dr. E. R. Sears and Dr. R. Riley. Seed of the different lines was grown for increase at either Columbia, Missouri, or Oxford, England, between 1970 and 1972. The minimum weight of seed extracted was 1 g, but the usual weight was 2.5 g. Seed was ground in a Wiley Mill fitted with a screen of size 40 and extracted with 70% ethanol in the proportion of 10 ml ethanol to 1 g seed. The flour was agitated periodically in the ethanol for at least four hours, after which the suspension was centrifuged at 10,000 RPM for 30 minutes at 4°C and the supernatant dialysed against distilled water at 4°C for 48 hours. The dialysate was further centrifuged at 10,000 RPM at 4°C for 30 minutes and the supernatant freeze dried. The protein powder is stored in a deep freeze.

The disc-electrophoretic technique has already been reported in detail (JOHNSON *et al.*, 1967). The running-gel columns were made from 15% acrylamide and 3 M urea, and an 0.8 mg protein-powder sample was added to the top of each column before layering with sample gel and buffer solution. The system has a running pH of 4.3 and the proteins migrate towards the cathode. Each column received 1 mA reversed current for 15 minutes, then 4 mA for 2½ hours. Gels were stained with amido black in 7% acetic acid overnight and were destained laterally (Canalco, Rockville, Maryland).

RESULTS AND DISCUSSION

The protein-electrophoretic profile of *T. aestivum* 'Chinese Spring', or of most other bread wheats, has bands that can be assigned to the AABB tetraploid wheat *T. turgidum* var. *dicoccon* and the DD genome of *Ae. squarrosa* (JOHNSON, 1972b) and Figure 1. The profile of Chinese Spring can be divided into two areas: the fast migrating, so-called albumin bands between 40 and 110 mm, and the more slowly migrating, so-called gliadin bands 0 and 40 mm on Johnson's standardized gel. This report is concerned primarily with an analysis of the albumin bands, which are more easily discernible. Moreover, this report is preliminary and tentative, for not all albumin bands have yet been assigned to chromosomes.

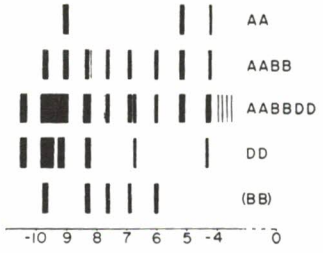

Figure 1. Diagram of protein-electrophoretic profiles of *Triticum monococcum* (AA), *T. turgidum* (AABB), *T. aestivum* (AABBDD) *Aegilops squarrosa* (DD) and a hypothetical pattern for the BB genome arrived at by subtraction of the AA from the AABB profiles. Data from JOHNSON, 1972a and b.

In that we are reasonably sure of the source of the DD genome of bread wheat, an analysis of the protein pattern of *Ae. squarrosa* indicates that there are at least five fast-migrating bands, the counterparts of which can be identified in the profile of *T. aestivum*. On the standard gel (JOHNSON, 1972b) these *squarrosa* bands are found to have migrated 105, 97, 91, 82 and 65 mm from the origin. If we now

compare the electrophoretic patterns of euploid Chinese Spring and all seven of the nullisomic-D, tetrasomic-A compensating lines of this cultivar (Fig. 2), it is evident that the *squarrosa* band at 105 mm is governed by a gene on chromosome 7D. The wide band which includes two *squarrosa* bands at 97 and 91 mm as well as two *argidum* bands at 97 and 90 mm (Fig. 1), is more difficult to analyze, but I have tentatively assigned the *squarrosa* bands to genes of chromosome 3D, although other D-genome chromosomes may also be involved. The next *squarrosa* band at 82 mm also appears to be controlled by a gene on chromosome 3D, while that at 65 mm is controlled by a gene on chromosome 4D. We can conclude from this analysis that these five *squarrosa* bands are controlled by genes on three of the seven haploid chromosomes of *Ae. squarrosa*.

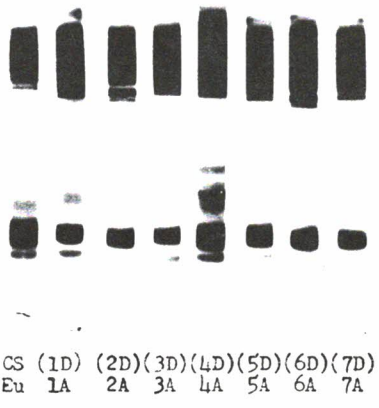

Figure 2. Protein-electrophoretic profiles of *Triticum aestivum* euploid Chinese Spring (CS/Eu) and all seven of the nullisomic-D, tetrasomic-A compensating lines. (1D)/1A is nullisomic 1D, tetrasomic 1A, etc.

Bands which appear to be affected by the tetrasomic A chromosomes in Figure 2 are the two fastest migrating gliadin bands (at 39 and 37 mm in Fig. 1), which are both controlled by genes on chromosomes 2A and 6A. The third of these four gliadin bands at 35 mm is absent in gels lacking both chromosomes 2D and 6D, and is presumably controlled by genes on these chromosomes. Chromosome 4A carries genes which control the complex bands at 83 and 69 mm, for components in both of these bands are denser in the tetrasomic-4A combinations.

The analysis of protein bands attributable to the AABB tetraploid wheats using the nullisomic-B, tetrasomic-A series of compensating lines (Fig. 3) is not yet complete, for sufficient seed of the (5B)5A line was not available. There appear to be eight fast-migrating bands which are characteristic of the AABB tetraploids (Fig. 1) running at 97, 90, 83, 76, 69, 60, 53 and 43 mm from the origin. In some genotypes the band at 90 mm migrates more quickly and fuses with that at 97 mm, and this may be so in Chinese Spring. The complexes of bands at 83 and 69 mm appear to be controlled in part by genes on chromosome 4A, for they are denser in the nulli-4B, tetra-4A combination (Fig. 3). The band at 76 mm is also formed of two bands, the wider of which is controlled by a gene on chromosome 3B, for it is absent in the nullisomic-3B combination. The bands at 90 and 97 mm have not yet been assigned to chromosomes, but from a survey of diploid wheat patterns, that at 90 mm appears to be on an A-genome chromosome. The bands at 60, 53 and 43 mm are elusive and have not yet been analyzed.

The gels of the nullisomic-2B, tetrasomic-2A and nullisomic-6B, tetrasomic-6A lines again demonstrate that chromosomes 2A and 6A carry

genes controlling the two fastest gliadin bands of Chinese Spring. In nullisomic-2B, tetrasomic-2A, the third band at 35 mm is missing, whereas it is not in nullisomic-6B, tetrasomic-6A gels. Presumably chromosome 2B also shares control of this band along with 2D and 6D.

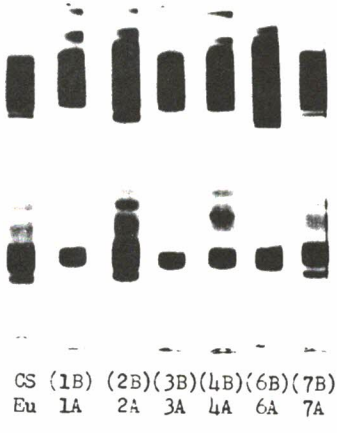

Figure 3. Protein-electrophoretic profiles of *Triticum aestivum* euploid Chinese Spring (CS/Eu) and six of the seven possible nullisomic-B, tetrasomic-A compensating lines in this cultivar, (5B)/5A being absent.

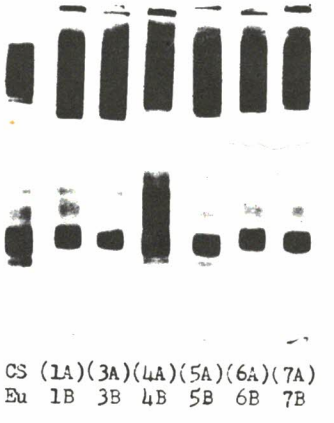

Figure 4. Protein-electrophoretic profiles of *Triticum aestivum* euploid Chinese Spring (CS/Eu) and six of the possible seven nullisomic-A, tetrasomic-B compensating lines in this cultivar, (2A)/2B being absent.

Seed of all nullisomic-A, tetrasomic-B lines was available for analysis, except that of the (2A)2B combination (Fig. 4). Genes on chromosome 4B control components of the complex bands at 83 and 69 mm, for these bands are denser in the tetrasomic-4B combination. The pattern for the nullisomic-6A, tetrasomic-6B line does not possess the gliadin bands at 39 and 37 mm, indicating that chromosome 6A does contribute to the control of these two bands.

Even though many bands in the Chinese Spring pattern have yet to be assigned to genes on particular chromosomes, the results to date indicate that genes on chromosomes 2A, 4A and 6A, 2B, 3B and 4B and 2D, 3D, 4D, 6D and 7D control bands in the electrophoretic profile. Some bands appear to be formed of dimeric or multimeric proteins, for they are controlled by genes on different chromosomes, for example, the bands at 39 and 37 mm by two chromosomes of the same genome, and that at 35 mm apparently by three genes in two different genomes (A and D). The complex bands between 60 and 83 mm which were originally thought to be characteristic of the unknown B genome, are demonstrated to be partly controlled by A-genome chromosomes. This possibility has already been suggested by JOHNSON (1972a), who hypothesized that the B genome is also derived from a diploid wheat. Certainly the characteristic protein profile for *T. monococcum*, which has none or

few fast-migrating albumin bands, is now known to be somewhat different from the A-genome profile embodied in the AABB tetraploid wheats. In that there are many similarities between the AABB and AAGG tetraploids with regard to their protein profiles, it might be inferred that the characteristic pattern of the *timopheevi* wheats is also partly controlled by genes on the A-genome chromosomes (JOHNSON, 1967).

ACKNOWLEDGEMENTS

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