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Cytogenetics in fruit breeding – localization of ribosomal RNA genes on chromosomes of apple (*Malus* × *domestica* Borkh.)

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Abstract The localization of rRNA genes was studied by fluorescent in situ hybridization (FISH) on chromosomes of the cultivated apple, *M.* × *domestica* 'Pinova' (2n = 34). The 18S/25S rRNA loci were detected in terminal positions of the short arms of two submetacentric and two metacentric chromosome pairs. One 5S rRNA gene locus was found in the proximal region of the short arm of a small metacentric chromosome pair.

Key words *Malus* · rDNA · 5S rDNA · FISH · Ag-NOR staining

Introduction

Malus × *domestica* Borkh., the cultivated apple, is a widely grown fruit crop in most regions of the world. The diploid karyotype of *M.* × *domestica* consists of 17 pairs of similar and rather small (1–3 μm) meta- to submetacentric chromosomes. Previous cytogenetic investigations on apple were mainly restricted to chromosome counts (Einset and Lamb 1951; Huckins 1977; Soloveva 1979; Guo-Lu 1987; Schuster and Büttner 1995). More detailed karyological investigations could help to identify individual chromosome pairs, to study the phylogenetic relationships within the genus and to characterize the chromosome complements of hybrids between related wild species and the cultivated apple.

Fluorescent in situ hybridization (FISH) is a useful tool for the physical mapping of defined nucleic acid sequences even in species with small chromosomes (Maluszynska and Heslop-Harrison 1991; Fukui et al. 1994; Schmidt et al. 1994), for the identification of chromosomes by means of specific DNA markers (Jiang and Gill 1993; Lubaretz et al. 1996) and for the differentiation of parental chromatin in interspecific hybrids and their progeny (Schwarzacher et al. 1992; Jacobsen et al. 1995; Keller et al. 1996).

Here we report on the in situ hybridization of 18/25S and 5S rDNA probes for identification of 5 of the 17 chromosome pairs of apple.

Materials and methods

Root tips from young seedlings of *M.* × *domestica* cv 'Pinova' were pretreated with 2 m*M* 8-hydroxyquinoline for 3 h at 4°C, fixed in ethanol–glacial acetic acid (3 : 1) and stored at −20°C. After digestion (20% pectinase and 2% cellulase in 7.5 m*M* KCl, pH 4.0, at 37°C for 60–90 min) the root tips were squashed in 45% acetic acid, frozen, dehydrated in 95% ethanol, air-dried and either immediately used for FISH or stored in glycerol at 4°C.

Silver staining of interphase nucleoli was performed according to Lacadena et al. (1984).

The plasmid VER 17 (Yakura and Tanifuji 1983, kindly provided by Prof. S. Tanifuji) containing the 5.8S, parts of the 18S and most of the 25S rRNA gene-specific region of *Vicia faba* was used as a rDNA-specific hybridization probe. It was labelled with Biotin-16-dUTP (Boehringer Mannheim) using the Nick Translation Kit N550 (Amersham) according to manufacturer's instructions. A 5S rDNA-specific probe was amplified and simultaneously labelled with digoxigenin-11-dUTP (Boehringer Mannheim) from the genomic DNA of *V. faba* via the polymerase chain reaction (PCR) using 5S rDNA-sequence-specific primers of *Glycine* (Gottlob-McHugh et al. 1990).

In situ hybridization was carried out according to Fuchs and Schubert (1995). Signals were detected using a ZEISS Axioskop with appropriate filter combinations. Each fluorochrome was captured separately using a cooled CCD camera system (Photometrics), pseudocoloured (Gene Join) and merged (Photoshop). The complete images were printed on a Tektronix Phaser IISDX.

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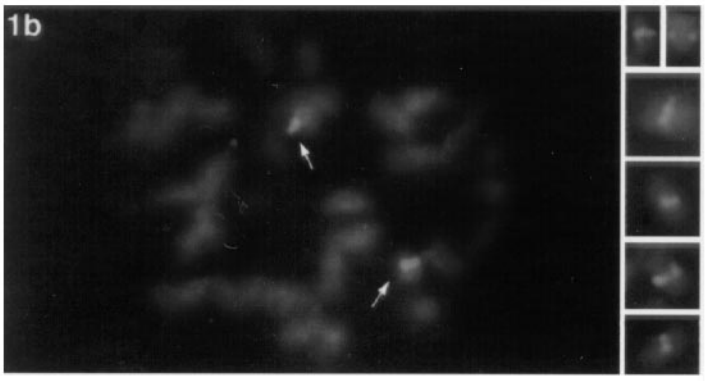

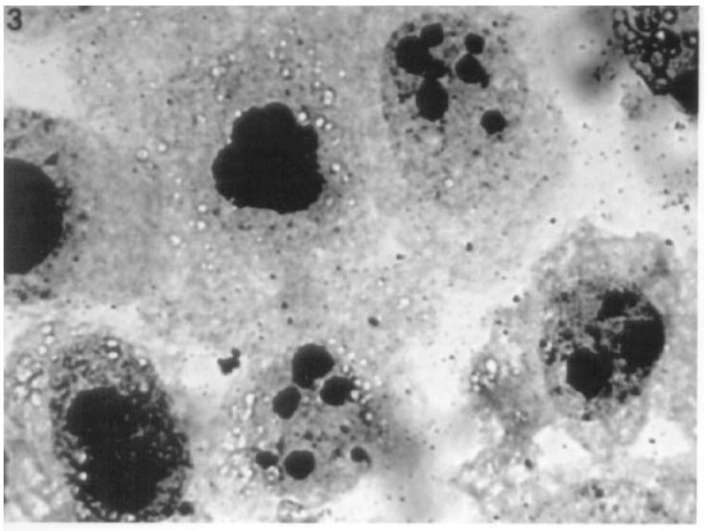

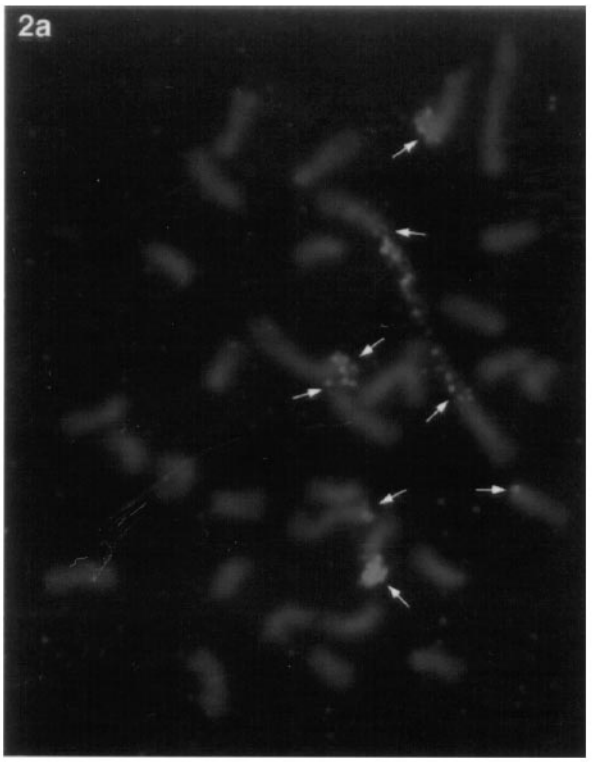

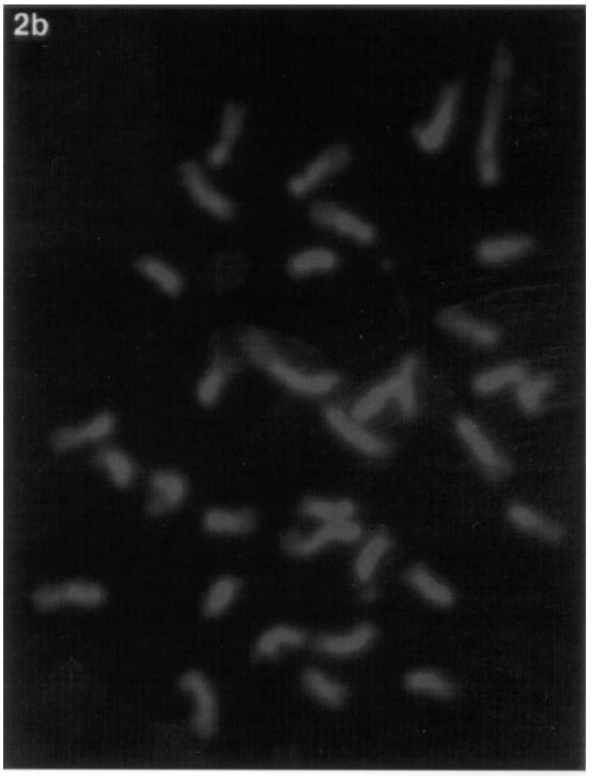

Fig. 1a, b Two complete metaphases of *M.* × *domestica* cv 'Pinova' after FISH with the 5S rDNA-specific probe. Arrows indicate the chromosome pair carrying the 5S rRNA genes. *Insert*: a sample of this chromosome type collected from different metaphases

Fig. 2 Metaphase chromosomes of *M.* × *domestica* cv 'Pinova' after FISH with the 18/25S rDNA-specific probe (*a*) and counterstaining with propidium iodide (*b*). *Arrows* indicate the eight hybridization sites

Fig. 3 Interphase nuclei of cultivated apple with up to seven dark nucleoli after silver nitrate staining

Results and discussion

In situ hybridization on apple chromosomes with the *Arabidopsis*-type telomeric sequence repeat (5′-TTTAGGG-3′, Richards and Ausubel 1988) had been performed previously by Fuchs et al. (1995). Signals of similar intensity were exclusively found on the chromosome termini. Due to the absence of signals at interstitial positions, this probe is not suitable for distinguishing apple chromosomes.

Data on physical mapping of the 5S rDNA have not yet been reported for apple. The 5S rRNA gene-specific probe hybridized exclusively on the proximal region of the short arm of a small metacentric chromosome pair (Fig. 1). The intensity of the signals was weak. Nevertheless, signals were detectable on at least one homologue of this chromosome pair in 10 out of 20 inspected metaphases.

In situ hybridization with the 18/25S rDNA probe revealed signals on 4 chromosome pairs of *M.* × *domestica*. The signals were located at terminal positions of the short arms of 4 submetacentric and 4 metacentric chromosomes (Fig. 2). In a recent paper (Gardiner et al. 1994) only 2 pairs of chromosomes with rDNA loci were reported for the apple rootstock 'MM 106'. This could be due either to differences between the varieties of apple used in these investigations or to the higher resolution of the FISH signals that were obtained by means of a cooled CCD camera in our experiments.

In order to test the activity of the eight rDNA loci, silver-nitrate staining was performed. Between one and seven nucleoli were detectable in interphase nuclei (Fig. 3). Approximately 200 interphase nuclei were inspected, but the maximum possible number of eight nucleoli was not observed. This could be due to either an early fusion of (minor) nucleoli or, if the cultivated apple in fact represents an allopolyploid species, to differential amphiplasty. Differential amphiblasty or nucleolar dominance means suppression of the NOR (nucleolus-organizing region) activity of one parental genome, which occurs frequently in interspecific hybrids (Rieger et al. 1979). Therefore, it would be of interest to test whether FISH with the genomic DNA of the presumed ancestor species (*M. sylvestris* Mill., *M. prunifolia* Borkh. or *M. baccata* Borkh.; Rehder 1962) could discriminate chromosomes of these ancestors of cultivated apple and whether the NOR-bearing chromosomes belong to different parental genomes.

The results demonstrated here show that probes derived from 5S rDNA and 18/25S rDNA can be used to discriminate 5 out of the 17 chromosome pairs of *M.* × *domestica*. By combining of FISH using additional suitable marker sequences and classical cytological methods (measurements of the chromosome/arm length and banding techniques) we should be able in the future to individualize more chromosomes of the apple karyotype.

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