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Identification of C-banded chromosomes in meiosis and the analysis of nucleolar activity in *Avena byzantina* C. Koch cv 'Kanota'

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Summary. The Giemsa C-banding technique was used to identify individual meiotic and somatic chromosomes in 21 monosomic lines of *Avena byzantina* C. Koch cv 'Kanota' (genome designation AACCDD). The hexaploid complement is composed of three sets of seven chromosome pairs. The heterochromatin in the putative diploid progenitors is located at the telomeres (genome A), at the centromeric and interstitial regions (genome C), or more evenly spread throughout the set (genome D). Comparisons based on C-banding between *A. byzantina* and its diploid progenitor species allowed us to allocate individual chromosomes into specific genomes. The C-banding technique may be useful for interspecific chromosome pairing analyses. Nucleolar activity and competition were studied using a silver-staining procedure. Only three chromosome pairs showed nucleolar organizer regions, thus indicating that nucleolar competition occurs naturally in hexaploid oats.

Key words: C-banding patterns – Nucleolar activity – *Avena byzantina*

Introduction

A. byzantina C. Koch is a cultivated oat with the allohexaploid constitution AACCDD ($6\times=42$). Each chromosome pair of the species has been assigned a number from 1 to 21 based on length and arm ratio (Rajhathy 1963). In addition, an idiogram of the chromosomes has been worked out by means of conventional staining procedures (Rajhathy and Thomas 1974; Morikawa 1985). An aneuploid series of the *byzantina* cv 'Kanota' has been a substantial help in providing the detailed information thus gathered. The techniques used, however have not been sufficiently discriminating as to enable us to relate each chromosome to one of the three progenitor genomes involved.

Giemsa or C-banding, which preferentially stains heterochromatin, has been shown to greatly facilitate the identification of individual chromosomes in wild species of *Avena* (Yen and Fillion 1977). Recently, the complete identification of somatic chromosomes of diploid and tetraploid oat species was reported by Fominaya et al. (1988a, b) using C- and Ag-NOR-banding. This investigation revealed differences between the heterochromatin pattern of the A and C genome on a diploid as well as a tetraploid level. The chromosomes of genome A mainly carry telomeric bands, whereas those of genome C show a higher chromatin condensation and several intercalary heterochromatin bands.

This observation stimulated us in the study presented here to attempt to use C-banding for further characterization of the 21 chromosome pairs of *A. byzantina*, with the additional goal of relating them to the A, C, or D genome. The identification should also include how many and which chromosomes show nucleolar activity.

Materials and methods

Root tips and anthers from twenty-one monosomic lines of cv 'Kanota' kindly supplied by Dr. T. Morikawa of the University of Osaka Prefecture (Osaka) were analyzed.

For mitotic examinations, seeds were germinated in petri dishes on moistened filter paper. Root tips were excised when 1–2 cm long and pretreated in ice water for 36 h, fixed in 3:1 ethanol-acetic acid, and stored at 0°–4°C for about 1–2 months. The C-banding technique used has been previously reported by Fominaya et al. (1988a). Silver staining (Lacadena et al. 1984) was used to determine the nucleolar organizer regions (NORs) on metaphase chromosomes.

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For meiotic studies, anthers in the first meiotic metaphase stage from monosomic plants were fixed in 3:1 ethanol-acetic acid and stored at 0°–4 °C for 6–8 months. The fixed material was flattened, frozen, and after removing the cover slip, the slides were stored in absolute ethanol for 24 h, incubated in 0.2 *M* HCl at 60 °C for 3 min, rinsed in tap water, and incubated in 5% $Ba(OH)_2$ at room temperature for 7 min. Slides were thoroughly washed in tap water and incubated in 2 × SSC at 60 °C for 1 h and stained with 1.5% Giemsa stain (R66 Gurr) in phosphate buffer pH 6.8 for the appropriate time (15–30 min). A minimum of 25 pollen mother cells having 20 bivalents plus 1 univalent at first metaphase were examined from two anthers of each monosomic.

Results and discussion

In order to trace all chromosomes as to their original genome, each individual univalent in critical monosomics was characterized by its C-banding pattern. Diagnostic C-bands were found for each chromosome as is shown in Fig. 1. This C-banding pattern was used as a reference when identifying individual somatic and bivalent chromosomes in other monosomic plants. Photographs of both meiotic and mitotic C-banded metaphase plates are shown in Fig. 2.

The karyotype of cv 'Kanota' has three pairs of satellite chromosomes, four pairs of metacentric chromosomes, seven pairs of submetacentric chromosomes, and seven pairs of subtelocentric chromosomes. With conventional staining methods it is often difficult to distinguish among members of each of the submetacentric and subtelocentric groups because of their similar chromosome size (Rajhathy and Thomas 1974; Morikawa 1985). However, using the C-banding patterns we could make clear the distinctions. Some chromosomes had bands that differed from the rest in their staining response. Such characteristic bands provided additional markers for use in chromosome identification. For example, chromosomes mono-10 and mono-11 can often be confused with each other as their sizes are similar. However, while mono-11 only showed interstitial bands on both arms, mono-10 consistently had centromeric bands as well. Similar differences were found when comparing other chromosomes.

Genome allocation of individual chromosomes is pertinent for studying the genome evolution of polyploid plant species. For this reason, information on C-banding patterns has been an important method for analyzing the phylogenetic relationship of species within the tribe *Triticeae* (Schlegel and Gill 1984; Morris and Gill 1987). Endo and Gill (1984) provided strong evidence for a common genome constitution when all the chromosomes of the *Elymus* and *Agropyron* diploid species showed similar C-banding patterns.

In 2x and 4x *Avena* species, the chromosomes of genomes A and C can be distinguished because they differ as to C-banding pattern (Fominaya et al. 1988 a). This information has proved to be useful for the allocation of specific chromosomes of hexaploid species with

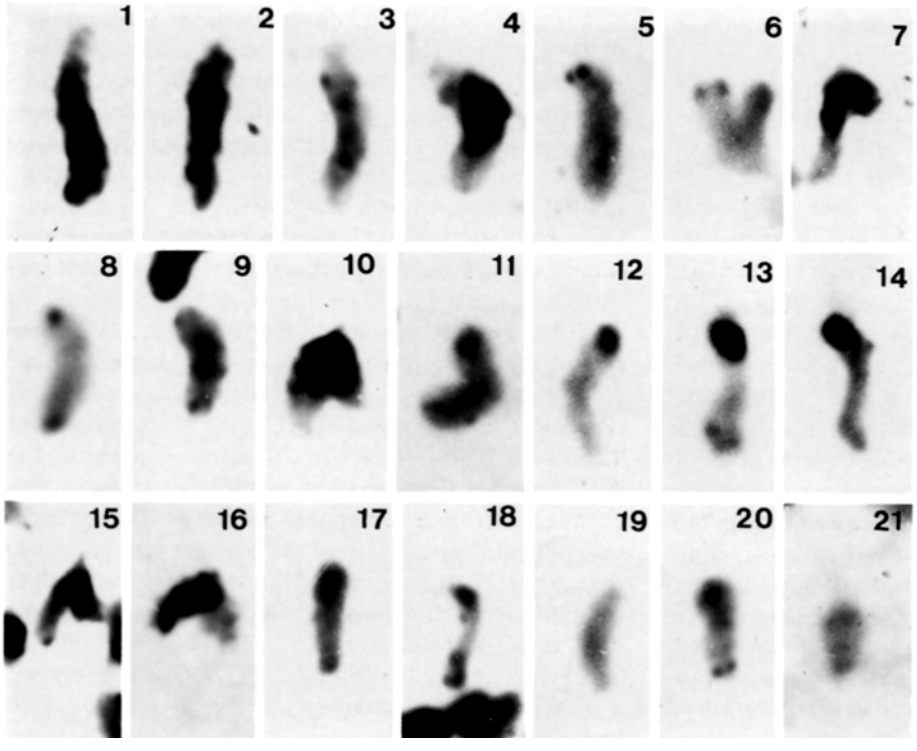

Fig. 1. C-banding patterns of twenty-one univalents in first meiotic metaphase from true monosomic plants

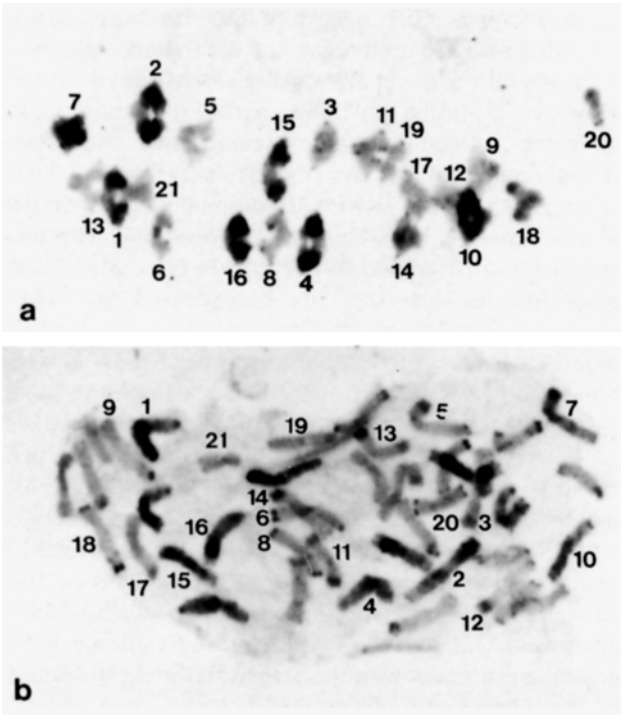

Fig. 2. C-banding meiotic (**a**) and somatic (**b**) metaphase cells of *A. byzantina*

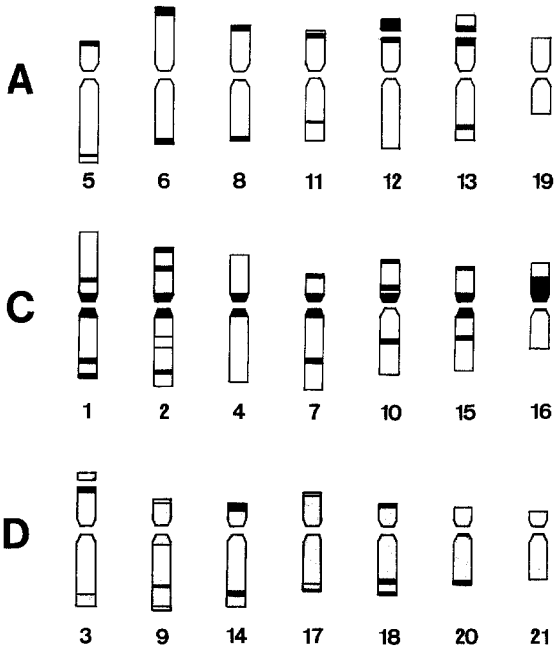

Fig. 3. Idiogram of the chromosomes of *A. byzantina* showing relative sizes and positions of C-bands (*solid regions*). *Top row* represents seven chromosomes belonging to genome A; *middle row* represents seven chromosomes belonging to genome C; *bottom row* represents seven chromosomes belonging to genome D

respect to their A and C genome. Since the 6x *A. byzantina* carries both these genomes, their characteristic chromosomes ought also to be recognized here. On the basis of our observations, the *byzantina* chromosomes can be divided into three groups according to their C-banding staining characteristics.

Seven chromosome pairs, namely mono-5, -6, -8, -11, -12, -13, and -19, showed a medium amount of heterochromatin. Euchromatin stained uniformly, the most prominent C-bands being located at the telomeres, while any interstitial bands that were present were either small or faint. Such a pattern conforms with that characteristic of the diploid A genome species (Fominaya et al. 1988 a), which suggests that the above-mentioned *byzantina* chromosomes should belong to genome A. Another set of seven pairs (mono-1, -2, -4, -7, -10, -15 and -16) carried a higher amount of heterochromatin in the centromeric and interstitial regions. Discrete and prominent C-bands were seen in the telomeres in one or both arms of all of these seven chromosomes. They showed a close resemblance to the corresponding chromosomes in the diploid species belonging to the C group (Fominaya et al. 1988 a), and suggest that they may be considered C genome chromosomes. The remaining seven pairs of chromosomes, mono-3, -9, -14, -17, -18, -20 and -21, showed dark euchromatin and exhibited very few heterochromatic bands, all of which were located in either the interstitial or telomeric regions. This chromosome group would then be assigned to the D genome. A tentative allocation of the chromosomes of *A. byzantina* into different genomes is depicted in Fig. 3.

On the basis of karyotypic observations, Rajhathy (1963) and Morikawa (1985) separated the chromosomes of the A genome from the chromosomes of the other two genomes (C and D). They argued that the longest chromosomes of four groups – satellite, metacentric, submetacentric, and subtelocentric chromosomes – should be assigned to the A genome. However, the C-banding results do not confirm this conclusion. At least the longest metacentric and longest submetacentric chromosome show C-banding patterns typical of C genome species.

It has been proposed that the tetraploid AACC species of the *A. maroccana* – *A. murphyi* complex have contributed to the AC genomes of the hexaploid species (Rajhathy and Thomas 1974; Leggett 1980; Murai and Tsunewaki 1986). When the C-banding patterns of the AACC species (Fominaya et al. 1988 b) and the *A. byzantina* pattern were compared, we found that the chromosomes of *A. murphyi* showed a greater correspondence to those of the AC genomes of the hexaploid species than did those from *A. maroccana*. Such a conclusion agrees with data on isoenzymatic analysis carried out by Sánchez de la Hoz and Fominaya (1989), who proposed a preference for *A. murphyi* as the AACC donor to hexaploid oat.

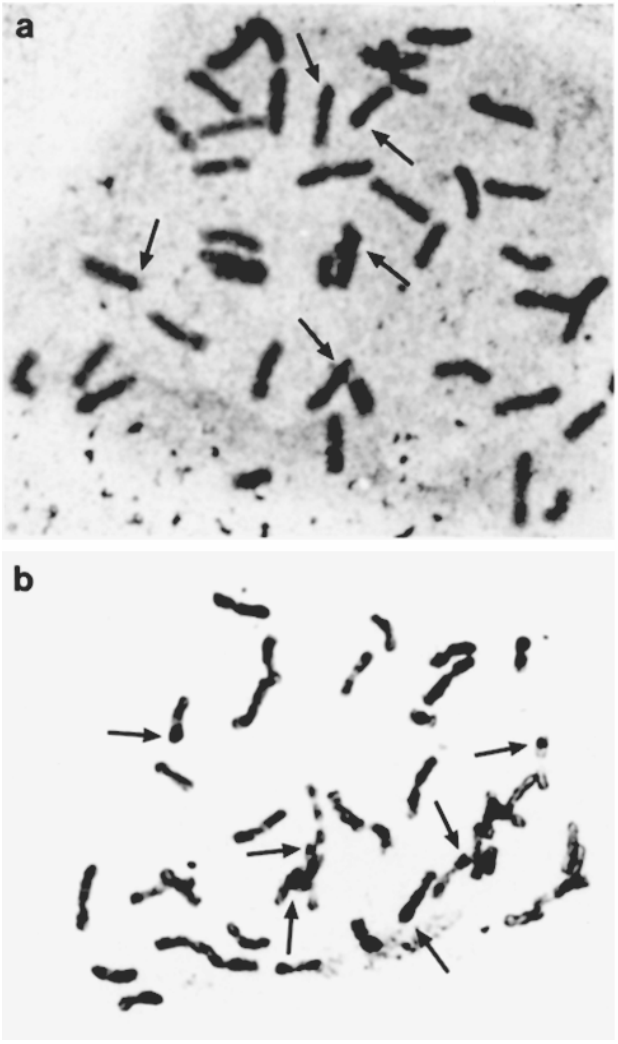

Fig. 4. Silver-stained somatic metaphase cells of (**a**) mono-12 with five silver-stained nucleolar organiser regions (NORs) and (**b**) mono-2 with six NORs

The possible origin of the D genome chromosomes remains unsolved. The significant differences observed between the bands of the D genome and the patterns found in diploid species carrying either genome A or C indicates that distant relationships exist. A possible explanation for the difficulty in tracing the D genome by C-banding analysis may be a structural differentiation of chromosomes that must have taken place during the evolution of the *Avenae*. Amplifications, deletions or rearrangements within those DNA sequences located at the heterochromatin regions are likely to have occurred during the divergent evolution of the diploid and polyploid species. A deeper understanding of the genomic relationships might come from chromosome pairing analysis of hybrids between the diploid and polyploid species and the identification of homoeologous groups.

Silver staining of *A. byzantina* showed the species to have six nucleolar organizer regions (NORs) in metaphase cells of 18 monosomic lines, while only five were observed in the remaining monosomic lines mono-3, -12, and -13 (Fig. 4). The C-banding patterns of the three chromosomes revealed that chromosomes 12 and 13 had abundant telomeric heterochromatin and thus most belong to genome A. Chromosome 3, which had very little heterochromatin at all, must belong to genome D. Since, with the exception of *A. ventricosa*, all of the diploid species analysed carried two pairs of SAT chromosomes, a maximum of 12 NORs might be expected (four for each genome). However, in the present study only 6 NORs were observed, which suggests inactivation of the nucleolus. Chromosome morphology and C-banding patterns indicate that both C genome NORs and one D genome NOR are supressed. The results support the observation of Rajhathy and Thomas (1974), who proposed the presence of three SAT chromosomes, two from A genome and one from D genome, in the standard karyotype of hexaploid oats. A similar competition between the NORs in several alloploid plants has been reported in cytological (Lacadena et al. 1984; Fominaya et al., 1988 b) or molecular examinations (Capesius and Appels 1989).

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