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SHORT COMMUNICATION

Centromeric position and genomic allocation of a repetitive sequence isolated from chromosome 18D of hexaploid oat, *Avena sativa* L.

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Abstract Cultivated oat (*Avena sativa* L.) is allohexaploid and contains three genomes (A, C, and D). By using fluorescence *in situ* hybridization, a 391 bp repetitive DNA fragment ('A336') isolated from oat chromosome 18D was predominantly localized in centromeric regions of mitotic metaphase chromosomes of oat accession 'CN64226'. Assays of simultaneous and sequential co-hybridizations with the C genome-specific repetitive DNA probe 'pAm1' isolated from tetraploid *A. murphyi* Ladiz. (AACC) and the application of different concentrations of the A336 probe revealed that the A336 DNA segment is more abundant in chromosomes of the A- and D-genomes than in chromosomes belonging to the C-genome. Our results provide information which may be useful in future cytogenetic studies and in aiding physical genome assembly.

Keywords *Avena sativa* · A336 · Centromere · 18D chromosome · FISH

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Introduction

Cultivated oats (*Avena sativa* L.) are allohexaploid (2n = 6x = 42) and the three genomes are postulated to represent the A, C, and D genomes of diploid *Avena* species (Rajhathy and Morrison 1959). The A-genome may originate from the direct ancestor of diploid *A. canariensis* Baum, Raj. et Samp. (2n = 2x = 14, A_cA_c) and the C-genome from the direct ancestor of diploid *A. ventricosa* Bal. ex Coss. (2n = 2x = 14, C_vC_v). The origin of the D-genome is not yet known (Loskutov 2008). A-genome chromosomes can be distinguished after *in situ* hybridization using a 114 bp repetitive DNA fragment ('pAs120a') isolated from the diploid oat *A. strigosa* Schreb. (2n = 2x = 14, A_sA_s) as a probe (Linares et al. 1998). *In situ* hybridization using a 464 bp repetitive DNA fragment ('pAM1') isolated from the tetraploid oat *A. murphyi* Ladiz. (2n = 4x = 28, AACC) as a probe labels oat chromosomes that are exclusive to the C-genome (Solano et al. 1992). However, no sequence has yet been discovered that hybridizes preferentially to the D-genome. Hence, Sanz et al. (2010) identified D-genome chromosomes indirectly by discriminating unlabelled chromosomes from labelled chromosomes after fluorescence *in situ* hybridization (FISH) using the A-genome- and C-genome-specific probes.

Previously, we (Luo et al. 2012) reported the characterization of a library of plasmid clones derived from chromosome 18D. These clones were isolated from the *A. sativa* L. cultivar 'Sun II' by Chen and

Armstrong (1995), who used microdissection to obtain intact metaphase copies of chromosome 18D (previously named 21), the smallest chromosome in hexaploid oat. Luo et al. (2012) reported the characterization of these plasmid clones and categorized three types of sequences from this library: known repeats, known genes, and unmatched fragments. The clone 'A336' was included in the latter category because its full sequence did not contain known repeats and had no similarity to sequence accessions in the National Center for Biotechnology Information (NCBI) database.

The objectives of this study were to locate A336 in the chromosomes of hexaploid oat and to explore whether its D-genome origin may allow it to be useable as a genome-specific probe in FISH experiments.

Materials and methods

Seeds of *A. sativa* L. accession 'CN64226' (a spring-type oat), selected based on local familiarity and availability, were germinated on sterile, wet filter paper at 20 °C under controlled light conditions (14 h light, 10 h dark). When 1.5–2.0 cm long, root tips were harvested to make cytological preparations for FISH. Using common procedures (*e.g.*, Shuai 2003), root tips were pretreated in ice water for 24–36 h, fixed in Farmer's fixative fluid (3:1 mix of 95 % ethanol and glacial acetic acid), then squashed in a drop of 45 % acetic acid. After freezing with liquid nitrogen, the slides with the squashes were uncovered, air-dried, and stored at −20 °C until use. FISH with multiple probes was performed as described by Hao et al. (2011). The pAm1 fragment (Linares et al. 1996) was used to identify chromosomes having a C-genome origin, and the A336 fragment (Luo et al. 2012) from chromosome 18D of Sun II was tested as a novel probe. Clones containing these fragments in pGEM-4Z were grown in LB liquid medium and the plasmids isolated using the High-Speed Plasmid Mini Kit protocol from IBI Scientific (Peosta, USA; Cat. No. IB47102). The concentration of pAm1 plasmid was 900 ng/μL, while three concentrations of the A336 plasmid were used: 600, 900, and 1,200 ng/μL. pAm1 was labelled with biotin-16-dUTP (Roche Diagnostics GmbH, Mannheim, Germany; REF 11745824910) and A336 was labelled with digoxigenin-11-dUTP (Roche Diagnostics Gmbh, Mannheim, Germany; REF 11745816910) according to the manufacturer's instructions. Streptavidin-Cy3 (SIGMA ALDRICH CHEMIE Gmbh, Steinheim, Germany; Pcod 1001238444) was used to detect the biotinylated probe and anti-digoxigenin-fluorescein (Roche Diagnostics GmbH, Mannheim, Germany; REF 11207741910) was used to detect the digoxigenin probe. The preparations were counterstained with 4, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Inc., Burlingame, USA). Slides were examined using an Olympus BX-51 microscope coupled to a Photometric SenSys Olympus DP70 CCD camera (Olympus, Japan). Raw images were processed using Photoshop ver. 7.1 (Adobe Systems Incorporated, San Jose, CA, USA).

Results

Images of mitotic metaphase plates of CN64226 visualized by FISH are illustrated in Fig. 1. Although it was not possible to confirm the identity of individual chromosomes using these methods, three additional replications (not shown) provided results that were consistent with the signal locations and genome identities reported here. Chromosomes of CN64226 showed eight pAm1-labelled translocations (yellow arrows in Fig. 1a, b, e), but only six chromosomes with C-genome translocations were labelled by A336. Three satellited chromosomes were labelled by A336 (white arrows in Fig. 1b, d, e). The most intense A336 signals were located in the centromeres of ten chromosomes and none of these originated entirely from the C-genome (red arrows in Fig. 1c, d, e). A336 signal was absent from the centromeres of ten other chromosomes (cyan arrows in Fig. 1c, d, e). The remaining 22 chromosomes had relatively weak A336 signals located in centromeres. Of the 22 signals, 6 of these were located in C-genome chromosome centromeres.

In preliminary work, several additional novel probes from chromosome 18D were also tested (data not shown) but only probe 336 showed evidence of having a centromeric location. In order to distinguish the A-genome from the D-genome, hybridizations were also attempted using the probe pAs120a (also not shown). Unfortunately, we were unable to replicate previously published results using this probe.

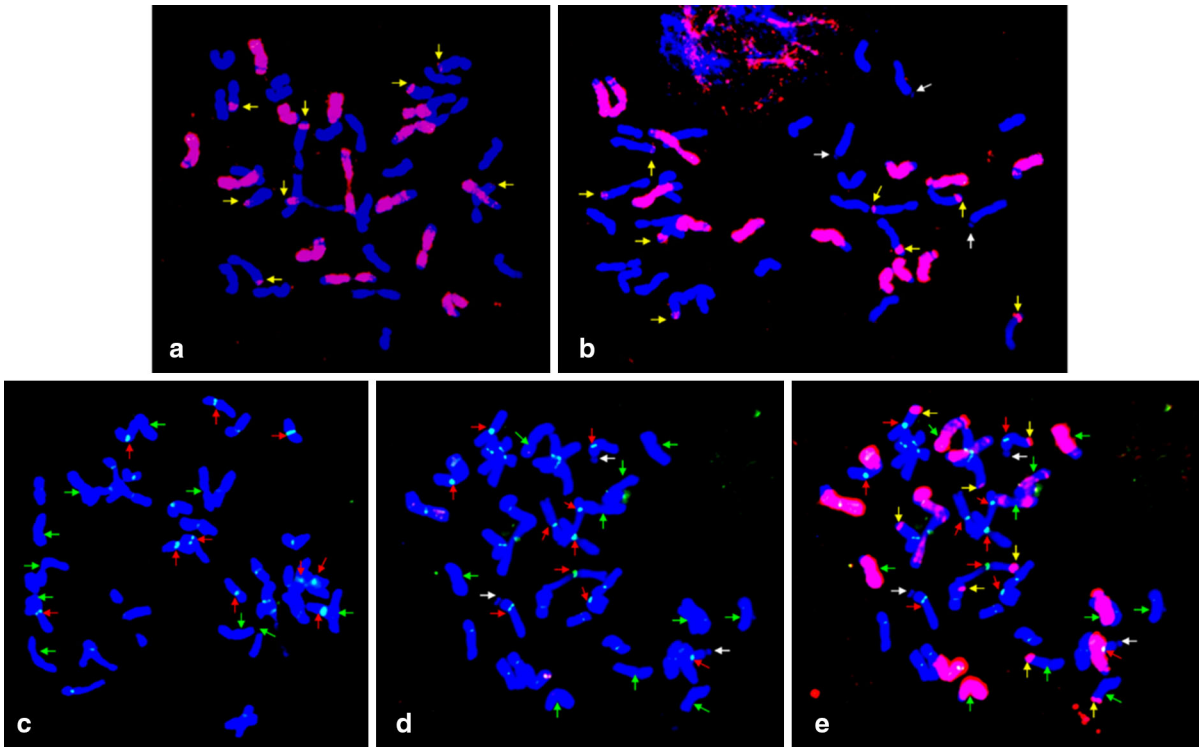

Fig. 1 Metaphase plates of *Avena sativa* L. accession 'CN64226' visualized after FISH. Chromosomes in **a**, **b**, and **e** were probed with biotin-labelled pAm1 (*pink*). **c**, **d**, and **e** show hybridization of the digoxigenin-labelled A336 probe (*green*). The concentration of probe used for both A336 and pAm1 was 900 ng/μL. All chromosomes were counterstained with DAPI. *Yellow arrows* show eight translocations labelled with pAm1 (**a**, **b**, **e**), while *white arrows* (**b**, **d**, **e**) highlight three chromosomes with satellites. *Red arrows* represent ten chromosomes with strong hybridization to A336 (**c**, **d**, **e**), whereas *cyan arrows* (**c**, **d**, **e**) highlight another ten chromosomes with centromeres not labelled by A336. (Color figure online)

Discussion

Sequences isolated from chromosome 18D are a potentially valuable resource for cytogenetic identification of the oat D-genome. In this study, molecular probes coupled with FISH analyses were used successfully to localize a novel sequence originating from chromosome 18D to a centromeric location.

The centromere is a chromosomal locus that regulates the proper pairing and segregation of the chromosomes during cell division (Ugarkovic 2009). Among plants, the centromeres of maize are the most well characterized using both genetic and molecular methods (Ma et al. 2007). The entire sequence and chromatin properties have also been elucidated for the centromere of rice chromosome 8 (Nagaki et al. 2004; Wu et al. 2004; Yan et al. 2005). A variety of satellite repeats of a few nucleotides each are present at the sites of *Drosophila* centromeres, with an organization that differs according to chromosome. Unlike *Drosophila*, maize centromeres have the same DNA elements present at all primary constrictions (Ananiev et al. 1998). Many tandem repeats are present at each centromere, but there is considerable variation in the copy number among different chromosomes and for the same chromosome across different lines (Kato et al. 2004).

To date, there is little information regarding the centromeres of oat. Our results demonstrate that, for at least one sequence, there is differential hybridization signal among the chromosomes. Differences in hybridization signal strength are likely related to the copy numbers of the repeat clusters around the centromeres, or possibly also to the degree of sequence homology and stringency conditions chosen during hybridization and post-hybridization washes of the slides. Since it is unlikely that single copies of the A336 fragment would be visible using the present

methodology, it is almost certain that the hybridization signals seen represent high copy numbers of the probe sequence. These are most likely arranged in tandem repeats, and it seems probable that differences among chromosomes are the result of varying copy number, as they are in maize.

Satellited chromosomes were in pairs, as usual. Nevertheless, the presence of only three satellited chromosomes in this work might be due to the loss of the fourth satellite when the metaphase cell spread was made. Chen and Armstrong (1995) also found only three satellited chromosomes in their preparations.

Results obtained using a suitable concentration of probe A336 suggested that the highest homology and/or greatest number of repeats were located in chromosomes of A- and/or D-genome origin. This supports previous findings that the C-genome is quite distinct from the A- and D-genomes (Fominaya et al. 1988; Linares et al. 1992; Jellen et al. 1994). We may speculate, but we cannot conclude, that the chromosomes with the strongest signal are those that originate from the D-genome. Although probe A336 demonstrated no apparent genome specificity, this probe will be useful for exploring the structure of oat centromeres.

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