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 sub-metacentric 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*

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).
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 (2\( x \), 4\( x \), and 6\( x \)) 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-phenylindole (DAPI) dissolved in the mounting antifade medium Vectashield (Vector Laboratories, Burlingame, CA, USA) and examined under a fluorescence microscope (BX-51, Olympus, Tokyo, Japan).

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).

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

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>2n</th>
<th>Genome size (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNPSP-005953</td>
<td>-</td>
<td>0.6367 ± 0.0094</td>
</tr>
<tr>
<td>DDH01</td>
<td>26</td>
<td>0.6581 ± 0.0029</td>
</tr>
<tr>
<td>TY01</td>
<td>-</td>
<td>0.6497 ± 0.0076</td>
</tr>
<tr>
<td>JT0035</td>
<td>-</td>
<td>0.6371 ± 0.0074</td>
</tr>
<tr>
<td>EBD01</td>
<td>-</td>
<td>0.6516 ± 0.0030</td>
</tr>
<tr>
<td>EBD02</td>
<td>-</td>
<td>0.6458 ± 0.0053</td>
</tr>
</tbody>
</table>

**Fig. 1.** Photographs of Rhododendron keiskei var. hypoglaucum from Korean natural population. A. Habit. B. Flower. C. Fruits.
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., 2x, 4x, and 6x) (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 \(\mu\)m, and the haploid karyotype length of 25.47 \(\mu\)m (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. ‘PxC6’ (1C =...
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 Rhododendron 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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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

LITERATURE CITED


Pellicer, J. and I. J. Leitch. 2020. The plant DNA C-values database (release 7.1): An updated online repository of plant


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

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>Reference</th>
<th>Korean name</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. aureum Georgi</td>
<td>26</td>
<td>Stepanov (1994)</td>
<td>노랑만병초</td>
</tr>
<tr>
<td>R. dauricum L.</td>
<td>26</td>
<td>Gurzenkov (1973)</td>
<td>산진달래</td>
</tr>
<tr>
<td>R. indicum (L.) Sweet</td>
<td>26</td>
<td>Rice et al. (2015)</td>
<td>영산홍</td>
</tr>
<tr>
<td>R. keiskei Miq. var. hypoglaucum Sutô &amp; T. Suzuki</td>
<td>26</td>
<td>This study</td>
<td>섬진달래</td>
</tr>
<tr>
<td>R. lapponicum (L.) Wahlenb.</td>
<td>26</td>
<td>Murin et al. (1984)</td>
<td>황산차</td>
</tr>
<tr>
<td>R. micranthum Turcz.</td>
<td>26</td>
<td>Ammal et al. (1950)</td>
<td>포리진달래</td>
</tr>
<tr>
<td>R. mucronulatum Turcz.</td>
<td>26</td>
<td>Gurzenkov (1973)</td>
<td>진달래</td>
</tr>
</tbody>
</table>