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Comparative Cytogenetic Characteristics and Physical Mapping of the 17S and 5S Ribosomal DNAs between *Atractylodes japonica* Koidz. and *Atractylodes macrocephala* Koidz.

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ABSTRACT : This study was carried out to compare chromosomal characteristics between *Atractylodes japonica* and *A. macrocephala*. Cytogenetic analysis was conducted based on karyotype analysis and physical mapping using fluorescence *in situ* hybridization. As a result of karyotype analysis by feulgen staining, somatic chromosome numbers of *A. japonica* and *A. macrocephala* were 2n=24. The length of the mitotic metaphase chromosomes of *A. japonica* ranged from 0.70 to 1.60 ㎛ with a total length of 12.11 ㎛ and the homologous chromosome complement comprised six metacentrics, five submetacentrics and one subtelocentrics. On the other hand, the length of the mitotic metaphase chromosomes of *A. macrocephala* ranged from 0.90 to 2.35 ㎛ with a total length of 16.58 ㎛ and the homologous chromosome complement comprised seven metacentrics and five submetacentrics. The total length of *A. japonica* chromosomes was shorter than that of *A. macrocephala*, but *A. japonica* had one subtelocentrics (chromosomes 4) different from *A. macrocephala* chromosomes. The FISH technique using 17S and 5S rDNA was applied to metaphase chromosomes. The signals for 17S rDNA were detected on the telomeric regions of chromosomes 4 and 5 in both *A. japonica* and *A. macrocephala*. The 5S rDNA signal was found in the short arm of chromosome 1.

Key words : *Atractylodes japonica*, *Atractylodes macrocephala*, karyotype, FISH, rDNA

INTRODUCTION

Packchul, *Atractylodes japonica* or *A. macrocephala*, is a very important Chinese herbal medicine in Asia and called "Sabju" in Korean botanical name. The pharmaceutical and botanical names of the Packchul are Rhizoma atractylodis and *Atractylodes japonica* or *A. macrocephala*, respectively. It has been reported that the Packchul has various health benefits, such as regulating the function of the stomach, replenishing the Qi, strengthening the spleen, resolving dampness, promoting water metabolism, stopping sweating and preventing miscarriage. The young stem and leaf have bitter taste and unique scent. These have been used as ingredients for Korean salads for a long time.

Cytogenetic analysis is useful for clarification of species phylogeny and evolution. Especially karyotype analysis has been successfully used to assist in the definition of species relationship. The *in situ* hybridization (ISH) technique, initially developed to map the rDNA sequences on mammalian chromosomes (Gall & Pardue, 1969; John *et al.*,

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1969), has become a powerful tool for detecting specific DNA sequences on genomes. Since the method for using non-radioisotope, biotin-labeled probes was introduced to facilitate plant genome analysis (Rayburn & Gill, 1985), ISH has been widely used for the study of the origin and evolution of specific genomes (Jiang & Gill, 1994).

Since the fluorescence *in situ* hybridization (FISH) technique was used to facilitate plant genome mapping, this technique has detected low and single copy sequences as well as rDNA and highly repetitive sequences in plants. Also, it is effective for detecting several probes simultaneously on the same metaphase chromosomal spread (Mukai *et al.*, 1993). In plants, there are several reports for the detection and mapping of genus using FISH; wheat (Mukai *et al.*, 1993), barley (Leitch & Heslop-Harrison, 1993), rye (Albino & Schwarzacher, 1992), tobacco (Kenton *et al.*, 1993) and rice (Kamisugi *et al.*, 1994).

In plants, the 18S, 5.8S, and 26S rRNA genes (45S rDNA) are organized in tandem arrays within the nuclear organizer regions (NORs). Each rDNA repeated unit includes a non-transcribed region, a transcribed spacer, and the coding regions for the three rRNAs and two internally situated transcribed spacers that flank the 5.8S rRNA gene (Gründler *et al.*, 1991; Wanzenböck *et al.*, 1997). In most higher eukaryotes, the 5S ribosomal RNA genes (5S rDNA) are arranged in tandem arrays at one or more chromosomal loci and mostly separated from the 45S rDNA (Fedoroff, 1979). The 5S rDNA consists of a coding sequence of approximately 120 bp and a non-transcribed spacer of several hundred bp. The coding sequences are highly conserved across a broad taxonomic range. This led to the expectation that coding sequences were valuable for phylogenetic analysis at higher taxonomic ranks. Because the 5S and the 45S rDNA loci are often different in number but are not strictly linked (Lapitan, 1992; Phillips *et al.*, 1988), they have been used as excellent markers for identifying chromosomes (Fuchs *et al.*, 1998; Schrader *et al.*, 1997).

The aim of this study is to find out the differences between two *Atractylodes* species, *A. japonica* and *A. macrocephala*, using molecular cytogenetic techniques.

MATERIALS AND METHODS

Plant materials

The medicinal plants, *A. japonica* and *A. macrocephala*, were used for cytogenetic analysis. *A. japonica* was collected from Gapyong, Gyeonggi province, Korea and *A. macrocephala* from China. The plant materials were cultivated in National Crop Experiment Station, Suwon, Gyeonggi Province, Korea from 2001 to 2002. Three-row plots with 22 plants per row were planted by 30 ㎝ × 20 ㎝ distance in a completely randomized block design. The total amounts of applied fertilizer were 15 ㎏ urea, 20 ㎏ fused phosphate, 5 ㎏ potassium chloride and 1000 ㎏ compost per 10a, respectively, as a basal dressing.

Karyotype analysis by feulgen staining

The seeds were germinated in petri-dishes at 25℃ for 10 days. The root tips of the plants were used for the chromosome analysis. Actively growing root tips were excised when they were about 1 cm in length and pretreated with distilled water at 4℃ for 12 hours. The treated root tips were then fixed in an acetic acid: ethanol (1:3, v/v) solution for one to two days. The samples were then hydrolyzed in 1N HCl for five minutes at 60℃. Afterward, squash mounts were prepared with feulgen. For a karyotype analysis, the chromosomes were classified according to the nomenclature of Levan *et al.* (1964).

Chromosome preparation

Fixed root tips were washed thoroughly in distilled water prior to the enzymatic treatment. The meristematic portion of the root tips was treated with the enzyme mixture (5% cellulase, 1% pectolyse, 1 mM EDTA, pH 4.5) at 37℃ for one hour for degradation of cell wall. After rinsing, the root tips were tapped carefully with the tip of a forceps on a glass slide with a few drops of the fixative, and then air-dried.

Probe preparation

The 17S rDNA, a 450 bp fragment of the 45S rDNA repeat, was obtained from genomic DNA of cucumber (Koo *et al.*, 2002). 5S rDNA probe was

obtained by PCR using total genomic DNA of *C. sativus* as a template DNA (Koo *et al.*, 2002). To label probes (5S and 17S rDNAs), PCR amplification was carried using Ex *Taq* poymerase (Takara) with 50% of dTTP replaced with digoxygenin-11-dUTP or biotin-16-dUTP (Boehringer Mannheim).

Fluorescence *in situ* hybridization

FISH was performed according to the method in Koo *et al.* (2002). Slides were immersed in 10 ㎎/㎖ RNase in 2x SSC for at 37℃ for 60 minutes and then washed in 2x SSC. Chromosomal DNA on the slides was denatured in 70% formamide at 70℃ for two minutes, and then dehydrated in a 70%, 85%, 95% and 100% ethanol series at −20℃ for three minutes each. The probe mixture contained 50% formamide (w/v), 10% dextran sulfate (w/v), 5 ng/㎕ salmon sperm DNA and 500 ng/㎖ of each probe DNA. For denaturing the probe, the mixture was heated at 90℃ for 10 minutes and kept on ice for five minutes. Seventy microliters of the probe mixture was applied to each denatured preparation and covered with glass cover-slip. The slides were then placed in a humid chamber at 37℃ for 18 hours. The probes were detected with avidin-FITC and anti-digoxigenin Cy3 (Roche). Chromosomes were counterstained with 1 ㎍/㎖ DAPI (Sigma). The signals were detected with a Cooled CCD camera (CoolSNAP, Photometrics). Images were recorded by software (Meta imaging series TM 4.6) using Leica epi-fluorescence microscope equipped with FITC-DAPI two-way or FITC-Rhodamine-DAPI three-way filter sets (Leica, Japan).

RESULTS AND DISCUSSION

Karyotype analysis by feulgen staining

The karyotype analysis has played an important role in the identification and designation of chromosomes in many plant species. However karyotype analysis for medicinal plants including *Atractylodes* species is rare. The results of karyotype analysis of *A. japonica* and *A. macrocephala* by feulgen staining are shown in Fig. 1.

Somatic chromosome numbers for *A. japonica* and *A. macrocephala* were 2n=24 (Fig. 1). There were chromosomal differences in karyotype analysis, especially on chromosome 4, 6, 7 and 12. The differences were measured and summarized in Table 1. The relative length (r) of the long arm (l) and short arm (s) is shown by the arm ratio (r = l/s). Based on arm ratio, Levan *et al.* (1983) grouped chromosomes in six categories; M (metacentric), m (metacentric), sm (submetacentric), st (subtelocentric), t (acrocentric) and T (telocentric).

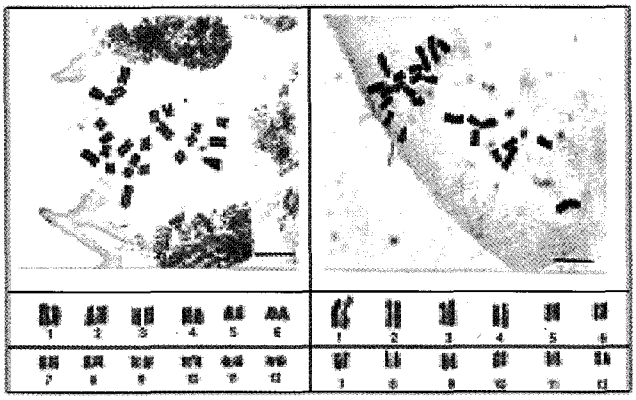

Fig. 1. Chromosome complement and karyotype of *A. japonica* (A) and *A. macrocephala* (B) (2n=24). The chromosomes were classified according to the nomenclature of Levan *et al.* (1964).

The length of the mitotic metaphase chromosomes of *A. japonica* ranged from 0.70 to 1.60 ㎛ with a total length of 12.11 ㎛ and the homologous chromosome complement comprised six metacentrics (chromosomes 1, 5, 7, 8, 9 and 10), five submetacentrics (chromosomes 2, 3, 6, 11 and 12) and one subtelocentrics (chromosomes 4). On the other hand, the length of the mitotic metaphase chromosomes of *A. macrocephala* ranged from 0.90 to 2.35 ㎛ with a total length of 16.58 ㎛ and the homologous chromosome complement comprised seven metacentrics (chromosomes 1, 5, 6, 8, 9, 10 and 12) and five submetacentrics (chromosomes 2, 3, 4, 7 and 11) (Table 1).

The total length of *A. japonica* chromosomes was shorter than that of *A. macrocephala*, but *A. japonica* had one subtelocentrics (chromosomes 4) different from *A. macrocephala* chromosomes.

Table 1. Analysis of somatic metaphase chromosomes of *A. japonica* and *A. macrocephala*.

Chromosome No.	Chromosome size (㎛)						Arm ratio (L/S)		Centromeric index	
	Long arm		Short arm		Total length					
	Aj	*Am*	*Aj*	*Am*	*Aj*	*Am*	*Aj*	*Am*	*Aj*	*Am*
1	1.00	1.35	0.60	1.00	1.60	2.35	1.67	1.35	m	m
2	0.85	1.20	0.45	0.70	1.30	1.90	1.89	1.71	sm	sm
3	0.80	1.25	0.45	0.55	1.25	1.80	1.78	2.27	sm	sm
4	0.87	1.25	0.25	0.45	1.12	1.70	3.48	2.78	st	sm
5	0.65	0.75	0.40	0.55	1.05	1.30	1.63	1.36	m	m
6	0.70	0.65	0.26	0.55	0.96	1.20	2.69	1.18	sm	m
7	0.50	0.75	0.43	0.40	0.93	1.15	1.16	1.88	m	sm
8	0.45	0.70	0.40	0.45	0.85	1.15	1.13	1.56	m	m
9	0.50	0.60	0.30	0.50	0.80	1.10	1.67	1.20	m	m
10	0.45	0.60	0.35	0.48	0.80	1.08	1.29	1.25	m	m
11	0.50	0.60	0.25	0.35	0.75	0.95	2.00	1.71	sm	sm
12	0.45	0.50	0.25	0.40	0.70	0.90	1.80	1.25	sm	m

m, metacentric; sm, submetacentric; st, subtelocentric
Aj, *A. japonica*; *Am*, *A. macrocephala*.

Physical mapping of 17S and 5S rDNAs using FISH

Locations of the rDNAs on the metaphase chromosomes were examined by multicolor FISH with the 17S and 5S rDNAs. Fig. 2 shows the location of 17S and 5S rDNA. The signals for 17S rDNA of *A. japonica* and *A. macrocephala* were equally detected on the telomeric regions of short arm in chromosomes four and five. Also, the signal for 5S rDNA was detected in the short arm of chromosome one. The 45S rDNA multigene families are located in a chromosomal nucleolus organizing region (NORs), which is cytologically visible as a secondary constriction that is associated with a nucleolus and distal satellite.

Although rDNA loci on chromosomes have been used as a chromosomal landmarker for phylogenetic analysis in many plant species, FISH results with 17S and 5S rDNAs could not confer any difference (Fig. 2). These results may suggest that FISH application depends on the type of species. Cuéller *et al.* (1999) reported that the results of physical mapping of the 5S rDNA and 45S rDNA showed the very close resemblance between *Helianthus annuus* (2n=34) and *H. argophyllus* (2n=34) at the chromosomal level and the need to search for suitable non-chromosomal markers. On the other hand, Sang and Liang (2000) reported that the position of rDNA loci varied from the interstitial to terminal region among the four accessions of the three *Sorghum* species; *S. bicolor* (2n=20), *S. halepense* (2n=40) and *S. versicolor* (2n=10) and the rDNA data were useful in investigating chromosome evolution and phylogeny.

Consequently, the result of karyotype study of both

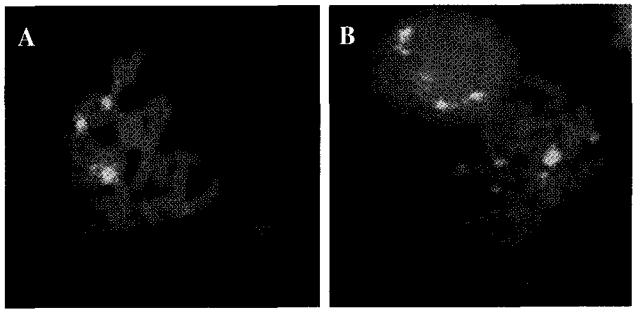

Fig. 2. Bicolor FISH pattern on the metaphase chromosomes of *A. japonica* (A) and *A. macrocephala* (B) using both 17S and 5S rDNA probes. Digoxigenen-labeled 17S rDNA probes was detected with antidigoxigenin rhodamine conjugate (red). Biotin-labeled 5S rDNA probe was detected with avidin-FITC conjugate (green).

A. japonica and *A. macrocephala* (Fig. 1 and Table 1) showed a little difference, implying that the cytogenetic study can be applied to differentiate relative species.

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