Intergenomic translocations of polyploid oats (genus *Avena*) revealed by genomic *in situ* hybridization

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(Received 21 March 2000, accepted 29 June 2000)

Wild and cultivated hexaploid oats share the same genomes (AACCDD) and display a considerable level of interspecific variation in both plant and chromosome morphology. The GISH was utilized to detect the interspecific genomic compositions in four hexaploid and two tetraploid oats using total genomic DNA of *Avena eriantha* (a C-genome diploid) as probe. Intergenomic translocations between A/D and C-genome chromosomes were frequently observed in hexaploid and tetraploid species. In the hexaploid, two pairs of A/D genome segments on C-genome chromosome (A/D-C) translocation and four to six pairs of C-genome segments on A/D genome chromosome (C-A/D) translocation were clearly identified whilst the number of A/D-C translocations was constant among species. In the tetraploid *A. maroccana* (AACC), a pair of A-C and four pairs of C-A translocations were observed. Moreover, the A/D translocation segments on chromosome 5C was detected only in *A. byzantina* and *A. maroccana*, whilst A/D-C translocations were observed on the 1C and 7C of *A. sativa*, *A. fatua* and *A. sterilis*. *A. byzantina* did however also carry the 1C rearrangement. This result shows that *A. byzantina* has retained a similar genomic constitution to the tetraploid ancestor of hexaploid oats, *A. maroccana*. Three pairs of A-C translocations were detected only in *A. murphyi* (AACC), and two pairs of those were the 1C and 7C as well as the three hexaploid species except *A. byzantina*.

INTRODUCTION

The genus *Avena* L. (Poaceae) comprises a polyploid series of diploid, tetraploid and hexaploid species with a basic chromosome number of seven. Based on chromosome number, genome constitution and morphology of the diaspore, thirty taxonomic species of this genus have been classified into 11 genomic groups (Leggett, 1992). The wild and cultivated hexaploid oats, *A. sativa*, *A. byzantina*, *A. fatua* and *A. sterilis*, are interfertile and occasionally cross in nature. Because of these close genetical and chromosomal affinities, these species are considered to belong to the a single biological species (Ladizinsky and Zohary, 1971) with the genomic designation AACCDD (Rajhathy and Thomas, 1974).

The identification of individual chromosomes of hexaploid oats has been reported using classical meiotic chromosome pairing and somatic karyotype studies (Rajhathy and Thomas, 1974; Nishiyama et al., 1989). By using the

C-banding analysis, a technique preferentially staining heterochromatin, the C-genome chromosomes were distinguished from the A- and D-genome chromosomes (Linares et al., 1992; Jellen et al., 1993), and the latter authors demonstrated C-banding polymorphisms among the hexaploid species studied.

Recently, genomic *in situ* hybridization (GISH) with fluorescent dye has proven to be a very useful tool for allocating chromosomes to genomes in allopolyploid species (Mukai et al. 1993). At the moment, the GISH was unable to separate the A- and D-genome chromosomes in hexaploid oats. But absolute discrimination between A/D- and C-genome chromosomes has been demonstrated by several oat workers (Jellen et al., 1994; Chen and Armstrong, 1994; Leggett and Markhand, 1995; Yang et al., 1999). These authors observed small C-genome segments translocated onto A- or D-genome chromosomes (abbreviated as C-A/D translocation) and vice versa (abbreviated as A/D-C) for A- or D-segments translocated onto C-genome chromosomes. The variation of number and position of the intergenomic translocations has been observed to be in parts of hexaploid and tetraploid genotypes.

Edited by Takashi Endo

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Fig. 1. Genomic *in situ* hybridization with biotinylated *A. eriantha* DNA to chromosomes of *A. sativa* (A), *A. byzantina* (B), *A. fatua* (C), *A. sterilis* (D), *A. maroccana* (E) and *A. murphyi* (F). Hybridization signals are visualized in yellow. The arrows show the A/D-C translocations. Scale bar is 10 μm.

So, it is possible to estimate the genomic evolution from the possible tetraploid ancestors to all the hexaploid species and intra-hexaploid differentiation depending on the number and position of the intergenomic translocations revealed by GISH.

The present paper reports interspecific genomic translocations in four hexaploid (AACCCDD) and two tetraploid (AACC) species having C-genome revealed by the GISH and discusses the genomic composition and chromosome evolution of polyploid oats.

MATERIALS AND METHODS

Plant materials Two cultivated hexaploid (*A. sativa* L. 'Sun II' and *A. byzantina* C. Koch 'Kanota'), two wild hexaploid (*A. fatua* accession 50-10 and *A. sterilis* accession 69-15) and two wild tetraploid oats (*A. maroccana* accession 69-14 and *A. muphyi* accession 74-17) were used for collecting root tips for chromosome preparations of the GISH. The C-genome diploid species, *A. eriantha* accession 69-5 was used for isolating total C-genomic DNA's for preparation of probe.

Chromosome preparation Chromosome preparations were carried out with a modification of the procedure of Matsuda and Kishigami (1981). Root tips were pre-treated in ice water for 24h at 0°C, fixed in Farmer's fixative (ethyl alcohol: acetic acid=3:1), and dehydrated through descending ethanol series. The root tips were then digested in the mixture of 0.5% Pectolyase Y-23 (Kikkoman) and 0.5% Cellulase "ONOZUKA" RS (Yakult) at 37°C for 50min. Meristematic regions of the root tips were spread on a slide glass in a few drops of Farmer's fixative and then air-dried.

In situ hybridization Hybridization and detection were carried out with a modification of the procedure of Mukai et al. (1993) and Schwarzacher et al. (1989). The probe DNA from *A. eriantha* was labelled by nick translation with biotin-14-dATP using the BioNick Labeling System (GIBCO/BRL). After hybridization, the signals were detected by fluorochrome fluorescein isothiocyanated avidin (FITC) and biotinylated anti-avidin-D. The slides were doubly stained with propidium iodide (PI) and 4,6-diamidino-2-phenylindole (DAPI). The FITC-avidin hybridization signals and PI are detected by their yellow and red fluorescence. Photographs were taken with Kodak Ektachrome ISO 400 color slide film.

Karyotype analysis The seven chromosomes belonging to the C-genome were identified by observing at least fifty good figure cell plates. According to chromosome length, each C-genome chromosome was numbered from the longest 1C to the shortest 7C chromosomes. And, the arm

ratio was carefully measured to clarify the difference of each chromosome. It was very easy to distinguish the longest, intermediate and shortest chromosomes, for instance, 1C, 5C and 7C.

RESULTS

Hexaploid species The GISH was performed using total genomic DNA derived from the C-genome diploid *A. eriantha* as probe to examine the C-genome chromosome structures in four hexaploid oats. Seven pairs of the C-genome chromosomes were successfully labelled and the remaining 14 pairs of chromosomes were unlabelled over all the hexaploid species indicating that these were A- or D-genome derived chromosomes (Fig.1-A,B,C and D). Moreover, the hybridization sites also clearly indicated the A/D-C and C-A/D intergenomic translocations. And the positions of all the translocations were at the terminal regions of the long arms of chromosomes.

Two pairs of C-genome chromosomes carrying an A/D-genome translocation were observed in all the hexaploid species, but different chromosomes were involved depending on the species. Based on karyotype analysis, the A/D-C translocations were located in terminal regions of the long arm of 1C and 7C of *A. sativa*, *A. fatua* and *A. sterilis* whilst in *A. byzantina* chromosome 7C did not carry a translocation but 5C did (Fig.2-A,B,C and D).

The number of C-A/D translocations also differed among the hexaploid species, four pairs being observed in *A.*

Fig. 2. Comparison of the C-genome chromosomes of *A. sativa* (A), *A. byzantina* (B), *A. fatua* (C), *A. sterilis* (D), *A. maroccana* (E) and *A. muphyi* (F), showing positions of intergenomic translocation sites shadowed.

Table 1. The genomic constitution of four hexaploid (AACCCDD) and two tetraploid (AACC) of *Avena* species.

Species	Ploidy	No. of intact chromosomes		No. of intergenomic translocations		
		A/D-genome	C-genome	C on A/D-genome	A/D on C-genome	Total
<i>A. sativa</i>	6X	18	10	10	4	14
<i>A. byzantina</i>	6X	20	10	8	4	12
<i>A. fatua</i>	6X	18	10	10	4	14
<i>A. sterilis</i>	6X	16	10	12	4	16
<i>A. maroccana</i>	4X	6	12	8	2	10
<i>A. murphyi</i>	4X	6	8	8	6	14

byzantina, five pairs in *A. sativa* and *A. fatua*, and six pairs in *A. sterilis*.

Tetraploid species In two tetraploid species, seven pairs of C-genome chromosomes were clearly labelled with total DNA's of *A. eriantha* and seven pairs of A-genome chromosomes were unlabelled (Fig. 1-E and F). The tetraploid species also had the same intergenomic translocations as the hexaploids. *A. maroccana* had a pair of A/D-C and four pairs of C-A/D translocations in terminal regions of chromosomes. However, *A. murphyi* had three pairs of A/D-C and four pairs of C-A/D translocations in both terminal and interstitial regions of chromosomes. The GISH plate of *A. murphyi* is presented here for the first time. The A/D-C translocation sites differed between the tetraploid species, a pair (5C) observed in *A. maroccana* (Fig.2-E) and three pairs (1C, 4C and 7C) in *A. murphyi* (Fig.2-F).

Number of A/D-C and C-A/D translocations The genomic constitution of the four hexaploid and two tetraploid species was summarized in Table 1. The A/D-genome chromosomes carried two to four times as many intergenomic translocation segments as the C-genome. The number of C-genome chromosome carrying translocated A/D genome segment was constant in hexaploid but varied in tetraploid species. The maximum total number of translocations observed was eight pairs in *A. sterilis* and the minimum was five pairs in *A. maroccana*.

DISCUSSION

The GISH technique allowed us to detect intergenomic translocations between A/D- and C-genome chromosomes in polyploid oats. And it seems reasonable to expect that intergenomic translocations between the A- and D-genome chromosomes also occur (Leggett and Markhand, 1995). Clearly, since oats are intrinsically an inbreeding species, natural populations are likely to have relatively stable genomes. However, the small proportion of outcrossing which is known to occur (1–2%) in oats, could give favourable environmental conditions, quite rapidly lead to karyotype restructuring. A number of authors have at-

tempted to identify individual C-genome chromosomes in hexaploid oats by GISH and FISH (e.g. Chen and Armstrong, 1994; Jellen et al., 1994; Leggett and Markhand, 1995; Yang et al., 1999). Fominaya et al. (1995) and Linares et al. (1996) performed FISH analysis using a repeated DNA sequence (pAm1) specific to C-genome chromosomes. When total genomic DNA from *A. eriantha* was used in this study, one to three pairs of C-genome chromosomes carrying A/D translocations were detected in four hexaploid and two tetraploid oats. In contrast, using pAm1, Linares et al. (1996) reported that it was possible to detect the A/D translocations on almost all the C-genome chromosomes in three hexaploid oats. Most of the translocations observed in *Avena* was detected on terminal position of chromosome which is rich with telomere repeat sequences. Thus, in the FISH analysis, the repetitive pAm1 probe detected much more translocations than total genomic DNA of *A. eriantha* because that the pAm1 probe involved a lot of variable flanking regions, like microsatellites.

Jellen et al. (1994) reported two pairs of A/D-C translocations whilst Chen and Armstrong (1994) and Leggett and Markhand (1995) reported three pairs in *A. sativa*. These translocations were usually located on the long arms of the chromosome. Though Jellen et al. (1994) reported a pair of A/D translocations to be located at an interstitial position on the long arm of a C-genome chromosome in *A. sativa*. The results of this study correspond to those of Jellen et al. (1994) except for that we did not observe an interstitial translocation in the hexaploid species but in the tetraploid, *A. murphyi* (Fig. 2-F-4C). In this study, the terminal A/D translocation segments of *A. byzantina* were identified as on the chromosomes 1C and 5C whilst Jellen et al. (1994) identified the rearrangements on the 1C and 7C. The A/D-C translocation on the 5C appears to be unique to *A. byzantina* and the tetraploid, *A. maroccana*, although the degree of chromosome homology could not be confirmed. We observed that the terminal A/D translocations in *A. fatua* occurred on the 1C and 7C. Yang et al. (1999) reported that the terminal A/D-C translocations on the 1C, 3C or 4C, and in addition, detected a terminal A/D translocation on each arm of chromosome 7C. In our study, a terminal A/D translocations in *A. sterilis* was observed on chromosomes 1C and 7C and

corresponded to the results of Jellen et al. (1994). Evidently the number of chromosome rearrangements observed in polyploid oats is dependent on species, accession and the detection technique used.

Although both tetraploid species, *A. maroccana* and *A. murphyi* were detected the translocation segment on medium length chromosome of seven pairs of C-genome chromosomes, two hexaploid species, *A. byzantina* (our result) and *A. fatua* (Yang et al., 1999) were also detected on medium length chromosome. It suggests that the chromosomes with the segment of two hexaploids are conservative. We considered that the 7C chromosome was derived from structural change of medium C-genome chromosome. To prove this hypothesis, C-banding or FISH method using repetitive DNA probes such as 5S-rDNA and 18S-rDNA are needed to identify the numbers of C-genome chromosome clearly, and to analyze the chromosome homology between the related species.

The intergenicomic translocations were not occurred reciprocally between C- and A/D-genomes. These translocations also do not lead to multiple chromosome pairings at meiosis, but lead to diploidization of polyploid oats. After that, a genetic control system suppressing multivalent formation was needed to keep a new stable genotype. The genomes of polyploid oats were remarkably reorganized through the intergenicomic translocations. The hexaploid oat is a typical allopolyploid species consisted with three genomes as well as hexaploid wheat. After polyploidization, however, mode of genomic evolution of oats was quite different from that of wheat.

Most of the chromosome rearrangements observed in both hexaploid and tetraploid oats involved the segments of C-genome chromosomes translocated onto A- or D-chromosomes. In additions, the C-genome chromosome segments increased in the hexaploid species (Table 1). This result suggests that the C-genome is changeable and has the tendency for heterochromatin of the C-genome to be transferred to the terminal regions of the A/D-genome chromosomes. The karyotype of the C-genome in hexaploid species consists of median and submedian chromosomes, whilst in the tetraploid they are median and subtelocentric chromosomes, the diploid species consisting of subtelocentric chromosomes (Hayasaki unpublished data). These diverse karyotypes of the C-genome at the different ploidy levels might well result from the chromosomal translocations from the C-genome to the A- or D-genome during speciation.

The authors are grateful to Dr. J. M. Leggett (Institute of Grassland and Environmental Research, Plas Gogerddan, U.K.) for his critical reading of the manuscript and encouraging

suggestions. The research was financially supported by the Sasagawa Scientific Research Grant from The Japan Science Society.

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