Korean Journal of Plant Taxonomy
52(2): 102–107, 2022

RESEARCH ARTICLE
https://doi.org/10.11110/kjpt.2022.52.2.102

A study of the chromosome number and genome size of the rare species *Rhododendron keiskei* var. *hypoglaucum* in Korea

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ABSTRACT: *Rhododendron keiskei* var. *hypoglaucum* (Ericaceae) was recently reported in Korea, with a disjunct distribution on the southern islands of the Korean Peninsula. Although chromosome numbers and ploidy variations are important traits in angiosperms, gaining a clear understanding the cytological features of *Rhododendron* has been hampered by the small size of its chromosomes. We herein report the chromosome number, karyotype structure, and genome size of *R. keiskei* var. *hypoglaucum* for the first time. The chromosome number of the investigated plants was $2n = 26$ with $x = 13$ as the base chromosome number, which is the one of the frequently encountered base chromosome numbers in *Rhododendron*. The karyotype of *R. keiskei* var. *hypoglaucum* is composed of metacentric and submetacentric chromosomes similar in length, which ranged from 1.39 to 2.40 μm. The DNA 1C-value in all examined accessions was small, ranging from 0.63 to 0.65 pg, further supporting the stable genome size in *Rhododendron*. These comprehensive cytological results provide a framework for detailed molecular, cytogenetic, and phylogenomic analyses that can be used to interpret the slow species diversification rate in *Rhododendron*.

Keywords: Chromosome number, flow cytometry, genome size, karyotype, *Rhododendron keiskei* var. *hypoglaucum*

RECEIVED 3 June 2022; **REVISED** 15 June 2022; **ACCEPTED** 21 June 2022

INTRODUCTION

The changes in chromosome number such as polyploidy and dysploidy play an important role in plant evolution, diversification, and eventually speciation (Lysak et al., 2006; Schubert, 2007; Soltis and Soltis, 2009). To date, chromosome numbers have been reported for approximately 25–30% of flowering plants (Weiss-Schneeweiss and Schneeweiss, 2013; Rice et al., 2015) and, along with molecular phylogenetic data (Pessoa et al., 2022) and genome size data (Choi et al., 2020; Mitrenina et al., 2021; Greimler et al., 2022), are still widely used in systematics.

The genome size (1C value) may vary considerably among closely related species, but is usually consistent within most species (Bennett et al., 2000; Greilhuber, 2005). Thus, it has important systematic implications (McCann et al., 2018; Choi et al., 2019, 2020), although significant variations in genome size have been observed among populations within the same species (Jang et al., 2018; Agudo et al., 2019; Becher et al., 2021). Classical karyotyping methods using Feulgen staining are challenging in highly polyploid species of Cyperaceae, Juncaceae and Ericaceae due to small size of the chromosomes, which hinders chromosomal determination in polyploids and aneuploids (Chung and Im, 2020; Chung and Chung, 2021; Choi et al., 2022; Redpath et al., 2022). Flow cytometry is an alternative method for estimating genome size and ploidy level (Choi et al., 2021; Sliwinska et al., 2021; Temsch et al., 2021).

Fig. 1. Photographs of *Rhododendron keiskei* var. *hypoglaucum* from Korean natural population. **A.** Habit. **B.** Flower. **C.** Fruits.

Rhododendron is the largest genus of the family Ericaceae, comprising about 1,000 species. The genus includes perennial woody plants distributed in Asia, Europe, North America, and Australia (Fang et al., 2005). Along with one recently recorded species (*R. keiskei* Miq. var. *hypoglaucum* Sutô & Suzuki; Yang et al., 2015), 12 taxa are currently recognized in Korea (Chang, 2007). The base chromosome number of *Rhododendron* is $x = 13$, and different ploidy levels ($2x$, $4x$, and $6x$) have been documented (Sax, 1930; Rice et al., 2015). Chromosome number and variation in genome size have been used in the systematics of Ericaceae (Khan et al., 2021). Information on chromosome number and C-values in Korean *Rhododendron* taxa is scarce (Pellicer and Leitch, 2020; Rice et al., 2015). At present, all Korean species are reported only as diploids (Appendix 1), and detailed karyotype analyses of these taxa are lacking. In our study, we investigated the chromosome number, karyotype, and genome size of the recently recorded species in Korea, *R. keiskei* var. *hypoglaucum*, for the first time.

MATERIALS AND METHODS

Six individuals from the recently recorded species *Rhododendron keiskei* var. *hypoglaucum* were collected from a natural population in Korea, and cultivated in the Plant Conservation Center (Korea National Park Research Institute) (Fig. 1, Table 1). Actively growing root tips from the cultivated plants were pretreated with 0.2 mM 8-hydroxyquinoline solution for 2.5 h at room temperature and then for another 2.5 h at 4°C before the samples were fixed in ethanol: acetic acid (3:1, v/v). Chromosome numbers and karyotypes were determined using the standard Feulgen staining technique as described by Choi et al. (2019) and Ikeda et al. (2021). The chromosomes of the investigated taxon are very small. Therefore, to improve karyotype resolution, the cells were additionally digested with enzymes as described by Jang et al. (2016). Briefly, meristematic root cells were digested at 37°C for 2.5 h with 1% cellulase Onozuka (Serva, Heidelberg, Germany), 1% cytohelicase (Sigma-Aldrich, Vienna, Austria), and 1% pectolyase (Sigma-Aldrich). Squash preparations were made in a drop of 60% acetic acid, and prepared slides were snap frozen in liquid nitrogen and cover slips were removed. All prepared slides were stained with 2 ng/μL 4′,6-diamidino-2-2phenylindole (DAPI) dissolved in the mounting antifade medium Vectashield (Vector Laboratories, Burlingame, CA, USA) and examined under a fluorescence microscope (BX-51, Olympus, Tokyo, Japan). Representative karyotypes were selected, cut out, and arranged in Adobe Photoshop CS6. Chromosome size was measured using Micromeasure, ver. 3.3 (http://www.colostate.edu/Depts/Boology/MicroMeasure/) as described by Jang et al. (2013).

Table 1. Chromosome number and genome size of *Rhododendron keiskei* var. *hypoglaucum.*

Accession no.	2*n*	Genome size (pg)
KNPSP-005953	-	0.6367 ± 0.0094
DDH01	26	0.6581 ± 0.0029
TY01	-	0.6497 ± 0.0076
JT0035	-	0.6371 ± 0.0074
EBD01	-	0.6516 ± 0.0030
EBD02	-	0.6458 ± 0.0053

The genome size of the six individuals of *R. keiskei* var. *hypoglaucum* was measured with flow cytometry (Sysmex CyFlow Cytometer, Sysmex Partec GmbH, Görlitz, Germany) performed on leaf materials. Fresh tissue from the cultivated plants growing in the Plant Conservation Center were used with *Solanum pseudocapsicum* L. (1C = 1.30 pg) (Temsch et al., 2010) as an internal standard. The detailed methodology used for the genome size measurement was described by Choi et al. (2021).

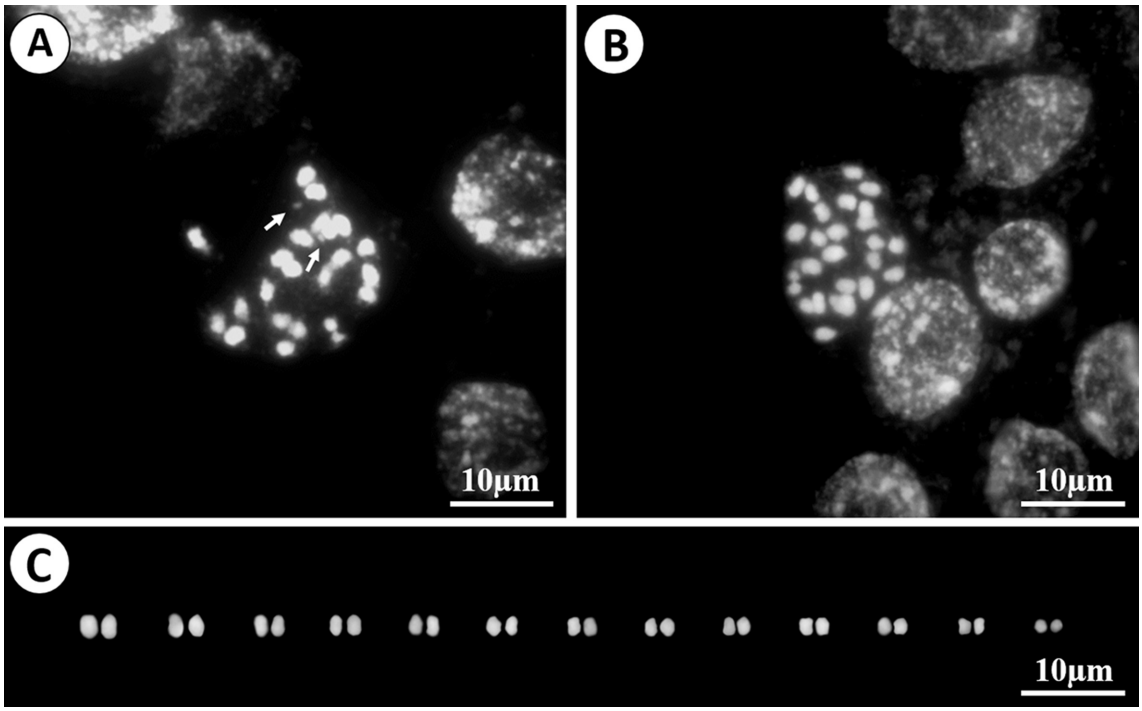

Fig. 2. Representative chromosomes of *Rhododendron keiskei* var. *hypoglaucum*. **A, B**. Metaphase in mitosis. **C.** Cut-out karyotypes of the metaphase in mitosis from Fig. 2B. Arrows indicate the satellite (NOR, nucleolar organizer region).

RESULTS AND DISCUSSION

Chromosome number and karyotype

The chromosome number determined for *Rhododendron keiskei* var. *hypoglaucum* is reported here for the first time (Table 1). Additional information on earlier chromosome reports for Korean *Rhododendron* taxa is given in Appendix 1. The base chromosome number of *R. keiskei* var. *hypoglaucum* was inferred to be $x = 13$ ($2n = 2x = 26$) (Fig. 2), in agreement with previous reports on chromosome numbers in other *Rhododendron* species (Gurzenkov, 1973; Murin et al., 1984; Stepanov, 1994; Atkinson et al., 2000). The previously reported chromosome numbers in 227 *Rhododendron* species included three different ploidy levels (i.e., 2*x*, 4*x*, and 6*x*) (Rice et al., 2015). Such relatively stable ploidy states and variations in chromosome number have been often documented in woody plants (Stebbins, 1938; Otto and Whitton, 2000), although little experimental research beyond comparative surveys on the chromosome number variations was conducted in angiosperms (Husband et al., 2013). Cut-out karyotypes were prepared for one individual of *R. keiskei* var. *hypoglaucum* to protect the taxa from the natural population (Figs. 1, 2). The karyotype of *R. keiskei* var. *hypoglaucum* is characterized by an asymmetry index (AsI) of 57.4%, predominantly metacentric and submetacentric chromosomes (Fig. 2B, C), the chromosome length in the range of 1.39 to 2.40 μm, and the haploid karyotype length of 25.47 μm (Fig. 2C). Similar to other *Rhododendron* taxa (Sax, 1930; Atkinson et al., 2000), identification of individual chromosome pairs was challenging. Due to this reason, only one pair of nucleolar organizer region was documented in the *R. keiskei* var. *hypoglaucum* karyotype (Fig. 2A), in accord with an earlier study (Atkinson et al., 2000).

Nuclear DNA content

We report for the first time the genome size of *Rhododendron keiskei* var. *hypoglaucum* (Table 1); flow histograms are shown in Fig. 3A. Genome size (1C) measured for six individuals ranged from 0.63 to 0.65 pg (Fig. 3B), which falls within the range of that reported in other species of the genus (e.g., 1C = 0.483–2.777 pg, but chromosome number unknown) (Khan et al., 2021). The genome size of *R. keiskei* var. *hypoglaucum* is smaller than that of *R. ponticum* var. *brachycarpum* (1C = 0.74 pg) (Bou Dagher-Kharrat et al., 2013) although both taxa have the same chromosome number $2n = 2x = 26$ (Ammal, 1950). This variation in genome size may be due to different internal reference standards: Bou Dagher-Kharrat et al. (2013) used *Petunia hybrida* (Hook) Vilm. 'PxPC6' (1C =

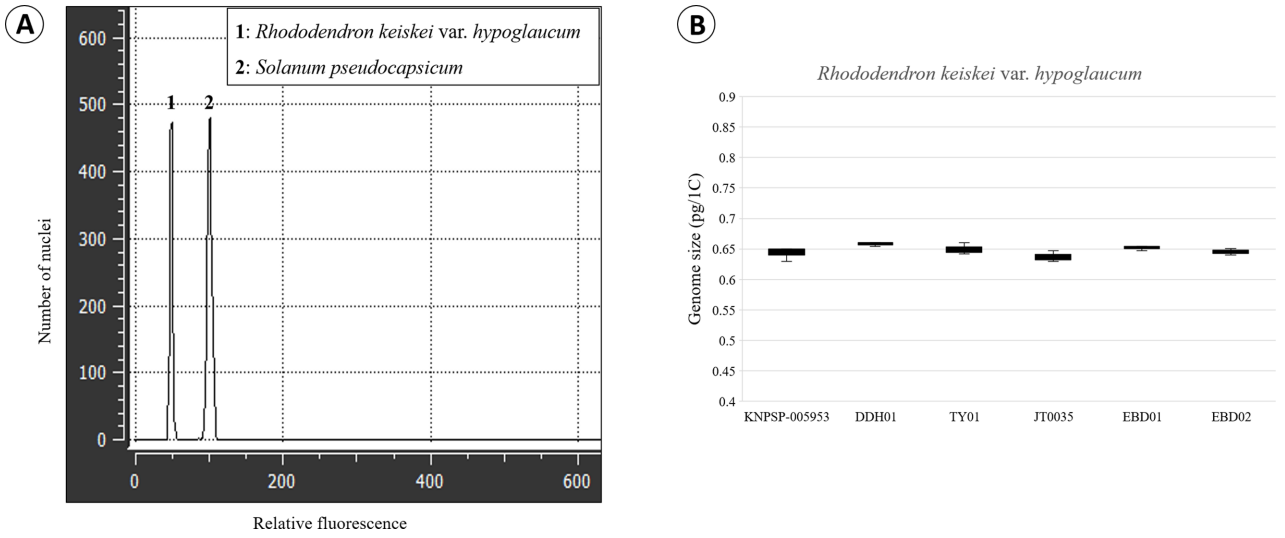

Fig. 3. Fluorescence histogram (**A**) and distribution of genome size data (**B**) of *Rhododendron keiskei* var. *hypoglaucum* with internal reference standard used for genome size estimation.

2.85 pg), whereas we utilized *Solanum pseudocapsicum* (1C = 1.30 pg/1C) (Temsch et al., 2010). The differences in genome size between the two species might be also due to differential accumulation of the non-coding repetitive DNA, as reported in other plant groups (Emadzade et al., 2014; Weiss-Schneeweiss et al., 2015; Jang et al., 2018). The genome size variation between species may thus provide an effective taxonomic marker (Jang et al., 2016; Choi et al., 2019, 2020).

In conclusion, this study is the first to report the chromosome number, karyotype, and genome size of the recently reported *R. keiskei* var. *hypoglaucum* from a Korean natural population. Although the results in this study show that cytological diversity based on genome size in the examined Korean *Rhododendron keiskei* var. *hypoglaucum* species is relatively low, more data incorporating additional species of *Rhododendron* populations to examine large-scale genomic differences among Korean *Rhododendron* species are necessary to better understand the genome evolution.

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ACKNOWLEDGMENTS

The authors would like to thank Prof. Hanna Weiss-Schneeweiss and Dr. Eva M. Temsch (University of Vienna) for kindly providing the seeds of the reference standard used in genome size estimation. We are also very grateful for Soyun Won at Dadohaehaesang National Park of Korea for their kind help with field survey. This work was supported by grants from the Korean National Park Research Institute, grant number NPRI 2021-09.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Appendix 1. Previously reported chromosome numbers of Korean *Rhododendron* species.

Species	2*n*	Reference	Korean name
R. aureum Georgi	26	Stepanov (1994)	노랑만병초
R. dauricum L.	26	Gurzenkov (1973)	산진달래
R. indicum (L.) Sweet	26	Rice et al. (2015)	영산홍
R. keiskei Miq. var. *hypoglaucum* Sutô & T. Suzuki	26	This study	섬진달래
R. lapponicum (L.) Wahlenb.	26	Murin et al. (1984)	황산차
R. micranthum Turcz.	26	Ammal et al. (1950)	꼬리진달래
R. mucronulatum Turcz.	26	Gurzenkov (1973)	진달래