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The complete chloroplast genome of the subtropical species *Camellia japonica* 'Huaheling'

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ABSTRACT

Camellia japonica 'Huaheling' is a rare subtropical *Camellia* species in China with high ornamental and medicinal value. The complete chloroplast genome of *C. japonica* 'Huaheling' is a 157,001-bp circular DNA molecule containing a large single-copy region (LSC, 86,704 bp), a small single-copy region (SSC, 18,393 bp), and two inverted repeat sequences (IR). Of the 131 genes identified, 86 are protein-coding genes, 8 are rRNA genes, and 37 are tRNA genes. A total of 54 simple sequence repeats (SSRs) were identified in the chloroplast genome. The phylogenetic analysis showed that *C. japonica* 'Huaheling' is clustered with *C. japonica*. This work provides valuable information for future study of the evolution and genetic diversity of *C. japonica* 'Huaheling.'

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Camellia genus plants are perennial woody plants that are mainly distributed in Zhejiang and Shandong Provinces in China; Honshu, Shikoku, and Kyushu Islands of Japan; and the west coast of the Korean Peninsula (Ueno et al. 1999; Gao and Parks 2005); they are of wide horticultural interest for their beautifully shaped flowers. The petals of *C. japonica* 'Huaheling,' whose origin is in southwest China, are egg-shaped, and more than 70 petals form a hemispherical flower bulb. The flowering period of *C. japonica* 'Huaheling' lasts from February to May, which is much longer in duration compared with other varieties; it also has high ornamental and medicinal value. Temperature is a major factor limiting the distribution of *C. japonica* 'Huaheling,' which grows optimally between 18 and 30 °C. Thus, studies of the cold resistance of *Camellia* species are needed.

Photosynthesis is essential for plant growth and development, and the photosynthetic rate of low-temperature-sensitive plants decreases rapidly after exposure to low temperature. This decrease in the photosynthetic rate stems from the destruction of the chloroplast structure and the decrease in chlorophyll content (Erdal 2012; Zhang et al. 2021). Chloroplasts are the sites of photosynthesis in plants, making them the 'nutrient factories' of plants. Because of the conserved mechanism and function of chloroplasts and their slow rate of evolution, chloroplast genomes are widely used to reconstruct plant phylogenies (Wu et al. 2014). To provide new insight into the phylogeny of *C. japonica*, we sequenced the chloroplast genome of *C. japonica* 'Huaheling'.

Healthy, fresh leaves of *C. japonica* 'Huaheling' were collected from the greenhouse of Qingdao Agricultural University College of Landscape Architecture (36°32′N, 120°17′E). The voucher specimens of *C. japonica* 'Huaheling' were deposited at the Germplasm Resource Center of Qingdao Agricultural University (accession CJ-24). Total genomic DNA was obtained by the CTAB (Doyle and Doyle 1987) method and paired-end sequenced on the Illumina Nova Seq 6000 platform. We obtained a total of 4.46G of raw data using Fast QC version 0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and filtered 0.14 G of low-quality data. We used SPAdes version 3.10.1 (Bankevich et al. 2012) to assemble the chloroplast genome and Velvet Optimizer version 2.2.5 to optimize the splicing results. The final genome was annotated with CpGAVAS2 (http://47.96.249.172:16019/analyzer/annotate) (Shi et al. 2019). BLASTp in NCBI was used to confirm the annotation of the protein-coding sequence. MISA version 2.1 (https://webblast.ipk-gatersleben.de/misa/index.php? action = 1) (Beier S et al. 2017) was used for simple sequence repeat (SSR) prediction. The complete and accurate chloroplast genome was uploaded to NCBI (MW602996).

The total length of the chloroplast genome of *C. japonica* 'Huaheling' is 157,001 bp and consisted of an 86,704 bp large single-copy (LSC) region, a 18,393 bp a small single-copy (SSC) region, and two 26,397 bp inverted repeat (IR) regions. The overall GC content is 37.3%. A total of 131 genes were annotated, including 86 protein-coding genes, 8 rRNA genes, and 37 tRNA genes (including *trnK-UUU*, *rps16*, *trnG-UCC*, *atpF*, *rpoC1*, *trnL-UAA*, *trnV-UAC*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, *trnI-GAU*, *trnA-UGC*, and *ndhA*) containing one intron. *clp*P and *ycf*3 genes have two introns, and the *rps12* gene shows

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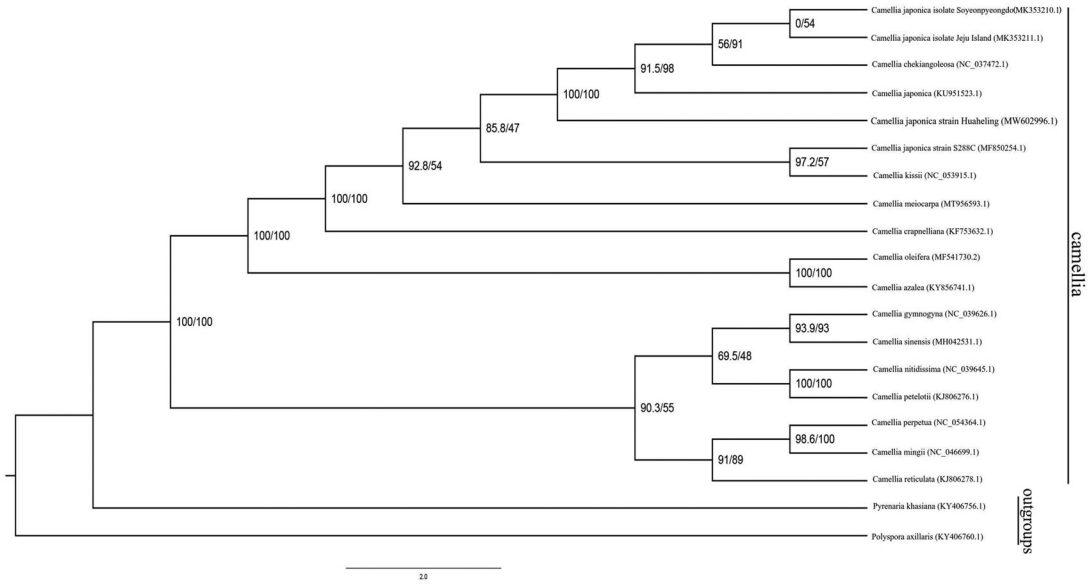

Figure 1. A phylogenetic analysis of the chloroplast genomes of 20 Theoideae species was constructed using maximum likelihood and with *Polyspora axillaris* and *Pyrenaria khasiana* as outgroups.

trans-splicing. A total of 54 SSR markers were detected in the chloroplast genome of *C. japonica* 'Huaheling'.

To explore the phylogenetic relationships of *C. japonica* 'Huaheling' in *Camellia*, the phylogeny of 5 species of *C. japonica* and 13 *Camellia* L. were studied; *Polyspora axillaris* and *Pyrenaria khasiana* were used as outgroups ((Figure 1)) . We used iQ-tree version 1.6.12 (Nguyen et al. 2015) based on the best model (TVM + F+R3) and 1000 bootstrap replicates. Eighteen plants of *Camellia* L. were divided into two families and *C. japonica* 'Huaheling' and *C. japonica* were most closely related.

Disclosure statement

The authors declare no potential conflict of interest.

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Data availability statement

The data that support the findings of this study are available in GenBank (https://www.ncbi.nlm.nih.gov/; GenBank accession number MW602996). The associated BioProject and BioSample numbers are PRJNA679998 and SAMN16869207, respectively.

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