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Review

Recent Progress on Studies of Chromosome Observation in Deciduous Fruit Trees

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Information on chromosomes is essential for the progress of genetic and biotechnological studies. In this paper, recent progress on studies of chromosome observation in deciduous fruit trees is reviewed. (1) An enzymatic maceration method, preparing good chromosome samples from plants with small chromosomes, was developed for *Prunus*, *Pyrus*, *Malus*, and *Diospyros*. (2) Some morphologically similar chromosomes could be distinguished by means of the banding technique using fluorochrome staining in *Prunus* and *Pyrus*. In addition, the divergence of chromosome configuration seems to be very low or non-existent in view of fluorescent banding patterns in both genera. (3) The number and location of 5S and 18S-5.8S-25S rDNA sites were detected by fluorescent *in situ* hybridization (FISH) in *Prunus*, *Pyrus*, *Malus*, and *Diospyros*. The sites of retrotransposons were also visualized by FISH in *Pyrus*. These results provided fundamental information on the chromosomes. Chromosomal location of the *S* (self-incompatibility) locus in *Malus* was also revealed by FISH. In *Diospyros*, the results of FISH as well as genomic *in situ* hybridization (GISH) offered new findings on the phylogenetic relationships in this genus and chromosome composition of somatic hybrids.

Key Words: CMA, FISH, karyotyping, peach, pear.

Introduction

Chromosome information is important to elucidate the stability of a plant at the cellular level and also for genetic and biotechnological studies. Chromosomes are the first dataset that leads to an understanding of the genetics of any species. Thus, chromosomal analysis is important for progress in breeding, genomic analysis, ploidy manipulation, and so on.

Analysis of plant chromosomes has progressed marked for the above-mentioned reasons since the 1970s. A new method for preparing chromosome samples established by Kurata and Omura (1978) in the field of chromosome research was significant. They developed the enzymatic maceration method to make clearly shaped chromosomes. This method is quite useful for preparing good chromosomes from plants with small chromosomes (Fukui, 1996).

Discrimination of morphologically similar chromosomes became possible because several banding techniques that are more effective than the conventional staining techniques with aceto-carmin and aceto-orcein were developed (Friebe et al., 1996). Of these methods, C-banding and fluorescent banding using a base-specific binding fluorochrome were applied to several plant species. Heterochromatin patterns were detected and some chromosomes could be identified by C-banding (Guerra, 1985; Tanaka and Hizume, 1980). The guanine-cytosine (GC)-specific chromomycin A_3 (CMA) and adenine-thymine (AT)-specific 4'-6-diamidino-2-phenylindole (DAPI) banding methods are quite reliable and useful for identifying chromosomes of various plants (Guerra, 1993; Hizume, 1991; Kondo and Hizume, 1982; Schweizer, 1976).

Chromosomal image analysis combining quantitative image data with numerical parameters of the length and arm ratio offered image parameters, condensation patterns, or chromosomal density profiles. Using this method, chromosomes of some plants were completely identified and quantitatively mapped (Fukui and Iijima, 1991; Fukui et al., 1998; Liu et al., 2004).

Fluorescence *in situ* hybridization (FISH), which detects the location on a chromosome of any gene is a very powerful tool for genome analysis (Fukui et al., 1994). This technique provides much chromosomal information using various genes as probes. Chromosome identification, chromosome configuration, evolution of species, and localization of useful genes have been elucidated by FISH (Hizume et al., 2002; Iwano et al., 1998; Murata et al., 1997; Ohmido et al., 1998; Pedersen and Langridge, 1997). Genomic *in situ* hybridization

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(GISH) using whole DNA as a probe also offers useful information about the chromosome configuration and evolution of species (Ji et al., 2004; Le et al., 1989; Ogawa et al., 2005).

In deciduous fruit trees, these studies are also essential for genetic improvement. Chromosomal counts of several deciduous fruit tree species have been conducted since the early 20th century (Janick and Moore, 1975), and improved techniques for counting chromosomes have been developed (Martínez-Gómez et al., 2005; Mary-Howell et al., 1988; Watanabe et al., 1990). In particular, these techniques are fundamental for polyploid breeding (Sanford, 1983).

The above-mentioned chromosome studies have been carried out in deciduous fruit trees over the last few decades mainly in species belonging to Rosaceae and *Diospyros*. In this review, the recent progress of chromosome studies of deciduous fruit trees, which regard to 1) chromosome sample preparation by means of the enzymatic maceration method, 2) chromosome banding using fluorochrome, and 3) *in situ* hybridization, is discussed.

1. Development of enzymatic maceration method for preparing chromosome samples

The early technique used for the observation of mitotic chromosomes was based on a sectioning method to cut tissues into thin slices (Darlington, 1928). Later, development of the squash method contributed to the improvement of chromosome observation. Compared with the sectioning method, samples for the squash method could be easily prepared while avoiding time-consuming sectioning procedures (Fukui, 1996). Chromosome numbers of various deciduous fruit trees were determined using this method (Kadota and Niimi, 2004; Notsuka et al., 2000; Oginuma, 1987).

A breakthrough in the chromosome preparation technique for fruit trees came with the establishment of the enzymatic maceration method. The main advantage of samples prepared by this method is that the chromosomes are free of cytoplasmic debris and are spread evenly on the slide glass. In particular, the enzymatic maceration method in combination with the air-drying method (EMA) allows observation of the fine structure of the chromosomes. These methods are quite effective for the preparation of good small chromosomes (around 1–3 μm) samples (Fukui, 1996).

Since important deciduous fruit trees belonging to Rosaceae and *Diospyros* possess small chromosomes, the EMA method has been applied to prepare their chromosome samples for more than two decades. Table 1 shows a list of the applications of the enzymatic maceration method in deciduous fruit trees. The conditions of the enzymatic treatment of *Prunus*, *Malus*, *Pyrus*, and *Diospyros* have been elucidated in several studies (Maghuly et al., 2010; Minamikawa et al., 2010; Omura, 1988; Schuster, 1996; Schuster et al., 1997; Yamamoto et al., 1999a, 2010a, b; Zhuang et al., 1990). In all species, cellulase in combination with pectinase or pectolyase was essential for chromosome sample preparation. An enzyme cocktail consisting of cellulase, pectolyase, and macerozyme was used in a few studies. Not only root tips but also shoot apical meristems were used as materials.

Figure 1 shows Giemsa-stained chromosomes prepared by the EMA method of *Prunus persica* (L.) Batch. 'Ohatsumomo' and *Pyrus pyrifolia* (Burm.f.) Nakai 'Osa Gold'. In both preparations, all 16 of *Prunus persica* and 34 *Pyrus pyrifolia* chromosomes were relatively extended and well spread without cytoplasm.

Good chromosome samples prepared by the EMA method allowed further subsequent studies on counting

Table 1. Several chromosome studies demonstrated enzymatic maceration methods in deciduous fruit trees.

Latin name	Common name	Material	Condition of enzymatic maceration			Reference
			Composition of enzyme	Tp.	Duration	
Prunus cerasifera Ehrh.	Myrobalan plum	Root tip	2% Cellulase Onozuka RS, 6% Pectinase	37°C	120–150 min	Omura (1988)
Prunus persica (L.) Batsch	Peach	Root tip	1% Cellulase Onozuka RS, 0.75% Macerozyme R200, 0.15% Pectolyase Y-23	37°C	60 min	Yamamoto et al. (1999a)
Prunus avium L.	Cherry	Root tip	1% Cellulase Onozuka R-10, 20% Pectinase 5-S	37°C	60–90 min	Schuster (1996)
Prunus spp.	Cherry rootstock	Root tip	2% Cellulase Onozuka R-10, 20% Pectinase	37°C	90–120 min	Maghuly et al. (2010)
Malus domestica Borkh.	Apple	Root tip	1% Cellulase Onozuka R-10, 20% Pectinase 5-S	37°C	60–90 min	Schuster (1996)
Malus domestica Borkh.	Apple	Root tip	2% Cellulase, 20% Pectinase	37°C	60–90 min	Schuster et al. (1997)
Malus domestica Borkh.	Apple	Root tip	2% Cellulase, 2% Pectolyase Y-23	37°C	20 min	Minamikawa et al. (2010)
Pyrus spp.	Pear	Root tip	4% Cellulase Onozuka RS, 1.5% Macerozyme R200, 0.3% Pectolyase Y-23	37°C	50–70 min	Yamamoto et al. (2010a)
Pyrus spp.	Pear	Root tip	4% Cellulase Onozuka RS, 1% Pectolyase Y-23	37°C	180 min	Yamamoto et al. (2010b)
Diospyros kaki L.	Persimmon	Shoot apical meristem	4% Cellulase RS, 1% Pectolyase	37°C	75–120 min	Zhuang et al. (1990)
Diospyros kaki L.	Persimmon	Root tip	4% Cellulase RS, 1% Pectolyase	37°C	60–70 min	Zhuang et al. (1990)
Diospyros spp.	Persimmon	Root tip	4% Cellulase RS, 1% Pectolyase Y-23	—	—	Choi et al. (2002)

the chromosome number (Zhuang et al., 1990), karyotyping (Omura, 1988), fluorescent staining (Yamamoto et al., 1999a, 2010a), FISH (Maghuly et al., 2010; Minamikawa et al., 2010; Schuster et al., 1997; Yamamoto et al., 2010b), and GISH (Choi et al., 2002, 2003b).

2. Banding technique by fluorescent staining

The GC-specific fluorochrome CMA and AT-specific fluorochrome DAPI banding methods are quite reliable and useful for identifying chromosomes (Hizume, 1991; Schweizer, 1976). These methods allow morphologically similar chromosomes to be distinguished (Hizume et al., 1989; Kondo and Hizume, 1982). In fruit trees, *Citrus* CMA/DAPI chromosome banding patterns were first reported by Guerra (1993). This and following studies revealed that CMA banding patterns of chromosomes could be classified into several types and each accession showed a characteristic CMA banding pattern. CMA banding patterns of chromosomes provide important information for phylogenetic, karyotyping, and breeding studies in *Citrus* (Befu et al., 2001, 2002; Cornello et al., 2003; Guerra, 1993; Miranda et al., 1997; Yahata et al., 2005; Yamamoto and Tominaga, 2003, 2004a, b; Yamamoto et al., 2007).

CMA/DAPI staining of the chromosomes of species belonging to *Prunus* and *Pyrus* has been demonstrated in deciduous fruit trees. In *Prunus persica*, six out of 16 chromosomes with CMA-positive (+) and DAPI-negative (–) bands were observed in the satellite positions of two chromosomes, telomeric positions of two chromosomes, and proximal positions of two chromosomes. Ten chromosomes did not have any CMA+/DAPI– bands (Fig. 2). CMA+/DAPI– bands detected in six out of 16 chromosomes were considered to be GC-rich sequences because CMA and DAPI were specific to GC- and AT-rich regions in chromosomal DNA, respectively. Detected numbers of CMA+/DAPI– bands of open pollinated seedlings of five cultivars from three species were stable in *Prunus* (Yamamoto et al., 1999a) (Table 2); however, CMA+/DAPI– bands were variable among seedlings in *Prunus mume* (Table 2).

In *Pyrus pyrifolia*, CMA+ bands were observed in telomeric positions of four chromosomes. In some samples, these CMA+ bands were observed at satellite positions. DAPI– bands were seen to correspond with CMA+ bands. Thirty chromosomes had no CMA+/DAPI– bands. No propidium iodide (PI) bands were observed in any chromosomes (Yamamoto et al., 2010a) (Fig. 3). The above-mentioned four telomeric CMA+/DAPI– bands were observed in ten cultivars from five species and one interspecific hybrid of *Pyrus* (Yamamoto

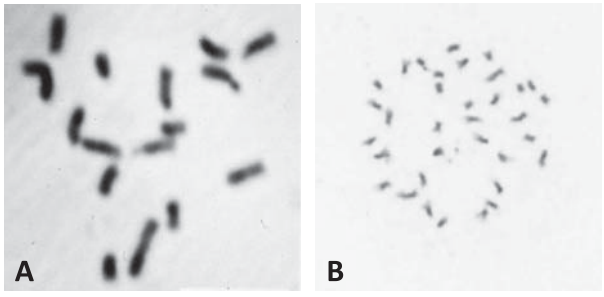

Fig. 1. Giemsa-stained chromosomes prepared by enzymatic maceration and air-drying method. A: *Prunus persica* 'Ohatsumomo' seedlings (2n = 16), B: *Pyrus pyrifolia* 'Osa Gold' seedlings (2n = 34). (Modified from Yamamoto et al., 1999a, 2010b)

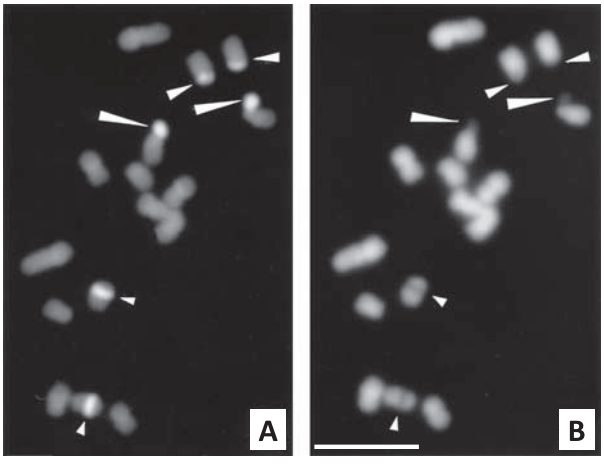

Fig. 2. CMA- and DAPI-stained chromosomes derived from seedlings of *Prunus persica* 'Akatsuki'. A: Stained with CMA, B: stained with DAPI. Long, medium, and short arrowheads indicate satellite, telomeric, and proximal regions with CMA-positive and DAPI-negative bands, respectively. Bar in B represents 5 μm for both figures. (Modified from Yamamoto et al., 1999a)

Table 2. Number of CMA-positive (+) and DAPI-negative (–) bands that appeared in chromosomes derived from open-pollinated seedlings of some *Prunus* species and cultivars.

Species	Cultivar	No. of CMA+/DAPI– bands
Prunus persica (L.) Batsch (Peach)	Ohatsumomo	6
	Akatsuki	6
	Momo Daigi Tsukuba 4 Go	6
P. salicina Lindl. (Japanese plum)	Honey Heart	6
P. armeniaca L. (Apricot)	Yamagata 3 Go	6
P. mume Siebold & Zucc. (Mume)	Yoseibai	5 to 8

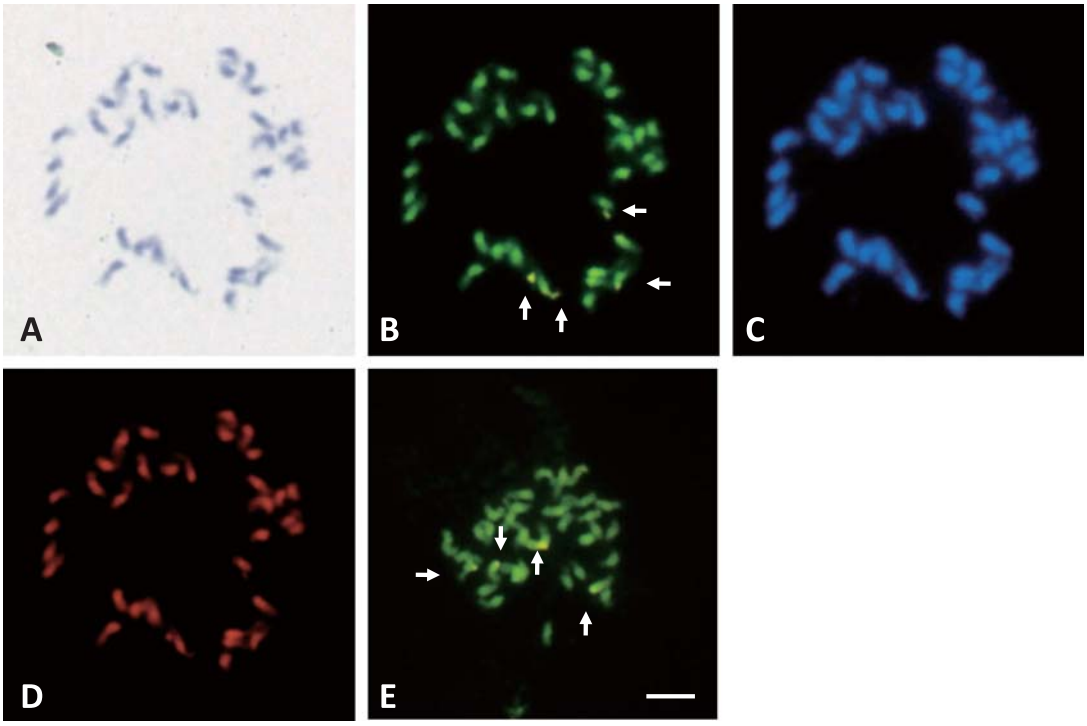

Fig. 3. Giemsa-, CMA-, DAPI-, and PI-stained chromosomes derived from seedlings of *Pyrus pyrifolia* 'Osa Gold'. A: Stained with Giemsa, B and E: stained with CMA, C: stained with DAPI, D: stained with PI. B: Two telomeric and two satellite regions with CMA-positive bands. E: Four telomeric regions with CMA-positive bands. Arrows indicate CMA-positive bands. Bar in E represents 5 μm for all figures. (Yamamoto et al., 2010a)

Table 3. Number of CMA-positive (+) and DAPI-negative (–) bands that appeared in chromosomes derived from open-pollinated seedlings of some *Pyrus* species and cultivars.

Species	Cultivar	No. of CMA+/DAPI– bands
Pyrus pyrifolia (Burm.f.) Nakai (Japanese pear)	Osa Gold	4
	Niitaka	4
	Saitama 8	4
P. communis L. (European pear)	La France	4
	Max Red Bartlett	4
P. bretschneideri Reder (Chinese pear)	Enli	4
	Yali	4
P. calleryana Decne. (Callery pear)	Aichi Mamenashi	4
P. pyrifolia × *P. ussuriensis* var. *aromatica* (Hybrid of Japanese and Iwateyamanashi)	Iwate 7 Go	4
P. mikawana Koidz. (Toyotomi Nashi)	Toyotomi Nashi	4

et al., 2010a, 2012) (Table 3). As mentioned above, the characteristics of the stains used are as follows: CMA and DAPI are GC- and AT-specific, respectively, and PI is not base-specific. The CMA+/DAPI– regions are considered to be GC-rich regions of chromosomes. In these studies, open-pollinated seedlings were used as samples. These seedlings, with the exception of 'Osa Gold', were probably generated by cross-pollination because of the self-incompatibilty of pear. The seedlings from 'Osa Gold' were probably generated by self-pollination because this cultivar is self-compatible. Although the genotype of each seedling was not identical to that of the original cultivar, almost all seedlings showed the same CMA+ bands.

In both *Prunus* and *Pyrus*, no or few species or varietal differences or individual (seedlings) variations of the CMA+/DAPI– banding pattern were observed. It could be concluded that the CMA+/DAPI– banding of *Prunus* and *Pyrus* chromosomes is very stable; however, the results for citrus demonstrated differences in CMA banding patterns among seedlings and species (Befu et al., 2001, Cornelio et al., 2003; Guerra, 1993; Miranda

et al., 1997; Yamamoto and Tominaga, 2003; Yamamoto et al., 2007; Yang et al., 2002).

3. *In situ* hybridization

In situ hybridization is a powerful tool for determining the chromosomal location of hybridized nucleic acids. Since fluorescence *in situ* hybridization (FISH) using fluorescein-labeled antibodies offers advantages over conventional autoradiographic detection, FISH has been used to detect the location of various genes in many plant chromosomes. Two or more sequences can be detected in the same preparation by using fluorochromes of different colors. This multi-color FISH (McFISH) should be useful in developing molecular cytogenetics (Mukai, 1996).

Table 4 shows a list of FISH studies of deciduous fruit trees (Choi et al., 2003a, 2003c; Corredor et al., 2004; Kim et al., 2011; Maghuly et al., 2010; Minamikawa et al., 2010; Nakao et al., 2005; Schuster et al., 1997; Yamamoto et al., 1999b; 2010b; 2012). Physical mapping of rDNA genes has been performed in some species, such as *Prunus*, *Malus*, *Pyrus*, *Diospyros*, and *Ginkgo*. McFISH studies detecting both 5S and 18S-5.8S-25S rDNA have been conducted in *Prunus*, *Pyrus*, and *Diospyros*. A retrotransposon gene of *Pyrus* and the *S* (self-incompatibility) locus of *Malus* has been detected on their chromosomes.

FISH using 18S rDNA as a probe of *Prunus persica* is shown in Figure 4 (Yamamoto et al., 1999b). Six signal sites were detected on the six chromosomes. The signals were located at satellites, and at telomeric and proximal regions of two chromosomes. The position of the rDNA sites corresponded to those of CMA+/DAPI− bands. Thus, all six 18S rDNA sites were considered to be GC-rich. This relationship between 18S rDNA sites and CMA+/DAPI− bands was in agreement with those obtained in other plants (Matsuyama et al., 1996). 18S-5.8S-25S rDNA was detected in telomeric regions of six chromosomes in *Prunus amylgdalus*, *Prunus subhirtella*, and *Prunus incica* × *serrula* (Corredor et al., 2004;

Table 4. Studies of fluorescent *in situ* hybridization (FISH) in deciduous fruit tree and detected genes.

Latin name	Common name	Gene	Reference
Prunus persica (L.) Batsch	Peach	18S ribosomal RNA	Yamamoto et al. (1999b)
Prunus amygdalus Batsch	Almond	5S and 18S-5.8S-25S ribosomal RNA	Corredor et al. (2004)
Prunus spp. (2 spcies)	Cherry rootstock	5S and 18S-5.8S-25S ribosomal RNA[z]	Maghuly et al. (2010)
Malus domestica Borkh.	Apple	5S and 18S-25S ribosomal RNA	Schuster (1997)
Malus domestica Borkh.	Apple	*S* (self-incompatibility) locus	Minamikawa et al. (2010)
Pyrus spp. (2 species)	Pear	18S-5.8S-25S ribosomal RNA	Yamamoto et al. (2010b)
Pyrus pyrifolia (Burm.f.) Nakai	Japanese pear	Ty1-*copia*-like retrotransposon	Kim et al. (2011)
Pyrus spp. (2 species)	Pear	5S and 18S-5.8S-25S ribosomal RNA[z]	Yamamoto et al. (2012)
Diospyros spp. (10 species)	Persimmon	18S-5.8S-25S ribosomal RNA	Choi et al. (2003a)
Diospyros spp. (11 species)	Persimmon	5S and 18S-5.8S-25S ribosomal RNA[z]	Choi et al. (2003c)
Ginkgo biloba L.	Ginkgo	5S and 26S-5.8S-18S ribosomal RNA[z]	Nakao et al. (2005)

[z] Multi-color FISH (McFISH).

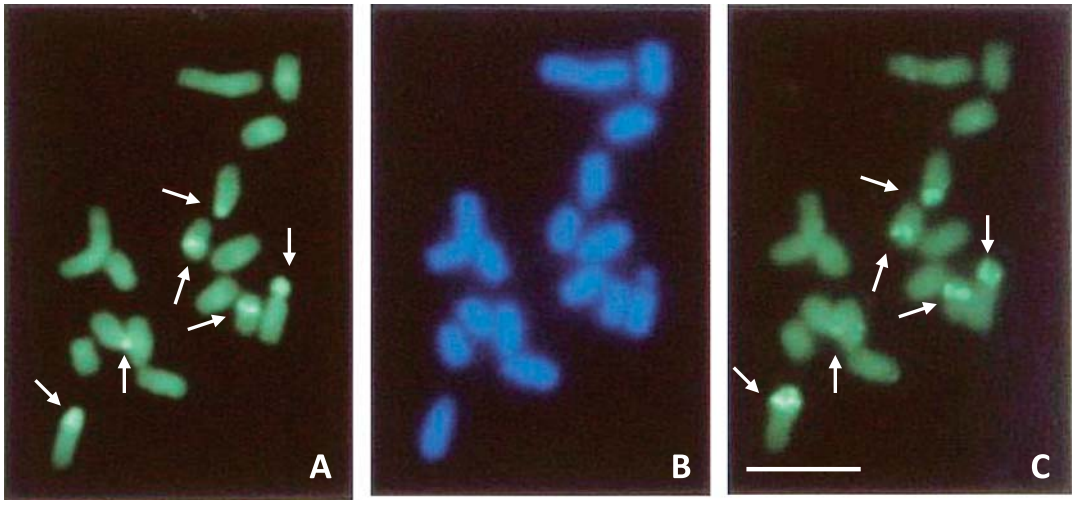

Fig. 4. Sequential application of CMA and DAPI staining and FISH with 18S rDNA probe in seedlings of *Prunus persica* 'Ohatsumomo' somatic chromosomes. A: Stained with CMA; B: stained with DAPI, C: FISH using 18S rDNA as the probe. Arrows indicate the six CMA positive bands and hybridization sites. The biotinylated probe in C was detected with FITC. No counterstaining was performed on the chromosomes. Bar in C represents 5 μm for all figures. (Modified from Yamamoto et al., 1999b)

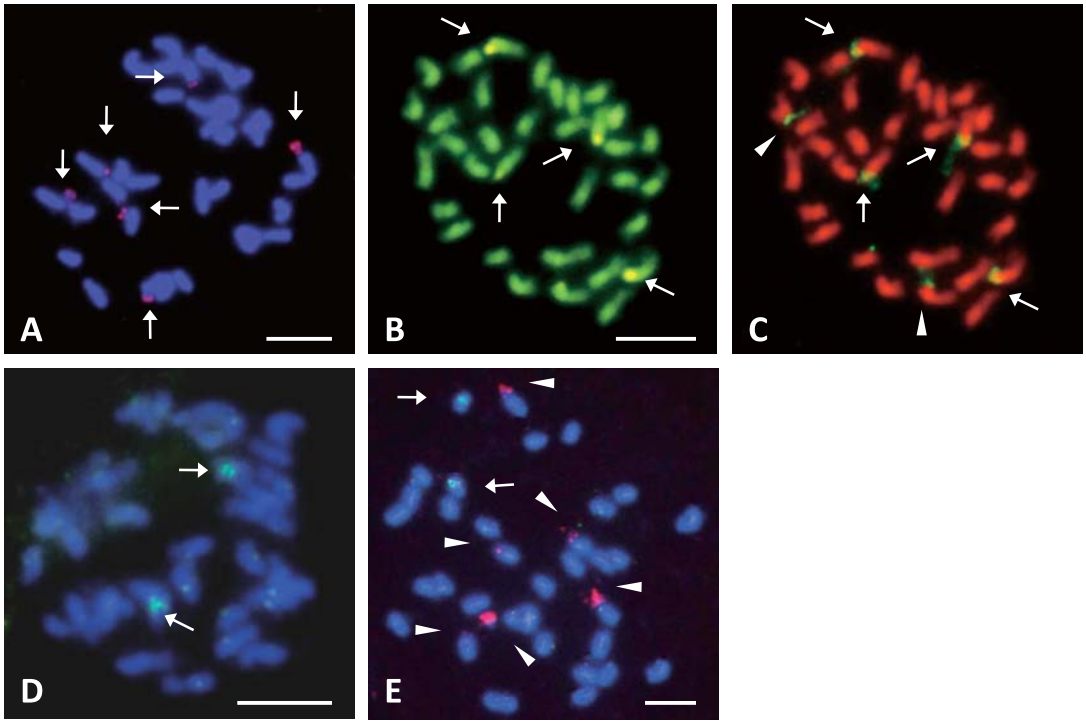

Fig. 5. FISH with 5S and 18S-5.8S-25S rDNA probes on somatic chromosomes and CMA-stained chromosomes in *Pyrus* seedlings. A and D: 'Osa Gold'; B, C, and E: Toyotomi Nashi. A and C: FISH with 18S-5.8S-25S rDNA, B: stained with CMA, D: FISH with 5S rDNA, E: McFISH with 5S and 18S-5.8S-25S rDNA. Arrows in A, B, C, and D indicate rDNA sites and CMA-positive bands. Arrows and arrowheads indicate 5S rDNA and 18S-5.8S-25S rDNA sites, respectively, in E. The digoxigenin-labeled probes in A and E were detected with Rhodamine/Texas Red (red signals). The biotinylated probes in C, D and E were detected with FITC (green signals). Chromosomes in A, D and E were counterstained with DAPI. Chromosomes in C were counterstained with PI. Bars represent 5 μm. (Modified from Yamamoto et al., 2012)

Maghuly et al., 2010). These results indicate conservation of the chromosome structure among *Prunus* species. Moreover, 5S rDNA of these species was found proximally located on four chromosomes (Corredor et al., 2004; Maghuly et al., 2010).

In *Pyrus*, *in situ* hybridization with the 18S-5.8S-25S rDNA probe revealed signals on six chromosomes. The six signal sites were located in telomeric regions of the six chromosomes. Four CMA+/DAPI– bands corresponded with 18S-5.8S-25S rDNA sites (Yamamoto et al., 2010b, 2012) (Fig. 5). This result agrees with the results of *Prunus* and *Ciser*, which showed CMA+ bands that corresponded with 18S-5.8S-25S rDNA sites (Galasso et al., 1996; Yamamoto et al., 1999b), although not all CMA+ bands were 18S-5.8S-25S rDNA sites in *Citrus* (Carvalho et al., 2005; Matsuyama et al., 1996). These results indicate that the rDNA sites of *Pyrus* are regions with high GC content (Schweizer, 1976). The 5S rDNA sites were detected in centromeric positions of two chromosomes. Two centromeric 5S rDNA and six telomeric 18S-5.8S-25S rDNA sites were located at different chromosomes, as determined from the results of McFISH (Yamamoto et al., 2012) (Fig. 5). In addition, the number and locations of 18S-5.8S-25S and 5S rDNA sites were stable among the seedlings of three and two *Pyrus* species, respectively.

The 18S-25S rDNA and 5S rDNA sites in *Malus* × *domestica* Borkh. were detected in telomeric regions of eight chromosomes and centromeric regions of two chromosomes, respectively (Schuster et al., 1997). The rDNA sites of *Pyrus* and *Malus* are quite similar. The similarity of linkage maps between *Pyrus* and *Malus* was also reported (Yamamoto et al., 2007). It could be

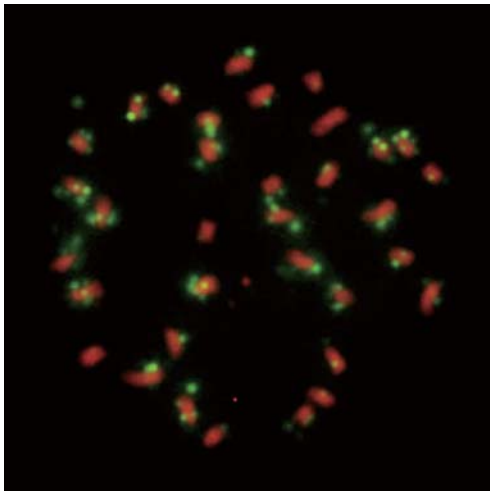

Fig. 6. FISH of *copia* retrotransposon probe (*Ppcrt*: green signals) to somatic chromosomes of *Pyrus pyrifolia* 'Osa Gold'. The biotinylated probe was detected with FITC. Chromosomes were counterstained with PI.

concluded that there is a syntenic relationship between *Pyrus* and *Malus* on the basis of these results, despite some divergence of chromosome configuration between them.

McFISH of 5S and 18S-5.8S-25S rDNA was performed to reveal phylogenetic relationships among *Diospyros* species (Choi et al., 2003c). More 5S and 18S-5.8S-25S rDNA sites were observed in species from the southern part of Africa than those from Asian species in diploid species. Among the Asian species, the number of both rDNA sites increased according to the ploidy of species.

Since male and female trees of *Ginkgo biloba* showed the same 5S and 26S-5.8S-18S rDNA sites, the discrimination of male and female trees by rDNA FISH was impossible (Nakao et al., 2005).

Besides the rDNA gene, the chromosomal distribution of Ty1-*copia*-like retrotransposons in the *Pyrus pyrifolia* genome was elucidated by FISH. These retrotransposons were dispersed throughout more than half of the *Pyrus pyrifolia* chromosomes (Kim et al., 2011) (Fig. 6). In addition, chromosomal location of the *S* locus, which controls self-incompatibility, in *Malus* × *domestica* Borkh. was reported by Minamikawa et al. (2010).

The technique using total genomic DNA as a probe is called genomic *in situ* hybridization (GISH). This method is very useful for the identification of parental chromosomes and genomic composition. Choi et al. (2003b) reported the relatedness of *Diospyros kaki* and *Diospyros glandulosa* on the basis of GISH results. Chromosome composition of somatic hybrids of *Diospyros kaki* and *Diospyros glandulosa* was revealed by multi-color GISH (McGISH) (Choi et al., 2002).

4. Conclusion and perspective

Chromosome studies of deciduous fruit trees have progressed during the last two decades; good chromosome samples could be prepared by means of the enzymatic maceration method, some morphologically similar chromosomes were distinguished by the banding technique using fluorochrome, and chromosome configurations were revealed by *in situ* hybridization. These results could provide fundamental information on chromosomes and are considered to contribute to the progress of breeding and genome studies in various species.

Recently, genome analysis of deciduous fruit trees has progressed markedly. Genome sequences of important species have been reported (Jaillon et al., 2007; Velasco et al., 2010). Cooperation between chromosome and genome studies develops our understanding of genome information and accelerates the breeding of new cultivars by biotechnological methods.

In addition, detection of the physical locations of useful gene loci has progressed in important crops and vegetables (Iwano et al., 1998; Ohmido et al., 1998). Therefore, physical mapping of useful genes, for instance, scab resistance and black spot disease susceptibility in pear (Terakami et al., 2006, 2007), should be conducted.

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