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Molecular analysis of the D-genome of the Triticeae

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Summary. The chromosome of three tetraploid *Aegilops* L. species containing the D-genome were analyzed by in situ hybridization with a repeated DNA sequence clone pAS1 isolated from *Aegilops squarrosa* and observed to be D-genome specific. This sequence is found on all seven D-genome chromosome pairs of *A. squarrosa* and hexaploid wheat. Two distinct D-genome patterns were observed in the tetraploid species. The D-genome of *A. cylindrica* was similar to hexaploid wheat. Seven pairs of chromosomes having large amounts and numerous sites of the sequence were observed. Five chromosome pairs with fewer and smaller sites of the repetitive sequence were observed in the D-genomes of *A. crassa* and *A. ventricosa.* In addition to these major repeated sequence differences, chromosomal modifications appear to have occurred between *T. aestivum* and *A. cylindrica* and between *A. crassa* and *A. ventricosa* resulting in changes with respect to location of the sequence between the respective species. D-genome divergence with respect to pAS1 sequence appears to have occurred at least in two forms, one characterized by the changes in amount of repetitive sequence and the second by changes in location of the sequence.

Key words: In situ hybridization – Biotin labeling – Chromosome evolution – *Aegilops*

Introduction

Polyploidy has played a fundamental role in the evolution of wheat. *Triticum aestivum,* hexaploid common wheat, consists of three separate genomes, A, B and D, with a basic genomic chromosome number of seven (2n = 6x = 42). Of the three genomes, the D-genome shares perfect homology with its ancestoral genome in the diploid species, *Aegilops squarrosa.* The B genome diploid species has yet to be identified and certain doubts are now being raised as to the totality of the A-genome in polyploid wheats (Dvořák 1983; Rayburn and Gill 1985 b). Indications are, however, that the D-genome has not remained stagnant throughout evolutionary time. Furuta et al. (1975) found significant DNA variation among accessions of *A. squarrosa* indicating changes at the molecular level in the diploid genome.

The D-genome is a pivotal genome in several additional polyploid species. Kihara (1954) determined by genome-analysis that *A. cylindrica* (2n = 28), *A. ventricosa* (2n = 28), *A. crassa* (2n = 28; 2n = 42) and *A. juvenalis* (2n = 42) all contained at least one D-genome. Kimber and Zhao (1983) on the basis of chromosome pairing studies in interspecific hybrids involving D-genome species determined that the D-genomes of these species could be divided into three clusters. One cluster consists of *A. squarrosa, T. aestivum, A. cylindrica* and *A. ventricosa;* the second consists of tetraploid and hexaploid *A. crassa;* and the third consists of *A. juvenalis* and *A. syriacum.* These genomes have been speculated to have diverged by chromosomal modifications. The recent isolation of a D-genome specific DNA sequence (pAS1) allowed molecular identification of D-genome chromosomes (Rayburn and Gill 1986 a, b).

The purpose of this study was the analysis of D-genome differentiation with respect to the sequence pAS1. Chromosomes of several polyploid species containing the D-genome along with the diploid *A. squarrosa* were in situ hybridized with pAS1 probe and karyotypes were determined with respect to those chromosomes which demonstrated significant labeling. The results of this analysis of D-genome differentiation involving this specific DNA sequence are presented here.

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Materials and methods

The species analyzed represent those tetraploid species which contain the D-genome of the Triticeae. The seeds are maintained at the Kansas State University Wheat Genetics Resource Center germplasm bank.

Clone pAS1 contains a 1 kb *Aegilops squarrosa* DNA insert. This sequence has been shown to hybridize to the D-genome chromosome of hexaploid wheat as well as all seven pairs of *A. squarrosa* chromosomes (Rayburn and Gill 1986a, b). The clone is described in more detail elsewhere (Rayburn and Gill 1986b).

pAS1 was nick translated and labeled with biotinylated UTP. Chromosomes were obtained from root tip squashes. The in situ hybridization was carried out at 37 °C for 6 h in 50% formamide, 2XSSC and 10% dextran sulfate. More precise descriptions of the hybridization conditions as well as the detection are found in Rayburn and Gill (1985a).

After hybridization, the chromosomes were observed under both bright field and phase optics. Photographs were taken and those chromosomes demonstrating sites of hybridization were karyotyped to represent the D-genome of each species.

Results

Two distinct D-genome patterns were observed in this study. The first pattern consisted of all seven pairs of the D-genome chromosomes showing heavy to moderately heavy sites of hybridization (Fig. 1a). In the second pattern, only five pairs of chromosomes showed light to moderately light sites of hybridization (Fig. 1c).

A. cylindrica (CD) was observed to have the first pattern (Fig. 1b). Seven pairs of chromosomes demonstrated sites of hybridization. These sites of hybridization were large and of very dark intensity. Eighteen sites of hybridization were observed in a haploid complement. All the chromosomes were observed to have multiple sites of the sequence. In so far as the labeled chromosomes were concerned, the plants appeared homozygous.

A. ventricosa (UnD) was observed to have the second pattern (Fig. 1d). Ten of the 28 chromosomes had hybridization sites of light intensity. Nine total sites were observed in a haploid complement. One chromosome was observed to have one site of hybridization with the remaining chromosomes containing multiple sites.

A. crassa (MD) also had the second pattern (Fig. 1c). Five of the seven chromosome pairs demonstrated hybridization sites of light intensity. Again nine sites of hybridization were observed. The karyotype of *A. crassa* was different from *A. ventricosa.* Both *A. crassa* and *A. ventricosa* appeared homozygous with respect to the labeled chromosomes.

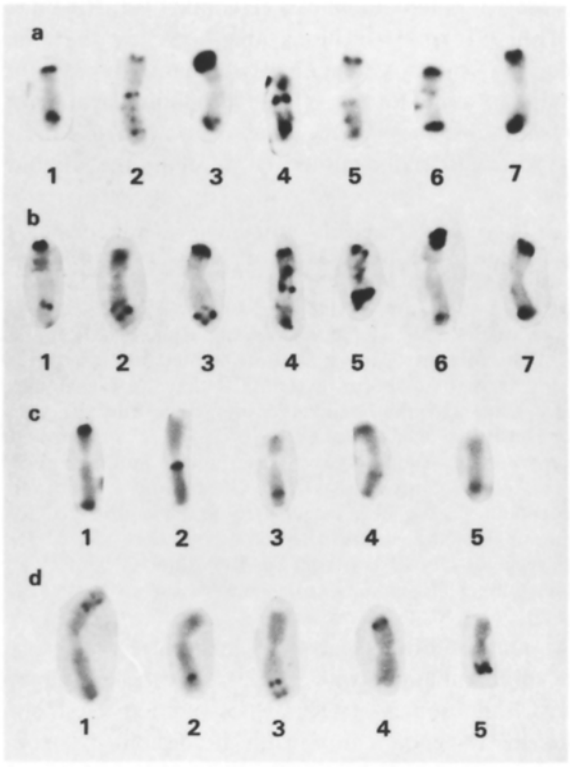

Fig. 1a–d. The D-genome chromosomes of the various polyploid *Triticum* and *Aegilops* species demonstrating hybridization sites of the repetitive sequence pAS1 isolated from *A. squarrosa:* **a** the seven D-genome chromosomes of *T. aestivum;* **b** seven *A. cylindrica* chromosomes; **c** five *A. crassa* chromosomes; **d** five *A. ventricosa* chromosomes

Discussion

Among the D-genomes of the polyploid species studied the genomes of *T. aestivum* and *A. cylindrica* appeared most similar. While the total number of sites is greater in *T. aestivum,* the number of large sites of hybridization was equal in both species. The difference in total sites seems to be the result of smaller sites of hybridization observed on chromosomes 2D and 5D (Fig. 1a) not being present in *A. cylindrica* (Fig. 1b). Three of the chromosomes of *A. cylindrica* (Fig. 1b: 1, 3, 7) appeared to have identical locations of the repetitive sequence as their common wheat counterparts (Fig. 1a: 1D, 3D, 7D). Chromosomes 2, 4 and 5 (Fig. 1b) appeared similar to chromosomes 2D, 4D, 6D respectively (Fig. 1a). Only chromosomes 6 (Fig. 1b) appeared to have no definitive counterpart in hexaploid wheat. These results suggest that the two D-genomes are basically unmodified with respect to amount and location of the D-genome specific repeated DNA sequence used in this study. These findings are in agreement with earlier findings which suggest that one of the genomes of *A. cylindrica* was indeed the D-genome (Kihara 1954). Additional studies have indicated that the D-genome of *A. cylindrica* and *T. aestivum* are basically similar (Chennaveiraiah 1960; Kihara 1963). Kimber and Zhao (1983) observed meiotic pairings in F_1

hybrids between *T. aestivum* and *A. cylindrica,* which substantiated this theory. Analysis at the repetitive DNA level is, therefore, in agreement with the previously reported data and provides further evidence as to the similarities of the two D-genomes.

The D-genomes of *A. crassa* and *A. ventricosa* are very different from the previously described genomes. Fewer chromosomes demonstrate sites of hybridization and fewer sites of hybridization are observed. In addition, the sites of hybridization are not as distinct as the sites observed in *T. aestivum.* These observations indicate that changes in amount as well as the location of the sequence occurred between the two D-genome patterns. Data from pairing studies have provided evidence for heterozygous translocations between the genomes of both *A. crassa* and *A. ventricosa* and *A. cylindrica.* Such translocations could account for some of the observed differences in location of the sites of hybridization between the two D-genomes.

The data presented here suggest that the D-genome of *A. ventricosa* is quite different from that of *T. aestivum* and *A. cylindrica* with respect to the location and amount of the repetitive sequence pAS1. From chromosome pairing data, the D-genome of *A. ventricosa* has been suggested to be unmodified with respect to the D-genome of *T. aestivum* (Kimber and Zhao 1983). Whether these changes are reflected in chromosome homology is not known. Chromosome pairing results may not be conclusive however. In previous studies, F_1 hybrids between *A. ventricosa* and *T. aestivum* have resulted in more univalents than predicted if complete homology of the D-genomes were observed. In addition, F_1 hybrids between *A. ventricosa* and *A. cylindrica* have demonstrated quinquevalents indicating translocation heterozygosity. A perplexing observation by Kimber and Zhao (1983) in the *A. ventricosa*×*A. cylindrica* hybrid is the decrease in the number of univalents. These results might be explained by residual homology of the C and Un genomes, irrespective of the D-genome. Although not conclusive, observations in this study suggest that the D-genome of *A. ventricosa* has become modified with respect to *T. aestivum.*

Data from pAS1 sequence location corroborate previous results on the differentiation of D-genome of *A. crassa* from that of *T. aestivum* (Kimber and Zhao 1983). The results from these studies indicate that the almost normal bivalent pairing observed in the amphidiploid produced by crossing tetraploid *A. crassa* with *A. squarrosa* (Kihara et al. 1965) may have been the result of chromosome differentiation between the two D-genomes. The chromosomes of the two species appear quite different with respect to the site of hybridization observed. Similarly with respect to pAS1 sequence, the D-genome of *A. ventricosa* is also differentiated from the D-genome of *T. aestivum.* However, these results are not in agreement with chromosome pairing analysis which indicates complete homology between D-genomes of *A. ventricosa* and *T. aestivum* (Kimber and Zhao 1983).

All the D-genome studied to date have been observed to contain the repeated DNA sequence pAS1. This sequence has yet to be observed in other genomes (Rayburn and Gill 1986a, b). Apparently, the pAS1 repeated DNA sequence was widespread in the ancestral D-genome. Furthermore, it is inferred that the ancestral D-genome consisted of seven pairs of chromosomes, all of which had locations of the repetitive sequence. This interpretation is supported by the observation of this pattern in various populations representing subspecies of *A. squarrosa* (Rayburn and Gill unpublished). Whether this hypothesis is true or not, the D-genome appears to have initially diverged at the DNA level. If the ancestral D-genome was as previously speculated, the D-genomes of *T. aestivum* and *A. cylindrica* have remained essentially unchanged with respect to amount of the sequences. The D-genomes of *A. crassa* and *A. ventricosa* appear to have lost, or partly deleted sites of the sequence. If the ancestral D-genome had the pattern similar to that of *A. crassa,* the modification would be the result of amplification of the sequence in the *T. eastivum* pattern. In either case, the divergence may be characterized by varying amounts of repeated DNA sequences.

The second divergence of the D-genomes may be characterized by chromosomal modifications. The differences between the D-genomes of *T. aestivum* and *A. cylindrica* may be explained by chromosomal changes as well as the differences between *A. crassa* and *A. ventricosa.* The genomes of *A. crassa* appear to have diverged to a greater extent than the D-genomes of *T. aestivum* and *A. cylindrica.* The divergence of the D-genomes in polyploid species appears to be complex involving changes at the molecular as well as the chromosomal level.

The results presented in this study indicate that the D-genomes observed could be characterized by two patterns. The first pattern consisted of D-genomes which had seven pairs of chromosomes having large amounts of the repeated DNA sequence and included *T. aestivum, A. cylindrica* and *A. squarrosa.* The second pattern consisted of five pairs of D-genome chromosomes having sites of the sequence and included *A. crassa* and *A. ventricosa.* The patterns seemed to basically agree with the clusters described by Kimber and Zhao (1983) but differed with *A. ventricosa* being more similar to *A. crassa.* Large differences in locations of the sequence were observed between these two species which could indicate chromosomal modifications which may partially explain the differences observed by Kimber and Zhao (1983).

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