

The complete chloroplast genome of *Dryas octopetala* var. *asiatica* (Dryadoideae, Rosaceae) and phylogenetic analysis

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ABSTRACT

Dryas octopetala L. var. *asiatica* (Nakai) Nakai 1918 is a dwarf shrub that mainly grow in alpine and arctic zones of the Northern Hemisphere, representing an endemic variety in Asia. In the present study, the complete chloroplast (cp) genome of *D. octopetala* var. *asiatica* was first characterized and used for its phylogenetic analysis. The cp genome span 158,271 bp with an overall GC content of 36.5%. A total of 129 genes were identified, including 84 protein-coding genes (PCGs), 37 tRNA genes, and 8 rRNA genes. In addition, repetitive sequences and microsatellites were detected within this species. Phylogenetic analysis involving 39 cp genomes from Rosaceae family indicated that *D. octopetala* var. *asiatica* was sister to the clade of Amygdaloideae. This study contributes fundamental insights into the cp genome of *Dryas octopetala* var. *asiatica*, which will have expanded its use in photosynthesis and evolutionary study.

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Introduction


Dryas octopetala L. var. *asiatica* (Nakai) Nakai 1918 is a dwarf shrub belonging to Dryadoideae, Rosaceae. Typically found in alpine and arctic zones of the Northern Hemisphere, this plant faces various environmental challenges such as low temperatures and limited nutrient availability that impact its

growth and reproduction. Known for forming ectomycorrhiza (ECM) and engaging in nitrogen-fixing root nodule symbiosis (Gardes and Dahlberg 1996; Bjorbækmo et al. 2010; Li et al. 2016; Billault-Penneteau et al. 2019), *Dryas* species play a significant role in mycology community ecology. Moreover, *D. octopetala* is of particular importance in research on climate



Figure 1. Reference image of *Dryas octopetala* var. *asiatica* taken by Limin Yang at Changbai Mountain of Jilin province, China. Note: *Dryas octopetala* var. *asiatica* is characterized by its prostrate growth habit, leathery leaves, showy white flowers, and its ability to thrive in harsh alpine conditions.

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change (McGraw et al. 2014; Panchen and Gorelick 2017). *Dryas octopetala* var. *asiatica* as the Asian endemic variety is sparsely distributed in alpine regions of northeastern Asia. A recent study has highlighted the sensitivity of photosynthesis to climate warming and its adaptability to temperature increase in *D. octopetala* var. *asiatica* (Zhou et al. 2019). Chloroplast (cp) is the site of photosynthesis, in which light

energy is converted to chemical energy and the oxygen and energy-rich organic compounds are produced. However, little is known about the whole cp genome for this variety. In this study, the cp genome of *D. octopetala* var. *asiatica* was first sequenced using next-generation sequencing. We mainly focused on the analyses of cp genome features, repeat sequences and phylogenetic reconstruction. This study not only offers the valuable cp genome data but also contributes to understanding the phylogenetic relationships within Rosaceae.

Table 1. Information of the chloroplast genome in *Dryas octopetala* var. *asiatica*.

Regions	Length(bp)	GC(%)	Number		
			protein-coding genes	tRNAs	rRNAs
Genome	158,271	36.5	84	37	8
LSC	86,980	34.1	62	22	0
SSC	18,461	30.2	12	1	0
IRA	26,415	42.5	5	7	4
IRB	26,415	42.5	5	7	4

Materials and methods

Dryas octopetala var. *asiatica* was collected from Changbai Mountain in Jilin province, China (Figure 1; GPS coordinates: 42°03'17"N 128°04'00"E). A voucher specimen (voucher

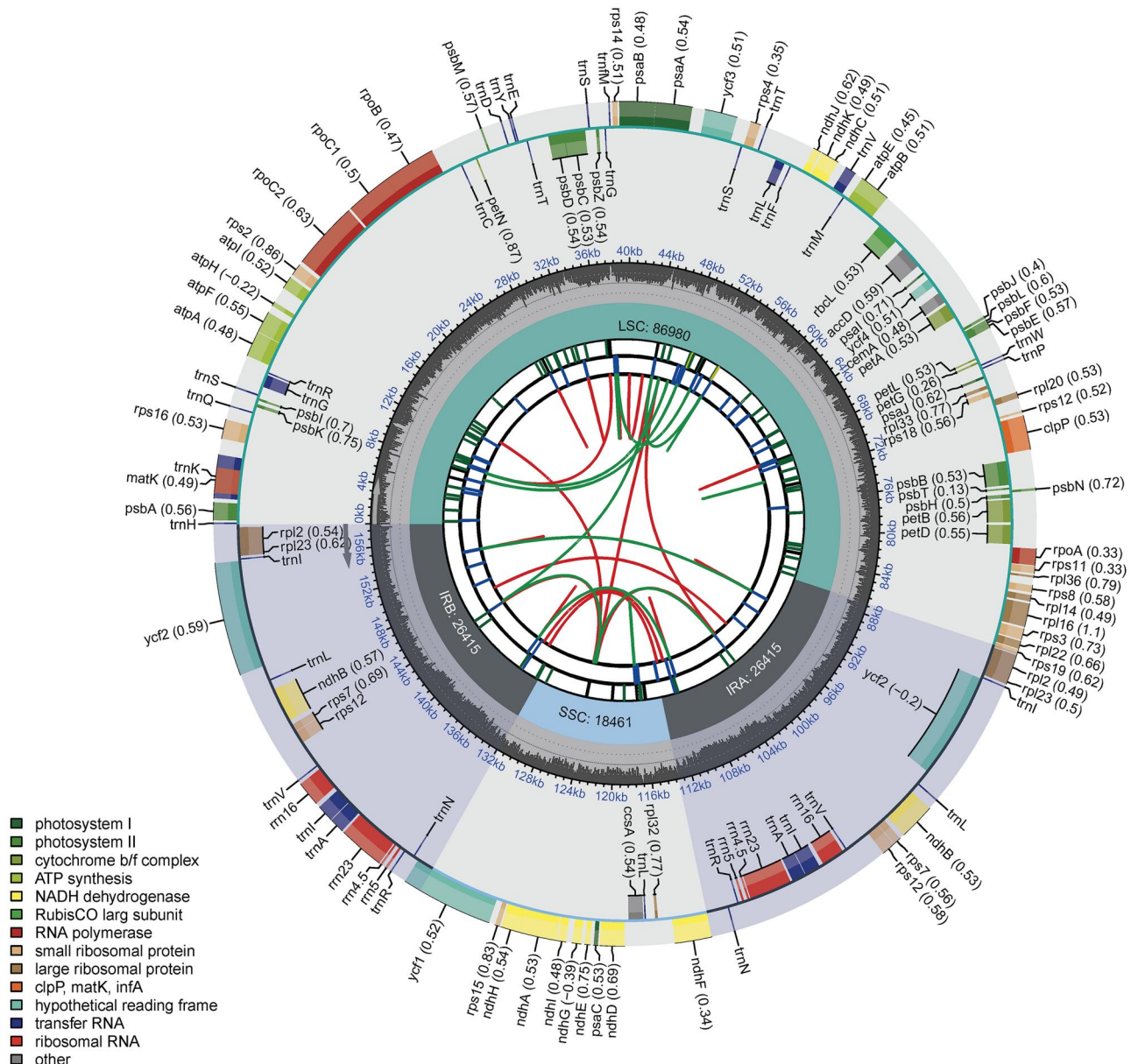


Figure 2. Chloroplast genome map of *Dryas octopetala* var. *asiatica*.

Note: Genes are depicted as differently sized and colored boxes on the outermost circle, with inner and outer boxes representing genes transcribed in clockwise and counter-clockwise directions. The middle circle illustrates changes in GC content at different positions, while the inner circle highlights the regions and lengths indicated by the tetrad structure (LSC, SSC, IRA, and IRB) in different colors.

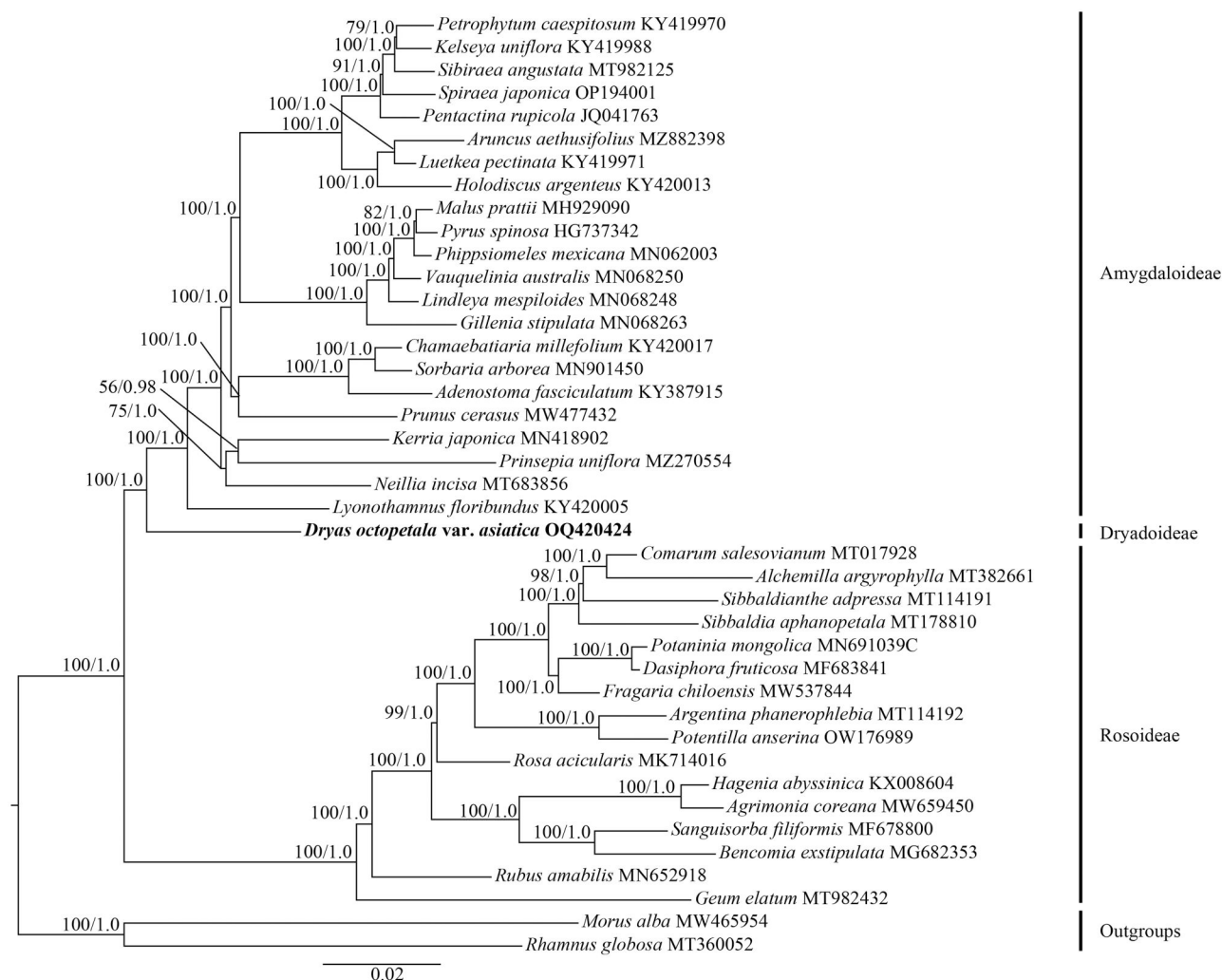


Figure 3. The phylogenetic tree based on the complete chloroplast genomes of 40 species of Rosaceae. Numbers at nodes correspond to maximum likelihood bootstrap percentage and the posterior probability of Bayesian inference. The sequences employed for the construction of the phylogenetic tree are detailed as follows: *Adenostoma fasciculatum* (KY387915, Zhang et al. 2017), *Agrimonia coreana* (MF135594, direct submission), *Alchemilla argyrophylla* (MT382661, direct submission), *Argentina phanerophlebia* (MT114192, Aogan 2020a), *Aruncus aethusifolius* (MZ882398, direct submission), *Bencomia exstipulata* (MG682353, direct submission), *Chamaebatiaria millefolium* (KY420017, Zhang et al. 2017), *Comarum salesovianum* (MT017928, Aogan 2020b), *Dasiphora fruticosa* (MF683841, Zhao et al. 2018), *Dryas octopetala var. asiatica* (OQ420424, this study), *Fragaria chiloensis* (MW537844, direct submission), *Geum elatum* (MT982432, direct submission), *Gillenia stipulata* (MN068263, direct submission), *Hagenia abyssinica* (KX008604, direct submission), *Holodiscus argenteus* (KY420013, Zhang et al. 2017), *Kelseya uniflora* (KY419988, Zhang et al. 2017), *Kerria japonica* (MN418902, Huo et al. 2019), *Lindleya mespiloides* (MN068248, direct submission), *Luetkea pectinata* (KY419971, Zhang et al. 2017), *Lyonothamnus floribundus* (KY420005, Zhang et al. 2017), *Malus prattii* (MH929090, direct submission), *Morus alba* (MW465954, direct submission), *Neillia incisa* (MT683856, direct submission), *Pentactina rupicola* (JQ041763, Kim and Kim 2016), *Petrophytum caespitosum* (KY419970, Zhang et al. 2017), *Phippsiomeles mexicana* (MN062003, Liu et al. 2019), *Potaninia mongolica* (MN691039, Cong and Jiang 2020), *Potentilla anserina* (OW176989, direct submission), *Prinsepia uniflora* (MZ270554, direct submission), *Prunus cerasus* (MW477432, direct submission), *Pyrus spinosa* (HG737342, Korotkova et al. 2014), *Rhamnus globosa* (MT360052, Xie et al. 2020), *Rosa acicularis* (MK714016, Chen et al. 2019), *Rubus amabilis* (MN652918, direct submission), *Sanguisorba filiformis* (MF678800, Meng et al. 2018), *Sibbaldia aphanopetala* (MT178810, Zhang et al. 2020), *Sibbaldianthe adpressa* (MT114191, Tian et al. 2020), *Sibiraea angustata* (MT982125, direct submission), *Sorbaria arborea* (MN901450, Sun et al. 2020), *Spiraea japonica* (OP194001, Zhang et al. 2023), and *Vauquelinia australis* (MN068250, direct submission).

number: ZhouHC1243) was deposited in the Herbarium of Kunming Institute of Botany, CAS (KUN, <http://www.kun.ac.cn/>, Tao Deng, dengtao@mail.kib.ac.cn). Genomic DNA was extracted from the silica gel-dried leaf using a modified CTAB method (Yang et al. 2015) and the constructed libraries were sequenced using Illumina NovaSeq PE150 platform. High-quality reads were filtered and *de novo* assembled with SPAdes software (Prijbelski et al. 2020). The cp genome of *D. octopetala var. asiatica* was annotated using PGA software (Qu et al. 2019) with manual adjustments and visualized with the online CPGView program (Liu et al. 2023). The annotated cp genome of *D. octopetala var. asiatica* was deposited in GenBank under accession number OQ420424. Phylogenetic reconstruction was performed with

one representative species from 39 genera of Rosaceae downloaded from NCBI GenBank database with *D. octopetala var. asiatica*. *Morus alba* from Moraceae and *Rhamnus globosa* from Rhamnaceae were used for the outgroups. Multiple sequence alignments of the complete cp genomes were carried out using MAFFT v.6.833 (Katoh et al. 2005) and used to construct phylogenetic trees with maximum likelihood (ML) and Bayesian inference (BI) methods as previously described (Zhang et al. 2017).

Results and discussion

The complete cp genome of *D. octopetala var. asiatica* was 158,271 bp long with an average sequencing depth of

2696 × and the minimum sequencing depth of 11 × (supplemental Figure S1). The overall GC content of this cp genome was 36.5% (Table 1). The cp genome exhibited a typical quadripartite structure, consisting of two copies of inverted repeats (IRs) and one large single-copy (LSC) and one small single-copy (SSC) region (Figure 2). The length of the four segments in this cp genome varied, with the LSC region being the largest at 86,980 bp, followed by the two IR regions at 26,415 bp each (Table 1). The GC contents of the four segments differed, with the IR regions having the highest GC content at 42.5% and the SSC region having the lowest at 30.2%. The IR regions were flanked by the LSC and SSC region, forming a circular structure (Figure 2). These results indicated the cp genome structure of *D. octopetala* var. *asiatica* is typical with most other land plants (Han et al. 2019; Huang et al. 2021; Zhang and Ling 2022).

The cp genome of *D. octopetala* var. *asiatica* was found to contain a total of 129 genes, including 84 protein-coding genes (PCGs), 37 tRNA, and 8 rRNA genes (Figure 2). The major functions of these genes were associated with photosynthesis and metabolic processes (Figure 2), with 20 genes specifically encoding proteins related to photosynthesis (Figure 2). The majority of PCGs were located in the LSC region, while 16 genes, including 7 tRNA genes, all 4 rRNA genes and 5 PCGs were duplicated in the IR region (Table 1 and Figure 2). Furthermore, 11 genes contained one intron and 2 genes had two introns (supplemental Figure S2), potentially undergoing cis or trans-splicing during the transcription process (supplemental Figure S2 and S3).

Repeats are often associated with genome rearrangement through gene inversion that reverses the order and orientation of multiple genes (Cavalier-Smith 2002). In this study, the dispersed repeats and long tandem repeats were identified and shown in the first two tracks (from the center going outward) (Figure 2). Of them, the majority were located in the LSC region, with a few repeats in the SSC region (Figure 2). Short tandem repeats or microsatellite sequences were shown in the third track and exhibited the similar distribution pattern. These repeat types were also found in other Rosaceae genera like *Sorbus* (Zhang and Ling 2022). Previous studies have indicated that repeat sequences have promoted the generation of substitutions and indels during chloroplast genome evolution (McDonald et al. 2011; Ahmed et al. 2012). Therefore, the repeats within *D. octopetala* var. *asiatica* cp genomes could potentially induce mutations, influencing mutation rates and the evolution of this species.

The phylogenetic position of *D. octopetala* var. *asiatica* as the representative species of Dryadoideae was investigated using 39 species from different genera of Rosaceae. Both BI and ML methods were employed, resulting in the identical topology (Figure 3). Rosaceae was resolved into three main clades representing the subfamilies: Amygdaloideae, Dryadoideae and Rosoideae. Amygdaloideae and Dryadoideae (the respective species, *D. octopetala* var. *asiatica*) had the closer relationship and formed two sister clades with strong supports (Figure 3), consistent with the previous studies (Potter et al. 2007; Zhang et al. 2017). In fact, Amygdaloideae and Dryadoideae have the similar chemical compounds, like

cyanogenic glycosides and sorbitol (Zhang et al. 2017). However, some analyses based on the partial sequences of cp genome (Zhang et al. 2017) suggested a sister relationship between Dryadoideae and Rosoideae. Given the presence of hybridization, apomixis and polyploidy present in Rosaceae (Vamosi and Dickinson 2006; Burgess et al. 2015), further research is needed to definitively resolve the phylogenetic relationships among these subfamilies.

Conclusions

In this study, we first sequenced and characterized the complete cp genome of *D. octopetala* var. *asiatica*. Our results revealed that the cp genome of *D. octopetala* var. *asiatica* follows a typical quadripartite and circular DNA structure. Various important features related to structure and sequence divergence such as genome size, gene/intron content, repeats and microsatellite, were analyzed in this species. Additionally, the phylogenetic analysis positioned *D. octopetala* var. *asiatica* as a representative species of Dryadoideae sister to the clade of Amygdaloideae. These basic knowledge of the cp genome of *D. octopetala* var. *asiatica* will enhance its utility in comparative studies and contribute valuable data for research on photosynthesis.

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Ethical approval

Dryas octopetala var. *asiatica* is not an endangered or protected species and collected without requirement of the specific permission from authorities. This plant used in the study is complied with national and international standards and local laws and regulations.

Author contributions

L.Z. L and S.D. Z designed the experiment and obtained the funding. L.Z. L and Y.G. Z performed laboratory work. L.Z. L, L.M. Y and S.D. Z performed bioinformatics analyses, wrote and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (<https://www.ncbi.nlm.nih.gov/>)

under the accession no. OQ420424. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA724898, SRR28753508, and SAMN41028577 respectively.

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