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Comparative cytological study of *Kirengeshoma koreana* and *K. palmata* (Hydrangeaceae)

Ha Yeon CHOI [1,2], Hye Rin KIM[1] and Kweon HEO[1]*

[1]*Department of Applied Plant Science, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 24341, Korea*
[2]*Department of Medical Science, College of Medicine, Yonsei University, Seoul 03722, Korea*

Corresponding author
Kweon HEO
E-mail: laurus@kangwon.ac.kr

ABSTRACT: Chromosome studies of *Kirengeshoma palmata*, a second-level endangered and rare species in China and Japan, have been creating misconceptions among researchers due to varying reports of $2n = 52$ and $2n = 54$ in relation to this species. Moreover, the karyotype of *K. palmata* has not been explained clearly thus far. Therefore, in this study, we aimed to confirm the exact number of chromosomes in *K. koreana* and *K. palmata* by securing this plant from Mt. Jirisan and Mt. Baekunsan on the Korean Peninsula and from Kochi and Miyazaki prefectures in Japan. In particular, enzyme digestion and scanning electron microscopy were utilized to determine most accurately the number of chromosomes for *Kirengeshoma*. Fluorescence *in situ* hybridization and flow cytometry were also undertaken to confirm the presence of polyploidy. The results of this study indicate that the controversial earlier finding of $2n = 52$ chromosomes in *Kirengeshoma* is indeed accurate. The findings here also show that the total chromosome length ranges from 40.37 to 46.90 μm in *K. koreana* and from 42.12 to 48.32 μm in *K. palmata.* The average total chromosome length ranged from 43.52 ± 2.37 in *K. koreana* and 45.07 ± 2.58 μm in *K. palmata*. However, secondary constrictions were not present in the two species of *Kirengeshoma*. In the fluorescence *in situ* hybridization study, 5S and 45S signals were detected in one pair each in *K. koreana* and *K. palmata*, and *K. koreana* according to flow cytometry results also showed diploid peaks. Therefore, the previously reported claim that *K. palmata* was auto-tetraploid is not supported. Rather, the results here confirm that the two species of *Kirengeshoma* are diploid, supporting earlier results from a recent study of population genetics. Our results also strongly support the contention that *Kirengeshoma* is closely related to the genus.

KEYWORDS: cytology, flow cytometry, fluorescence *in situ* hybridization, *Kirengeshoma*, polyploidy

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INTRODUCTION

The *Kirengeshoma* Yatabe is a genus of Hydrangeaceae containing two species, *K. koreana* Nakai and *K. palmata* Yatabe. Both species are perennial plants in China, Korea, and Japan, with palm-shaped leaves, slender stems, and yellow bell-shaped beautiful flowers (Yatabe, 1890; Hwang and Wei, 2003; Lee, 2003; Qiu et al., 2009). These plants grow mainly in shady and humid environments. The reproductive characteristics of *Kirengeshoma* include the features: 5 petals and 15 stamens having different lengths. Flowers of *Kirengeshoma* bloom from July to August at the tip of its stem (Lee, 2003). To date, the native population of *Kirengeshoma* has been reported in four regions of eastern China (Zhang et al., 2006; Qiu et al., 2009) and three regions of Japan, including Chugoku, Shikoku, and Kyushu (Murata, 1986; Iwatsuki et al., 2001). In Korea, *Kirengeshoma* is prevalent only in Mts. Baekunsan and Jirisan in the southern provinces, and is protected as a critically endangered species by the Ministry of Environment (Korea National Arboretum , 2021). In Japan, it is also native to the northwest of Chugoku in Honshu, Shikoku, and Kyushu, which are attractive tourist sites with them growing thousands of plants under the Cedar Forest in private botanical gardens (Murata, 1986; Iwatsuki et al., 2001). Meanwhile, *Kirengeshoma* is prevalent at an altitude of 800 m or higher in Anhui and Zhejiang provinces in the eastern

region of the Yangtze River in China and is protected as an endangered species across the nation as per the Chinese Plant Red Data Book (Fu, 1992; Hwang and Bartholomew, 2001).

Kirengeshoma was first treated as a new genus taxonomically by Yatabe (1890). Later, Nakai treated the collected specimens of *Kirengeshoma* from Korea as a new species, *K. koreana*. Therefore, Chinese and Japanese *Kirengeshoma* were termed as *K. palmata*, and *Kirengeshoma* prevalent in Korean peninsula was called *K. koreana* (Nakai, 1935). However, recently, botanists have also reported views suggesting integration of *K. koreana* into *K. palmata* (Hwang and Wei, 2003; Ko, 2007).

Meanwhile, a cytological study is very useful in all fields of plant sciences, such as genetics, taxonomy, breeding, and species identification of genetic resources. Notably, with cytology, it is possible to check the number of chromosomes, perform karyotype analysis, check polyploidy status, identify chromosomal composition pattern, and confirm the presence of satellite chromosomes in a species (Abraham and Prasad, 1983; Kim, 1992). In particular, when a species cannot be identified due to unclear morphological characteristics, it can be very efficiently identified based on chromosome karyotyping using molecular cytology as in fluorescence *in situ* hybridization (FISH) and genomic *in situ* hybridization (Bennett et al., 1992; Jiang and Gill, 2006; Markova et al., 2007; Han et al., 2013). However, cytological studies of the relatively endangered species of *Kirengeshoma* in the literature are very limited in number and scope. Until now, cytological studies of *K. palmata* have been conducted by Hamel (1951) who reported that the chromosome number is $2n = 52$, and provided a pictorial evidence for *Kirengeshoma* to be autopolyploid, but it can be said to be a result of lack of objectivity. Afterwards, Funamoto and Nakamura (1989)

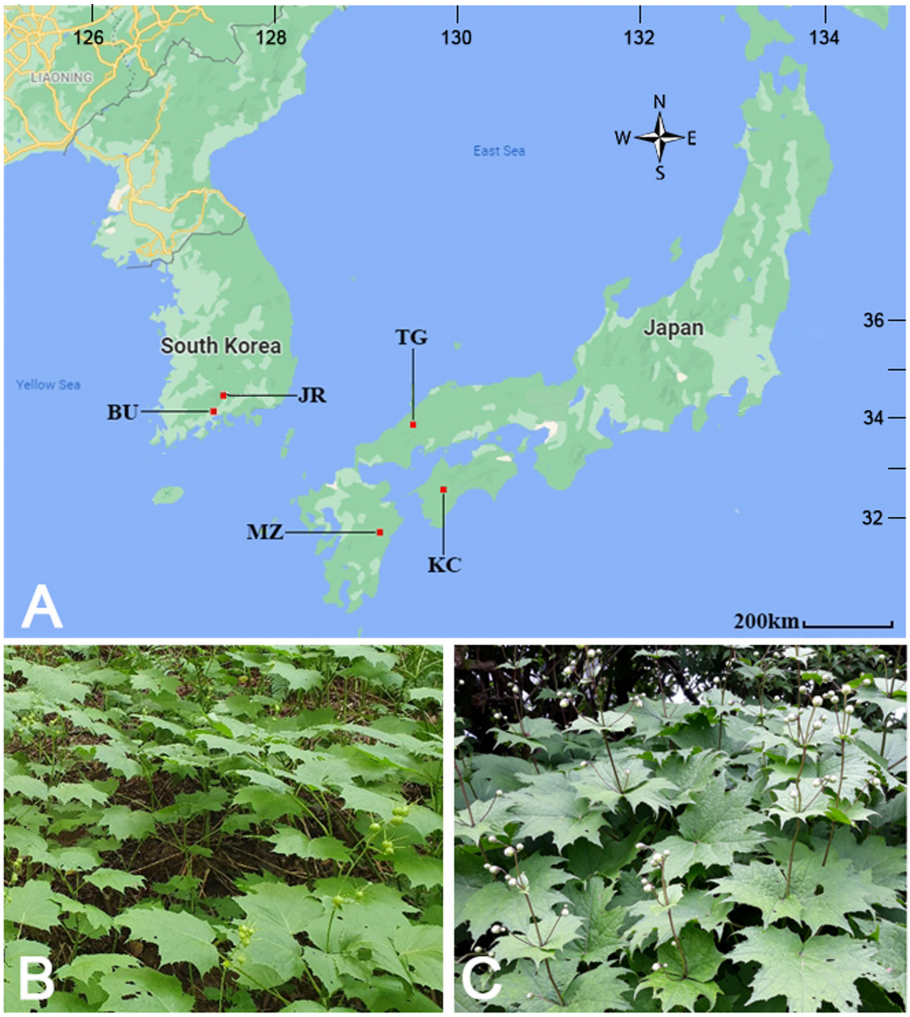

Fig. 1. The distribution map (**A**) of *Kirengeshoma koreana* (**B**) and *K. palmata* (**C**) populations used in this study. BU, Mt. Baekunsan; JR, Mt. Jirisan; KC, Kochi, Hyakuichi Bot. Garden of Japan; MZ, Miyazaki prefecture of Japan; TG, collection site by Funamoto and Nakamura, 1989.

Table 1. Collection information of plant materials used in this study.

Taxa	Collection information
K. koreana Nakai	KOREA. Jeollanam-do, Gwangyang, Mt. Backunsan, elev. 1,100 m, 22 Aug 2017, *K. Heo 201752* (KWNU)
K. koreana Nakai	KOREA. Gyongsangnam-do, Sancheong, Mt. Jirisan, elev. 1,050 m, 23 Aug 2017, *K. Heo 201755* (KWNU)
K. palmata Yatabe	JAPAN. Kochi Pref. Yusuhara, Purchased from Hyakuichi Garden, 29 Jul 2019, *K. Heo s.n.* (KWNU)
K. palmata Yatabe	JAPAN. Hyogo Pref. Kobe, Rokko Alpine Botanical Garden, 4 Dec 2017, *S. Mitsuyama s.n.* (KWNU). Original plants were collected from Miyazaki Prefecture and planted at Rokko Botanical Garden.

reported that the chromosome number of *K. palmata* was 2*n* = 54 in native Japanese population from the Togouchi site of Hiroshima prefecture. In this regard, even the Chromosome Counts Database (CCDB) remains unresolved in case of *K. palmata*, and these inaccurate results have caused controversy in its cytological study. Therefore, this study was conducted to derive accurate results using squash, enzyme digestion, DAPI (4',6-diamidino-2-phenylindole) staining, and FISH technique on Korean and Japanese *Kirengeshoma* plant materials to confirm the correct chromosome number and polyploidy status of *K. koreana* and *K. palmata*.

MATERIALS AND METHODS

Plant materials

Taxonomically, *Kirengeshoma* has been commonly known as *K. koreana* Nakai in Korea and *K. palmata* Yatabe in China and Japan. In this study, *Kirengeshoma* plants were obtained from Mts. Baekunsan and Jirisan in Korea, and from Kochi and Miyazaki prefectures in Japan (Fig. 1, Table 1). Unfortunately, Chinese population of *Kirengeshoma* was not considered in this study.

Preparation of chromosome slides

For the observation of chromosomes, root tips of young *Kirengeshoma* plants grown for about a month were taken, soaked in 2 mM 8-hydroxyquinoline, and pre-treated for 6 h. Subsequently, the root tips were fixed in Farmer's fluid (Sass, 1951) for 24 h, and long-term stored in a refrigerator with 70% ethanol until used.

The fixed root tips were washed in distilled water and treated in 4% enzyme mixture at 37°C for 75 min. The enzyme mixture was then removed, 80 µL of ethanol and acetic acid 3:1 solution was added, and the resulting solution was vortexed and centrifuged. Then the supernatant of the solution was discarded, 60 µL of acetic acid and ethanol 9:1 solution was added to the remaining solution, and the root tips were broken with round tip needle. The obtained solution was then dispensed on a glass slide in a water bath, and the glass slide was dried and stained with DAPI to identify the chromosome.

Additionally, to confirm the exact number of chromosomes, the enzyme-treated slides were washed with absolute ethanol, coated with ion-coater, and observed under a scanning electron microscope at 3 kV (Supra 55VP FE SEM, Zeiss Co., Oberkochen, Germany).

Fluorescence *in situ* hybridization (FISH)

To confirm the polyploidy in *Kirengeshoma*, flow cytometry and FISH were conducted on it. The 5S rDNA was labeled with biotin-16-dUTP (Roche, Mannheim, Germany) by nick translation and detected with streptavidin/avidin-Cy3. 45S rDNA was labeled with digoxigenin-11-dUTP (Roche) and detected with anti-dig-fluorescein isothiocyanate (FITC). All polymerase chain reaction (PCR) amplifications were carried out in a volume of 20 µL containing 2 µL of 10× Ex Taq buffer, 2 µL of dNTP, 10 pmol of each primer, 0.2 µL of Ex Taq polymerase, and 3 µL of DNA template. Amplifications of a specific region were performed under the following reaction condition: initial denaturation at 95°C for 5 min, 30 cycles of 30 s at 95°C, 30 s at 43°C (for 5S) and 30 s at 63°C (for 45S), 30 s at 72°C, and terminal extension at 72°C for 10 min. After the 5S and 45S PCR products were labeled by nick translation, each product was incubated at 15.6°C in PCR for 1 h. Finally, 0.5 M EDTA was added, and the resultant was incubated at 65°C for 10 min. On completion of this procedure, to do hybridization, the hybridization mixture was denatured at 90°C for 10 min and directly placed on ice for 5 min. Then, the mixture was added to 24 × 50 mm coverslips and inversely covered the glass slides and was denatured at 80°C for 5 min on a slide warmer. Finally, hybridization was carried out in a humid chamber at 37°C for 16–20 h. After hybridization, the glass slides were incubated in 20% Formamide at 42°C for 10 min, then transferred to a 1× detection buffer at room temperature for 10 min. Biotin-labeled 5S rDNA and digoxigenin-labeled 45S rDNA were detected with Cy3-conjugated streptavidin and anti-digoxigenin-FITC at 37°C for 1 h. Subsequently, the glass slides were washed three times for 5 min in 1× detection buffer. The glass slides were dehydrated in ethanol series at room temperature for 3 min and air-dried in the dark. Then,

the slides were counter-stained with vecta-shield contained DAPI and observed under a fluorescent microscope (Axio image II, Zeiss Co.).

Karyotype analysis

Karyotype analysis of *K. koreana* was conducted using the Zen 3.0 program (Zeiss Co.) and edited with Adobe Photoshop CS6. Since the chromosome size of *Kirengeshoma* is very small, it was difficult to distinguish between the short and the long arms. Therefore, chromosome length was calculated by measuring the total length. Notably, the two well-spread cells from each site were selected and measured. For statistical analysis, the standard deviation was calculated from the mean value of the well-spread metaphase cells.

Flow cytometry

The fresh young leaves of the *K. koreana* were cut to 3 × 3 cm and placed in a petri dish for flow cytometry. Then, 500 µL of cold lysis buffer was added to the petri dish, and the leaves were chopped with a razor. The chopped leaves were rotated in the lysis buffer for 20–30 seconds. The resulting solution was filtered using a filter suspension and kept on ice. Then, 140 µL of the filtered liquid was put into a new 1.5 mL amber tube, and 1 µL of RNase A (1–10 mg/mL) was added to the liquid. The solution after the addition was incubated at 37°C for 30 min. Finally, 350 µL of PI staining solution was added to the solution, the obtained solution is incubated at room temperature for at least 1 h, and then the peak position was checked using a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA, USA.).

RESULTS

Somatic chromosome number

In this study, the number of chromosomes of *K. koreana* and *K. palmata* were observed in more than 10 cells by enzyme digestion and DAPI staining. Finally, two well-spread cells of each site were selected and calculated. In Fig. 2, A to D represent Korean samples, and E to H represent Japanese samples. In squash, it was impossible to count the accurate chromosome number because of the small chromosome size of *Kirengeshoma*. When chromosomes were observed by DAPI staining after enzyme treatment, the number of

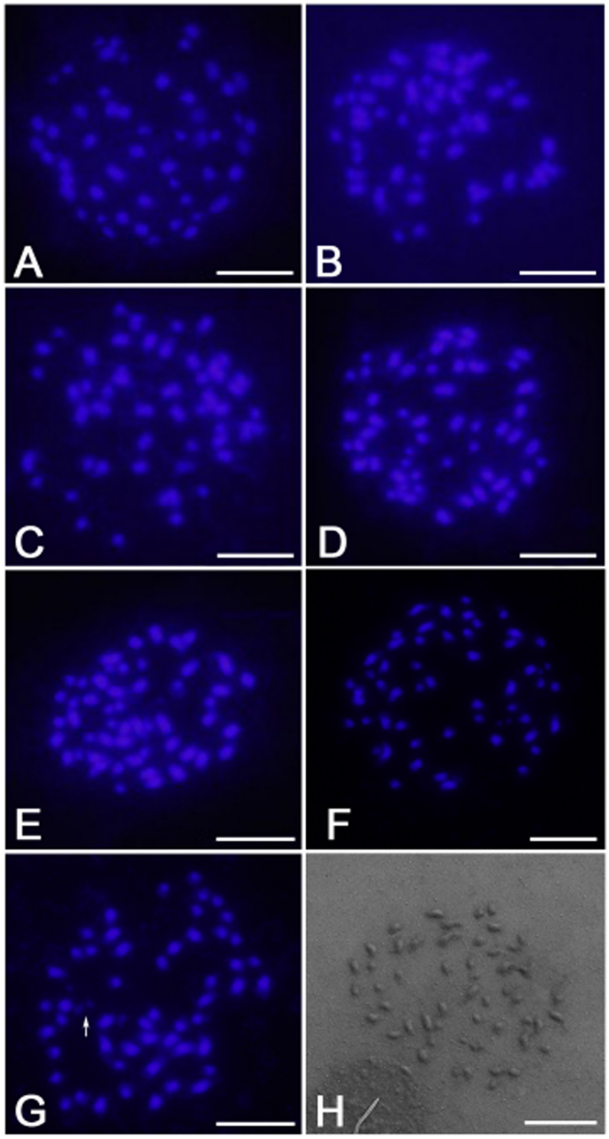

Fig. 2. The somatic chromosomes of *Kirengeshoma koreana* (**A–D**) and *K. palmata* (**E–H**) observed by enzyme digestion methods. **A, B.** Baekunsan. **C, D.** Jirisan. **E, F.** Kochi prefecture. **G, H.** Miyazaki prefecture. Scale bars = 10 µm.

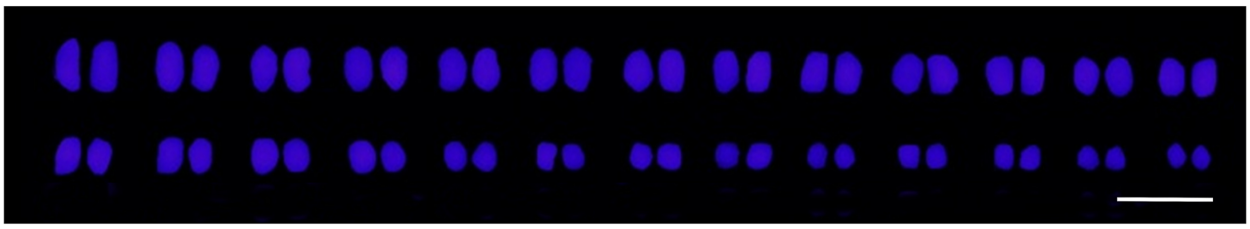

Fig. 3. The karyotype of *Kirengeshoma koreana* from Backunsan ($2n = 52$). Scale bar = 5 µm.

chromosomes was clearly determined to be $2n = 52$ (Fig. 2). Notably, all cells appeared to be with $2n = 52$. Very rarely, there was $2n = 56$ appearing as $2n = 52$ plus four faint small points (Fig. 2G arrow). However, we could not confirm whether these points were normal chromosomes, B chromosomes, or fragments of autosomes. Therefore, these four small points were not included into the measured values (Table 2). In essence, this situation is a subject for further discussion. Additionally, we performed scanning electron microscopic observations on *K. palmata* to confirm its exact chromosome number. These observations also confirmed that $2n = 52$ (Fig. 2H). Meanwhile, the chromosomes were very small that it was difficult to perform karyotype analysis, but it was possible to measure the total chromosome length by pairing homologous chromosomes (Fig. 3, Table 2). Therefore, the longest chromosome was 3.08 μm and the shortest chromosome was 0.89 μm. The average size of the chromosome ranged from 2.64 to 1.01 μm. In particular, there were no secondary constrictions at all cells as reported in previous studies (Hamel, 1951; Funamoto and Nakamura, 1989) (Fig. 3, Table 2).

Table 2. Chromosome total length and standard deviation of *Kirengeshoma koreana* and *K. palmata,* collected from Korea and Japan populations.

Chromosome number	*K. koreana* (μm)					*K. palmata* (μm)				
	BU 1	BU 2	JR 1	JR 2	Mean ± SD	KC 1	KC 2	MZ 1	MZ 2	Mean ± SD
1	2.34	2.62	2.53	2.59	2.52 ± 0.11	3.04	3.08	2.33	2.55	2.75 ± 0.32
2	1.97	2.28	2.28	2.51	2.26 ± 0.19	2.70	2.71	2.29	2.38	2.52 ± 0.19
3	1.94	2.24	2.17	2.40	2.19 ± 0.17	2.49	2.58	2.21	2.27	2.39 ± 0.15
4	1.85	2.16	2.12	2.35	2.12 ± 0.18	2.29	2.41	2.10	2.15	2.24 ± 0.12
5	1.78	2.05	2.09	2.29	2.05 ± 0.18	2.25	2.35	2.07	2.10	2.19 ± 0.11
6	1.77	2.01	2.06	2.21	2.01 ± 0.16	2.22	2.28	2.04	2.05	2.15 ± 0.10
7	1.76	1.99	2.03	2.14	1.98 ± 0.14	2.19	2.24	1.98	2.02	2.11 ± 0.11
8	1.74	1.93	1.99	2.10	1.94 ± 0.13	2.16	2.21	1.95	1.99	2.08 ± 0.11
9	1.71	1.91	1.97	2.06	1.91 ± 0.13	2.12	2.13	1.92	1.89	2.02 ± 0.11
10	1.68	1.88	1.90	2.03	1.87 ± 0.13	2.07	2.08	1.87	1.85	1.97 ± 0.11
11	1.66	1.86	1.85	1.99	1.84 ± 0.12	2.03	2.02	1.78	1.77	1.90 ± 0.13
12	1.63	1.79	1.85	1.91	1.80 ± 0.10	1.97	1.93	1.75	1.74	1.85 ± 0.10
13	1.58	1.71	1.79	1.87	1.74 ± 0.11	1.85	1.88	1.67	1.69	1.77 ± 0.09
14	1.53	1.65	1.76	1.82	1.69 ± 0.11	1.80	1.79	1.62	1.66	1.72 ± 0.08
15	1.53	1.62	1.70	1.72	1.64 ± 0.07	1.73	1.71	1.57	1.58	1.65 ± 0.07
16	1.49	1.56	1.64	1.66	1.59 ± 0.07	1.69	1.67	1.52	1.54	1.61 ± 0.08
17	1.46	1.43	1.52	1.61	1.51 ± 0.07	1.56	1.59	1.45	1.50	1.53 ± 0.05
18	1.41	1.35	1.41	1.42	1.40 ± 0.03	1.43	1.47	1.32	1.35	1.39 ± 0.06
19	1.34	1.22	1.28	1.40	1.31 ± 0.07	1.31	1.41	1.28	1.27	1.32 ± 0.06
20	1.29	1.19	1.25	1.37	1.28 ± 0.07	1.26	1.36	1.23	1.25	1.28 ± 0.05
21	1.22	1.12	1.22	1.34	1.23 ± 0.08	1.23	1.35	1.19	1.18	1.24 ± 0.07
22	1.16	1.09	1.20	1.32	1.19 ± 0.08	1.18	1.33	1.12	1.13	1.19 ± 0.08
23	1.15	1.07	1.19	1.27	1.17 ± 0.07	1.14	1.27	1.05	1.08	1.14 ± 0.08
24	1.14	1.05	1.17	1.26	1.16 ± 0.08	1.08	1.21	0.98	1.05	1.08 ± 0.08
25	1.13	0.99	1.12	1.19	1.11 ± 0.07	1.03	1.18	0.94	1.00	1.04 ± 0.09
26	1.11	0.91	1.05	1.07	1.04 ± 0.08	1.02	1.08	0.89	0.95	0.99 ± 0.07
Total length	40.37	42.68	44.14	46.90	43.52 ± 2.37	46.84	48.32	42.12	42.99	45.07 ± 2.58

BU, Mt. Baekunsan of Korea; JR, Mt. Jirisan of Korea; SD, standard deviation; KC, Hyakuichi Botanical Garden of Japan; MZ, Rokko Alpine Garden of Japan.

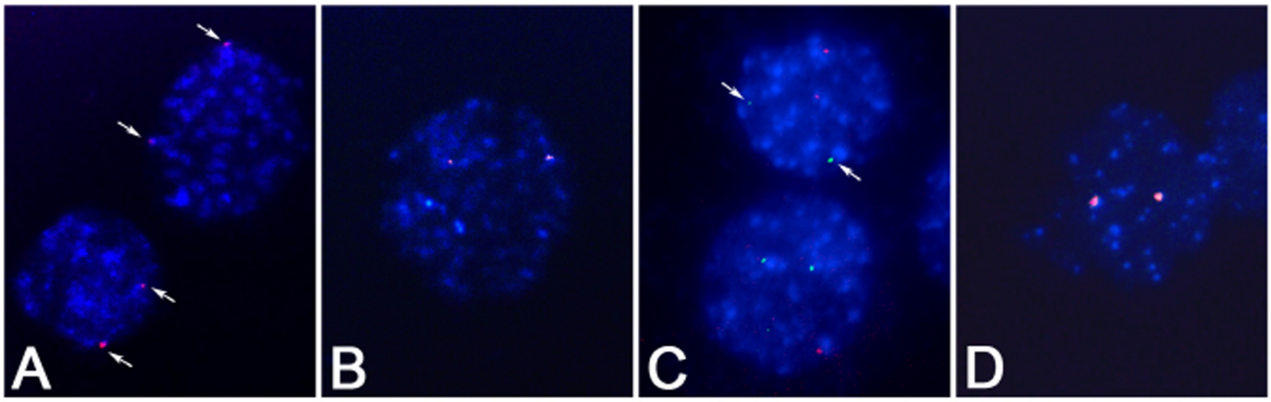

Fig. 4. Fluorescence *in situ* hybridization patterns of the prophase chromosomes of *Kirengeshoma koreana* from Mt. Backunsan (**A**), Mt. Jirisan (**B**) and *K. palmata* from Kochi Hyakuichi Bot. Garden (**C**), Miyazaki prefecture (**D**) using rDNA probes. Arrows indicate 5S red signal (**A**) and 45S green signal (**C**). **A** to **D** were taken at X1,000 magnification.

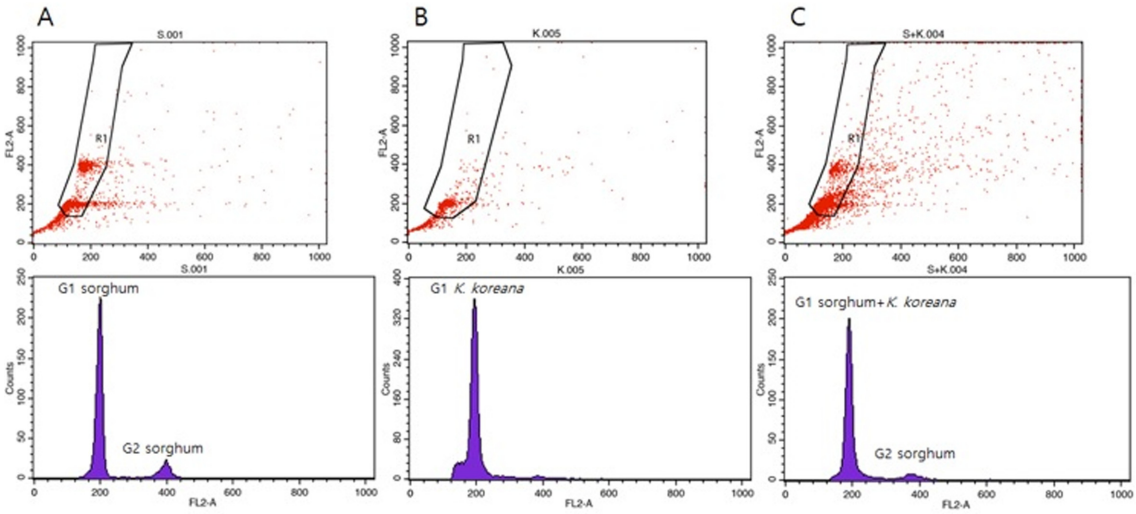

Fig. 5. Flow cytometry histogram of *Kirengeshoma koreana*. **A.** *Sorghum bicolor*. **B.** *K. koreana*. **C.** *Sorghum bicolor* + *K. koreana.*

Fluorescence *in situ* hybridization

To estimate the signals of 45S rDNA and 5S rDNA in *Kirengeshoma*, FISH technique was followed. The results of the estimation showed that 45S rDNA signal (green) was found in one pair of homologous chromosomes (Fig. 4C) and 5S rDNA signal (red) was observed in one pair of homologous chromosomes. In particular, the results of the experiment focusing on the 5S signal showed that a pair of 5S signals appeared in both the Korean and Japanese populations of *Kirengeshoma* (Fig. 4A, B, D).

Ploidy analysis

Sorghum bicolor ($2n = 2x = 20$) was used as the control plant for the accurate ploidy level determination in *Kirengeshoma koreana*. Notably, the flow cytometry histograms of PI-stained nuclei revealed distinct peaks. The first two peaks represented the G1 and G2 peaks of *Sorghum* (Fig. 5A). Also, the G1 nuclei peak of *K. koreana* observed under the same conditions was the same as the peak of *Sorghum* (Fig. 5B), and the peak positions of *Sorghum* and *K. koreana* were together (Fig. 5C). Finally, the peak positions of *S. bicolor* and *K. koreana* were the same. As a result, *K. koreana* was also confirmed to be a diploid (Fig. 5A–C).

DISCUSSION

Generally, each plant species has its own chromosomal characteristics, such as the number, size, and shape of each chromosome, which is useful in identifying the species, as well as in estimating the tendency of evolution of the plant (Kim, 1992). In this study, the chromosome number and total chromosome length of the native Korean and Japanese

populations of *Kirengeshoma* were measured based on DAPI staining and FISH technique. The chromosome studies of *K. palmata* were previously reported by Hamel (1951) and Funamoto and Nakamura (1989).

Hamel (1951) suggested that the chromosome number of *K. palmata* is $2n = 4x = 52$, having two pairs of 1.7 μm, eight pairs of 1.2 μm, six pairs of 1.1 μm, two pairs of 1.0 μm, four pairs of 0.9 μm, and four pairs of 0.8 μm with drawings of the chromosomes. This means that *K. palmata* has metacentric, sub-metacentric, and telocentric chromosomes. Furthermore, Funamoto and Nakamura (1989) have also studied the chromosomes of *K. palmata*. The plant materials for their study seemed to have been taken from the Togouchi village of Hiroshima prefecture of Honshu. They argued that the number of chromosomes in *K. palmata* was $2n = 54$, a result that contradicts the results of Hamel (1951). In addition, they supported their claim with photographs of the chromosome, but the findings did not suggest a definite karyotype, polyploidy, and any of B chromosomes, chromosome breakage, or aneuploidy issues.

In this study, although it was not possible to investigate the Chinese population of *Kirengeshoma palmata*, the results suggested that $2n = 52$ individuals were identified and dominant among the individuals of *K. koreana* and Japanese populations of *K. palmata* considered. In addition, very rare individuals were identified to have $2n = 54$ or 56 with faint small points in the Japanese population, *K. palmata* (Fig. 2G). In this case, it can be assumed that the $2n = 56$ individual is the result of a $2n = 52$ individual's due to B chromosome occurring (Chen et al., 2022), chromosome breakage, or aneuploidy. B chromosome is often observed in angiosperms, and there are cases where individuals with and without B chromosome exist even within the same species of Asteraceae (Houben et al., 1999). Generally, B chromosomes are conspicuously smaller than the smallest autosomes (Peppers et al., 1997; Douglas and Birchler, 2017). In addition, small B chromosomes exhibit a tendency to be mitotically unstable and thus may vary in number from cell to cell within the same individual (Camacho, 2022).

As for the ploidy of *Kirengeshoma palmata*, it was previously reported as $2n = 52$ (Hamel, 1951) and as an auto-tetraploid plant with a base chromosome number of $n = 13$. However, the cytogenetic evidence to support this is unclear and lacks information. Also, Funamoto and Nakamura (1989) reported the chromosome number of *K. palmata* to be $2n = 54$, which is a number that cannot be agreed upon as tetraploid as mentioned by Hamel (1951). However, Yuan et al. (2014) investigated the gene flow and drift on the six populations genetic structure of the endangered *K. palmata* from East China and South Japan. In their study, they showed that 12 loci of the six populations had no more than two alleles per locus. The study also indicated that the genome of *K. palmata* was structured as a diploid rather than as a polyploidy through gene silencing or genomic rearrangements (Wolfe, 2001; Levin, 2002). Microsatellite loci in such ancient polyploidy but diploidization species can be treated as co-dominant diploid markers (Ashley et al., 2003). Also, molecular cytogenetics using 5S or 45S rDNA is useful for species identification and polyploidy determination (Specht et al., 1990; Han et al., 2013). In our FISH results, 45S rDNA was observed in one pair of chromosomes, and 5S rDNA was found to appear in a pair of chromosomes. When focusing only the 5S rDNA signals, it appeared at one pair of examined cells of *K. koreana* and *K. palmata* (Fig. 4). This result suggested that *K. koreana* and *K. palmata* are a diploid rather than a tetraploid. Also, when *Sorghum bicolor* and *K. koreana* were compared by flow cytometry, *K. koreana* showed equivalent peak position under the same conditions as *Sorghum bicolor*. This indicates that *K. koreana* is not a tetraploid but a diploid like *Sorghum bicolor*.

In phylogenetic tree, Hydrangeaceae has been divided into two tribes, Hydrangeae and Philadelpheae (Hufford, 2004). *Kirengeshoma* is placed in tribe Philadelpheae with the closely related genus *Deutzia* (Hufford et al., 2001). *Deutzia* has polyploidy in chromosome number, $n = 13$, 26, 39, 52, 65, 78, and 91 (Chatterjee et al., 1989; Hembree et al., 2020). Therefore, the basic chromosome number of *Deutzia* is $x = 13$. *Kirengeshoma* has the closest relationship with *Deutzia*, forming same clade in phylogenetic tree (Kim et al., 2015).

The results of this study provide clear evidences for the uncertainties previously reported in the cytogenetic study of *K. koreana* and *K. palmata*. The study could further be used as a basis for cytogenetic research in Hydrangeaceae. Taxonomically, *Kirengeshoma* prevalent in Korean peninsula is named as *K. koreana* (Nakai, 1935), and that native to China and Japan is named as *K. palmata* (Yatabe, 1890; Hwang and Wei, 2003). However, the results of the cytological study showed that the chromosome number of the two species was found to be $2n = 52$, which, along with other morphological characteristics.

ORCID: Ha Yeon CHOI https://orcid.org/0000-0002-1206-2490; Hye Rin KIM https://orcid.org/0000-0002-1415-9675; Kweon HEO https://orcid.org/0000-0003-3785-3974

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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