Physical mapping of 5S, 45S, *Arabidopsis*-type telomere sequence repeats and AT-rich regions in *Achillea millefolium* showing intra-chromosomal variation by FISH and DAPI

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ABSTRACT. *Achillea millefolium* L. collected in Altai, Russia showed $2n=54$ as a hexaploid chromosome number. The interphase and metaphase chromosomes had FISH signals and DAPI bands: FISH yellow-colored signals of the 5S and 45S rDNA and the *Arabidopsis*-type telomeres were obtained after the hybridization of the biotin labelled probes of the 5S, pTa71 and the *Arabidopsis*-type telomere sequence repeats. The 5S rDNA signals consisted of 11 yellow-color signals at the secondary constriction of the sat-chromosomes, two large interstitial signals on the long arm of two chromosomes, and very small four signals on the long arm of four chromosomes. The interphase nucleus showed 11 deep and two faint yellow color signals of 5S rDNA sites. Eleven yellow color signals of the 45S rDNA sites were detected on the short arm of 11 chromosomes. The *Arabidopsis*-type telomere signals were located on the terminal region on the both arms of chromosomes, while some were located far from the chromosome arm on the external part of the satellites in the sat-chromosomes, three terminal signals in some chromosomal arms one signal instead of two in the other chromosomes. DAPI-positive bands were found on interphase nucleus and 42 chromosomes, of which ten were terminal on both arms, four had two bands on a single arm, two had two bands on centromeric and terminal regions and 26 had single bands on a single arm. The 11 signals of the 5S and 45S rDNA sites and the the *Arabidopsis*-type telomere signals and DAPI bands performed variation in size and intensity

KEYWORDS: *Achillea millefolium*, *Arabidopsis*-type telomere sequence repeats, DAPI, FISH, 5S rDNA, 45S rDNA

Achillea millefolium L., yarrow or thousandleaf: grassy balds, roadsides, disturbed areas; common, April–November. The species has been taxonomically treated with several subspecies and varieties such as *A. millefolium* ssp. *millefolium*; *A. millefolium* ssp. *lanulosa* (Nuttall) Piper; *A. lanulosa* Nuttall; *A. millefolium*; *A. millefolium* var. *millefolium*; *A. millefolium* var. *occidentalis* DC. (Weakly 2007). It is often used as traditional and folklore medicine (Pleah *et al.* 2002). The name *Achillea millefolium* has been applied to a complex of barely distinguishable species or subspecies, which have evolved by polyploidy. This has been resulted in some confusion in nomenclature. The name *Achillea* was used to mean *A. millefolium*, without differentiation between the different subspecies (Ross 2003). *Achillea* is a variable temperature herb with a very wide range of potential actions and uses. This is associated with its unusually wide range of active constituents, including essential oils, sesquiterpene lactones, flavonoids, alkaloids, polyacetylenes, sterols, phenolic acids, coumarins, and tannins. *Achillea* can contain 0.2–1% essential oil, which may contain 0–50% chamazulene (Ross 2003).

Polyploidy is an intriguing phenomenon in plants that has provided an important pathway for evolution and speciation of the species (Soltis and Soltis 2000). The widespread occurrence of polyploidy has been attributed to the potential of polyploid species to adapt to a wider range of habitats and survive better in unstable climates

than their diploid progenitors. The types of changes in chromosomes/genomes that have occurred during species evolution, has a direct impact on the basic and applied botany and to the aims of plant researchers and breeders in understanding genome evolution and genetics makes this area worthy of further investigation (Heslop-Harrison 2000). In nature, closely related taxa might make cross-hybrids, which play an important role in the chromosome evolution of the species (Kondo *et al.* 1994).

Therefore, several chromosome analyses of polyploidy *Chrysanthemum* and its allied genera have been carried out extensively in the Japanese, Chinese and Russian species (e.g., Tanaka *et al.* 1989; Abd El-Twab and Kondo 2003a,b, 2006, 2007a; Hoshi *et al.* 2003; Kondo *et al.* 1994; Zhmyleva and Kondo 2006; Pellicer *et al.* 2007).

Fluorescent banding methods such as 4-6-diamidino-2-phenylindole (DAPI) to determine the A-T (Adenine-Thymine) rich segments along the chromosomes of various plants could provide more details and critical chromosomal data for phylogenetic relationships of species (Sumner 1990; Hizume *et al.* 1997). Therefore, the fluorochrome banding techniques have been employed to some chromosome complements of Japanese and Chinese species of *Chrysanthemum sensu lato* (Kondo *et al.* 1995; Khaung *et al.* 1995; Abd El-Twab and Kondo 2003a).

In higher eukaryotes, rDNA's are organized into two distinct gene classes of the major rDNA cluster encoding 45S rDNAs; and the minor rDNA cluster encoding 5S

rDNA. The minor rDNAs are usually found in loci that are separate from those of the major rDNAs, and is repeated in tandem comprises a highly conserved 120-nucleotide coding sequence and non-transcribed spacer and not involved in the nucleolus formation (Inafuku 2000). Another highly conserved sequence is found at the physical ends of chromosomes of nearly most of plant species. Multiple copies of a motif similar to the seven nucleotide unit TTAGGG are added by an enzyme, telomerase, incorporating an RNA template, and function in stabilizing the chromosome ends and enabling semi conservative replication of the DNA up to the telomerase added region (Fuchs and Schubert 1995).

Non-radioactive *in situ* hybridization is a valuable, effective and safety method for studying the chromosomal distribution of DNA sequences and copy numbers at different sites, and to follow evolutionary changes in their physical organization in the genome (Harrison and Heslop-Harrison 1995; Kondo *et al.* 1996; Abd El-Twab and Kondo 2008). As the pioneer among molecular cytogenetics techniques, fluorescence *in situ* hybridization (FISH) allows identification of specific sequences in a structurally preserved cell, in metaphase or interphase. This technique, based on the complementary double-stranded nature of DNA, hybridizes labeled specific DNA (probe). The probe, bound to the target, will be developed into a fluorescent signal. The fact that the signal can be detected clearly, even when fixed in interphase, improves the accuracy of the results (Mühlmann 2002). FISH became a valuable method for studying the chromosomal distribution of DNA sequences and copy numbers at different sites, and to follow evolutionary changes in their physical organization in the genome (Harrison and Heslop-Harrison, 1995). FISH makes chromosomes of plant species providing the information of molecular characters of nucleolar organizing region (Heslop-Harrison *et al.* 1991; Kondo *et al.* 1996; Kondo and Abd El-Twab 2002; Abd El-Twab and Kondo 2003b, 2008). In plants, physical localization of the tandemly repeated genes encoding the 5S ribosomal RNA (5S rDNA) and 18S-5.8S-25S ribosomal RNA genes (45S rDNA) and *Arabidopsis* type of telomere repeats has provided a group of valuable chromosome landmark markers. The classes of rDNA and telomeres are a major type of repetitive sequence in the higher plant genome. The chromosomal localization of the 5S, 45S rDNA and *Arabidopsis*-type telomere sequence repeats via FISH in the chromosome complements of several species in *Chrysanthemum sensu lato* was reported (Kondo *et al.* 1996; Khaung *et al.* 1997; Honda *et al.* 1997; Abd El-Twab *et al.* 1999; Abd El-Twab and Kondo 1999, 2002, 2003a,b, 2004, 2007a, b, 2008).

In our laboratory, *Achillea millefolium* is used as both pollen and ovule parent to produce the artificial inter- and intra-generic hybrids among the group species in *Chrysanthemum sensu lato*. Therefore, in the present work, we aimed to investigate the chromosome number and the

physical location of the 5S, 45S rDNA sites and telomeric repeats by FISH and AT regions by DAPI on the somatic chromosomes of *A. millefolium*, which is considered a very important and valuable landmark for identifying specific chromosomes of this species in the artificial produced hybrids.

MATERIALS AND METHODS

The plants used in this study were *Achillea millefolium* (sn: Russ-1, 2 and 3) were cultivated in the Laboratory of Plant Chromosome and Gene Stock, Graduate School of Science, Hiroshima University.

Chromosome preparation Following Kondo *et al.* (1996) growing root tips were collected and pretreated in 0.002 M 8-hydroxyquinolin at 18°C for 1.5 h. They were fixed in 1:3 of glacial acetic acid and 95% ethanol at 4°C for 2 h. Fixed roots were excised and washed in distilled water many times to remove the fixative. Five to ten tips 2-5 mm long each from root tips were placed in 1.5 ml microcentrifuge tube containing the enzymatic buffer of 5% cellulase (Yakult), 2% pectolyase Y-23 (Kikkoman). They were incubated at 37°C for 20 min, and then, their soft meristematic tissues were washed in distilled water to remove the enzymatic solution and were squashed in 45% acetic acid. The cover-slip was removed by the dry-ice freezing method and the preparation was dried at room temperature.

DAPI fluorescent staining after FISH Following Abd El-Twab and Kondo (2003a), the slides were removed their hybridized probe, the slides after FISH were heated at 85°C for 10 min in washing buffer (0.1XSSC, 0.5% SDS), washed in 100% alcohol for 1h, then air-dried at room temperature and finally applied DAPI. The slides were washed in MacIlvain buffer at pH 7.0 at room temperature for 30 min and they were dropped 50 µl of 0.1 mg/ml Actinomycin D (Sigma) dissolved in MacIlvain buffer at pH 7.0. They were washed in MacIlvain buffer at pH 7.0 at room temperature for ten min, and were stained in 10 µg/ml DAPI dissolved in MacIlvain buffer at pH 7.0 at room temperature for 30 min. The slides were washed in MacIlvain buffer at pH 7.0 at room temperature for 10 min and were mounted with coverslips using a drop of 50% glycerol. The slides were observed on an Epifluorescence microscope using UV filter (UV-2A; Nikon).

Ribosomal DNA probes: 5S rDNA probe The 5S rDNA probe was produced by the polymerase chain reaction (PCR) method as described by Hizume (1993) and Abd El-Twab and Kondo (2002).

45S rDNA probe The probe of pTa71 (45S rDNA) consisted of a 9 kb Eco RI fragment of rDNA derived from *Triticum aestivum* L. (Gerlach and Bedbrook 1979), which was recloned into pUC19 plasmid. It was comprised of the coding sequence for the 18S, 5.8S and 26S genes

and the non-transcribed spacer sequences.

Probe of the Arabidopsis-type telomere sequence repeats

The probe of the Arabidopsis-type telomere sequence repeats was synthesized by using the polymerase chain reaction (PCR). PCR was carried out in the absence of template using the primers (TTTAGGG)₅ and (CCCTAAA)₅ (Schwarzacher and Heslop-Harrison 1991; Cox *et al.* 1993; Abd El-Twab and Kondo 2007a). Each 50 µl reaction mixture included 50 mM Tris-HCl, pH 9.0 (25°C), 50 mM KCl, 0.1 mM EDTA, 0.1% (v/v) Triton X-100, 1.25 mM MgCl₂ 2.5 units of Taq polymerase (TOYOBO), 200 µM each dNTP and 200 nM each primer. Temperature cycling was performed as follows: The first step of one cycle each took for 1 min at 94°C, and 30 sec at 55°C and 1 min at 72°C; the second step of 35 cycles each for 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C; and the final step for 3 min at 72°C. The product was purified by PCI (phenol:chloroform:isoamylalcohol=25:24:1) treatment, CIA treatment and ethanol precipitation method. The oligonucleotide purified, the Arabidopsis-type telomere sequence repeats were dissolved in 5 µl of TE buffer (10.0 mM Tris.Cl at pH 8.0 and 1.0 mM EDTA at pH 8.0). The concentration of the products was measured with the absorption meter (Ultrospec 3000). Then, the Arabidopsis-type telomere sequence repeats were used for labeling reaction.

Labeling of the probes The probes were labeled with biotin-14-dATP nick translation kit (Gibco BRL), labeling of each probe was carried out according to the manufacturer's protocols.

The procedure of FISH and detection of the probe followed the methods described by Abd El-Twab and Kondo (1999). FISH that fluoresced the yellow-color was visualized by FITC after the hybridization with the biotin labeled probes and red-color was visualized by the counter stain of propidium iodide (PI). Fluorescence signals were examined with an epifluorescence microscope with Nikon B-2A filter cassette and microphotographs were taken on CCD camera (Pixera Pengium 600CL). Analysis of hybridization signals and karyograms were produced using Adobe Photoshop 7.

RESULTS AND DISCUSSION

Achillea millefolium collected during the field trip of 2004 in the Altai Mountains, Russia showed the chromosome number of $2n=54$ (hexaploid). The interphase and metaphase chromosomes in *A. millefolium* had FISH signals and DAPI bands (Figs. 1 and 2). Respective interphase nucleus had 11 yellow-color signals of 45S rDNA and positive DAPI bands (Fig. 1A and B). Each interphase nucleus showed four nucleolus cavities that had 14 yellow color signals of the 45S rDNA (Fig. 2A) instead of 11 (Fig. 1A), that showed two signals inside the left nucleolus cavity, five signals on the border of the other three nucleolus cavities, and the other four signals were found outside the

cavities in the interphase nucleus. Eleven yellow-color signals of 45S rDNA were detected on the short arm of 11 chromosomes hybridized with the biotin labeled probe of pTa71 (Fig. 1D and Fig. 2C). Eleven yellow signals of 5S rDNA at the sites of sat-chromosomes, two large interstitial signals on the long arm of two chromosomes, and very small four signals (not visible constantly in all plates) on the long arm of four chromosomes, which hybridized with the biotin labeled probe of the 5S rDNA (Fig. 1E). Only Eleven strong and two faint yellow color signals of 5S rDNA sites in the interphase (Fig. 1C) were detected after hybridization with the probe. The plate of metaphase chromosome complement counterstained-red by PI was DAPI-positive bands on 42 chromosomes, of which ten chromosomes had terminal bands on both arms; four chromosomes had two bands on a single arm; two chromosome had two bands centromeric and terminal; 26 chromosomes have single positive DAPI bands on a single arm (Fig. 1E and F). The signals of the Arabidopsis-type telomere sequence repeats were yellow color on the terminal region on both arms of the metaphase chromosomes. The sat-chromosomes showed the signal of the Arabidopsis-type telomere sequence repeats far from the chromosome arm on the external part of the satellite, some chromosomal arms showed three terminal signals and the others showed one signal instead of two that were hybridized with the somatic chromosomes, as appeared in the karyogram of the chromosome complement, which showed chromosome pairing that had similar terminal telomere of yellow color signals (Fig. 2D and E), which hybridized with the biotin labeled probe of the PCR amplified telomeric repeats.

Achillea millefolium L. has been taxonomically well-studied with several different synonymous names such as *A. lanulosa* Nutt., *A. magna* auct., *A. millefolium* subsp. *borealis* (Bong.) Breitung, *A. millefolium* subsp. *lanulosa* (Nutt.) Piper, *A. millefolium* subsp. *millefolium*, *A. millefolium* var. *occidentale* DC. (see Germplasm Resources Information Network (GRIN) Taxonomy for Plants: <http://www.ars-grin.gov/>).

Achillea millefolium has been subjected to intensively investigate its chromosome numbers such as $2n=18$, diploid (Daniela 1997), $2n=27$, triploid (Maffei *et al.* 1986), $2n=36$, tetraploid (eg. Hommel and Wieffering 1979; Löve and Löve 1982), $2n=45$, pentaploid (Androshchuk and Kostinenko 1981), $2n=54$, hexaploid (eg., Morton 1977; Arohonka 1982; Kuzmanov *et al.* 1989; Huber and Baltisberger 1992; Druskovic and Lovka 1995), $2n=72$, heptaploid (Magulaev 1982). Additionally, the species showed also several different haploid sets such as $n=9$, 18, 22, 27, 29, 36 and 58) and aneuploid chromosome numbers such as $2n=34$, 35 and 44) (Gervais 1977; Keil and Stuessy 1977; Pireh and Tyrl 1980; Gupta and Gill 1981; Morton 1981). but very little in chromosome character in the most standard references.

Achillea lanulosa which was considered a synonym of

Fig. 1. Interphase (A-C) and metaphase chromosomes (D-G) have FISH signals (A-E) and DAPI bands (B and G) in *A. millefolium* (sn: Russ-1, 2004). Yellow-color was fluoresced and visualized by FITC after the hybridization with the biotin labeled probes and red-color was visualized by the counter stain of PI. A-B. Same interphase nucleus has eleven yellow-color signals of 45S rDNA sites (A) and positive DAPI bands (B). C. Eleven strong and two faint yellow color signals of 5S rDNA sites hybridized with the biotin labeled probe of the PCR amplified 5S rDNA. D. Eleven yellow-color signals of the 45S rDNA sites that hybridized with the biotin labeled probe of pTa71. E. Eleven yellow-color signals of 5S rDNA sites at the same sites of the NOR regions of the sat-chromosomes, two large interstitial signals on the long arm of two chromosomes, and very small four signals on the long arm of four chromosomes, which hybridized with the biotin labeled probe of the 5S rDNA. F-G. Same plate of metaphase chromosome complement counterstained-red by PI (F) and DAPI-positive (G) bands on 42 chromosomes, of which ten chromosomes have terminal bands on both arms; four chromosomes have two bands on a single arm; two chromosome have two bands centromeric and terminal; 26 chromosomes have single positive DAPI bands on a single arm. Bar=10 μ m.

A. millefolium regarding morphological variation in leaf shape among different populations (Gurevitch 1991). The chromosome number reported for *A. lanulosa* was tetraploid with $2n=36$ (Löve and Löve 1980, 1982) while it was the haploid chromosome set with $n=36$ (Keil and Stussy 1977) and $n=18_{II}$ (Löve and Löve 1980). However, the chromosome report of another synonym *A. magna* could not be found. It was interesting to find that differences in chromosome number between the *A. millefolium* subspecies were often accompanied by important chemical

differences (Ross 2003), which, in their turn, may lead to differences in temperature, actions, and uses. The percentage of chamazulene and the general composition of the essential oil fraction vary greatly with the chromosome number of the plant material. The tetraploid plants had sesquiterpenes, mainly chamazulene (ca 25%), while the hexaploid plants of *A. millefolium* ssp. *millefolium* had no chamazulene (Ross 2003). However, it could not be supported simply that the other species and subspecies of *A. millefolium* were just synonyms of the species. The

Fig. 2. FISH yellow color signals on mitotic interphase (A-B) and metaphase (C-E) chromosomes in *A. millefolium* (sn: Russ-2, 2004). Yellow-color was fluoresced and visualized by FITC after the hybridization with the biotin labeled probes and red-color was visualized by the counter stain of PI. A. Interphase nucleus showing four nucleoli cavities; fourteen signals instead of eleven, which show two signals inside the left nucleolus cavity, five signals on the border of the other three nucleoli cavities, and the other four signals outside the cavities. B. Yellow color signals of the telomeres that hybridized with the biotin labeled probe of the PCR amplified telomeric probe. C. Eleven signals of the 45S rDNA on the short arm of eleven chromosomes that hybridized with the biotin labeled probe of pTa71. D. Terminal yellow signals of the telomeres that hybridized with the biotin labeled probe of the PCR amplified telomeric probe. E. Karyogram of the chromosome complement showing the chromosome pairing that has similar terminal telomere signals. Bar=10 μ m.

chromosome numbers in the species were correlated with variation of natural products with respect to the ploid level (Ross 2003).

Divergence and speciation are often accompanied by reorganization in the chromosomes. However, the karyotypes of *Chrysanthemum* species and related genera are highly conservative and it is difficult for them to be differentiated closely related species, such as species of the same ploidy level by karyotyping (Tanaka *et al.* 1989). In contrast, rDNA FISH gives much higher resolution for detecting chromosome variations among closely related species. Our present results and other studies (Kondo *et al.* 1996; Khaung *et al.* 1997; Honda *et al.* 1997; Abd El-Twab and Kondo 2003b, 2007b, 2008) have shown that within the genus *Chrysanthemum* and closely related genera the number and positions of major rDNA sites on chromosomes varied among the species in *Chrysanthemum sensu lato*. This variation should have a phylogenetic implications, since the more similar rDNA FISH patterns are likely to be in the most closely related taxa (Abd El-Twab and Kondo 2003b). The distribution patterns of rDNA loci among the chromosomes vary markedly among *Chrysanthemum*

sensu lato.

In diploid species the number of major 45S rDNA sites typically ranged between one and four pairs (*e.g.*, Abd El-Twab and Kondo 2003b). The highest % of FISH signal number of the 45S rDNA per chromosome complement among the wild species of *Chrysanthemum* was 44.4% in the diploid *D. lavandulifolia* while the highest % among the wild hexaploid species was 33.3% in *C. occidentali-japonense* (Abd El-Twab and Kondo 2003b) and that was varied approximately 25.9-35.2%, in the wild *C. zawadekii* indicating genome complex (Abd El-Twab and Kondo 2006) and was the highest 55.5% in the cultivars of *Chrysanthemum* (Abd El-Twab and Kondo 2004). In the present study signal numbers were not even number but odd number and percentages of the FISH signal number of the 45S rDNA were approximately 20.4 % (Table 1), also the 11 signals of the 5S, 45S rDNA sites and the telomeric signals and DAPI bands showed variation in size and intensity indicating this *A. millefolium* studied up to date might have a complex genome.

Since, a prediction of chromosomal locations of satellites would be that closely related satellite sequences

Table 1. FISH signals of 45S rDNA sites in somatic chromosomes of several species in *Chrysanthemum sensu lato* up to date

Species	2n	FISH signals of pTa71		References of 45S rDNA sites in <i>Chrysanthemum</i>
		Of 45S rDNA sites	No. %	
<i>Achillea millefolium</i>	54	11	20.4	present study
<i>Ajania remotipinna</i>	18	4	22.2	Abd El-Twab and Kondo 2007b
<i>Chrysanthemum boreale</i>	18	6	33.3	Honda <i>et al.</i> 1997
<i>C. horaimontanum</i>	18	2	11.1	Abd El-Twab and Kondo 2003
<i>C. japonicum</i>	18	4	22.2	Kondo <i>et al.</i> 1996
<i>C. lavandulifolium</i>	18	8	44.4	Khaung <i>et al.</i> 1997
<i>C. chanetii</i>	36	8	22.2	Abd El-Twab and Kondo 2007b
<i>C. indicum</i> (Japan)	36	8	22.2	Khaung <i>et al.</i> 1997
<i>C. indicum</i> (China)	36	12	33.3	Abd El-Twab and Kondo 2003
<i>C. wakasaense</i>	36	8	22.2	Kondo <i>et al.</i> 1996
<i>C. yoshinaganthum</i>	36	10	27.8	Khaung <i>et al.</i> 1997
<i>C. occidentali-japonense</i>	54	4	11.1	Honda <i>et al.</i> 1997
		10	18.5	Abd El-Twab and Kondo 2003
<i>C. vestitum</i>	54	18	33.3	Abd El-Twab and Kondo 2003
		10	18.5	Abd El-Twab and Kondo 2003
<i>C. grandiflorum</i> cv. 'Shuho-no-chikara'	54	30	55.6	Abd El-Twab and Kondo 2004
<i>C. grandiflorum</i> cv. 'Golden Shark'	54	12	22.2	Abd El-Twab and Kondo 2004
<i>C. grandiflorum</i> cv. 'Monaco'	52	7	13.5	Abd El-Twab and Kondo 2004
<i>C. grandiflorum</i> cv. 'Sei Alpes'	54	12	22.2	Abd El-Twab and Kondo 2004
<i>C. morii</i>	72	10	13.8	Abd El-Twab and Kondo 2003
<i>Leucanthemella linearis</i>	18	6	33.3	Abd El-Twab and Kondo 2007a
<i>Nipponanthemum nipponicum</i>	18	2	11.1	Abd El-Twab and Kondo 2007a

showed similar chromosome distributions. Closely related satellite repeats occupy adjacent regions of the chromosomal DNA. Such, topological constraints might arise from either functional restrictions or from mode of origin, one from the other (Lohe and Roberts 1988). It also helps to clarify genetic maps and to assign linkage groups to physically marked chromosomes. By comparing the rDNA FISH patterns identified homologous chromosomes among the species. It was found that *A. millefolium* in the present study had different shapes and sizes of the of the 5S and 45S rDNA sites on the NOR regions of the sat-chromosomes, which were two groups; first group consists of eight chromosomes have large region of yellow color signals of the 45S rDNA sites, and extended outside the short arms of the chromosomes, the second group consists of three chromosomes have three large 45S rDNA attached with chromosomes. Based on the number and place of the FISH signals of the 45S rDNA sites in the interphase nucleoli we could speculate that various numbers of the FISH yellow color signals of the 45S rDNA sites in the interphase nuclei might due to extension of the sat-chromosome inside the nucleus due to active sites. The signals inside or around the nucleolus border might indicate gene activity and active sat-chromosomes, while the signals outside the nucleoli cavities might indicate that the sites of 45S rDNA are inactive gene in the interphase nucleus. The co-localization of the 5S and 45S rDNA sites gave us now a preliminary outline about the relation of the *Chrysanthemum sensu lato*, since two major groups of 5S rDNA were given to be able to divide the species into at least two or three genetically different groups by using the probe of 5S rDNA amplified from *C. boreale* and *C. vestitum* and two main features such as the interstitial signals of the 5S site (Abd El-Twab and Kondo 2002) and the co-localized 5S and 45S sites on different chromosomes in this study and else where (Abd El-Twab and Kondo 2004, 2007a). This study may be extended to several different materials in *Chrysanthemum sensu lato* using the probe of 5S rDNA to justify our speculation about the taxonomic and genetical relationships among the species group of *Chrysanthemum sensu lato*, with special references to the Japanese and Chinese species.

Studies on origin and chromosome constitutions of the native polyploid species of *Chrysanthemum sensu lato* may contribute to satisfactory phylogenetic and taxonomic treatment of species relationships. In the course of evolution, the species genome appears to have undergone structural reorganization and recombination and, thus, formed unique chromosome complement characteristics as observed here. Knowledge of the relative physical locations and the number of multicopy rDNA gene loci are important and useful both for the construction of physical maps of chromosomes and for phylogenetic studies. Our data of the FISH provided useful information on the presence of the 5S and 45S rDNA sites, telomere and AT-rich regions by DAPI as well as their relative positions on chromosomes

of the species, which are considered a very important landmark for identifying specific chromosomes of this species in the artificial produced hybrids in our laboratory.

World populations of *Achillea millefolium* and its synonyms may be very important for investigating the genome characterization, changes, interaction and evolution among the collections of the world wide distributed and medicinally important species.

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