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RESEARCH NOTE

Complete chloroplast genome of *Atractylodes japonica* native to the Korean Peninsula and *Atractylodes* species identification challenges

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Abstract

H.I. Choi, G. Lee, J. Ryu, J.W. Park, and S.H. Kim. 2023. Complete chloroplast genome of *Atractylodes* japonica native to the Korean Peninsula and *Atractylodes* species identification challenges. Int. J. Agric. Nat. Resour. 111-115. *Atractylodes japonica* Koidz. ex. Kitam. (Asteraceae) is a perennial plant, of which the dried rhizome has been utilized as a traditional herbal medicine in East Asia. Here, we sequenced and annotated the complete chloroplast genome of *A. japonica* native to Korea. The circular genome was 153,207 bp in length and was composed of a large single-copy region (84,256 bp), a small single-copy region (18,657 bp), and a pair of inverted repeat regions (25,147 bp each). The annotation and manual curation of genes predicted a total of 114 genes, of which 80 were classified as protein-coding genes, 30 were transfer ribonucleic acid (RNA) genes, and 4 were ribosomal RNA genes. Phylogenetic analysis using 13 genomes from 6 *Atractylodes* species showed intermingled clustering of 9 genomes from 4 species, *A. chinensis*, *A. japonica*, *A. koreana*, and *A. lancea*, indicating ambiguity in their taxonomic identification.

Keywords: Asteraceae, phylogeny, plastid, taxonomic problem.

Introduction

Atractylodes, a genus of the family Asteraceae, consists of six recognized species of perennial plants dispersed and cultivated in East Asia, encompassing regions such as Korea, China, and Japan (Wang et al., 2021). The rhizomes of *Atractylodes* species can effectively treat gastrointestinal disorders and have been used in herbal medicine for > 1000 years (Lee et al., 2002; Kim et al., 2018). The taxonomic classification of *Atractylodes* species is controversial due to their similar medicinal uses and morphology, particularly with respect to four species, *Atractylodes japonica* Koidz. ex. Kitam., *Atractylodes chinensis* (DC.) Koidz., *Atractylodes koreana* (Nakai) Kitam., and *Atractylodes lancea* (Thunb.) DC. (Wang et al., 2021).

A. japonica is a native plant to the Korean Peninsula, and its dried rhizome is called Baekchul

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in Korean, Baizhu in Chinese, and Byakujutsu in Japanese. The classification of the herbal origins of Baekchul is discordant among Korea, China, and Japan (Kim et al., 2018; Wang et al., 2021). In this study, we acquired and characterized the chloroplast genome of *A. japonica* native to Korea and analyzed its phylogenetic relationship with previously reported *Atractylodes* species for taxonomic identification.

Materials and methods

Plant materials

Seeds of *A. japonica* were provided by the Department of Herbal Crop Research under the National Institute of Horticultural and Herbal Science (Eumseong, Republic of Korea). The plants were grown in the experimental field at Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongeup, Republic of Korea). Its voucher specimen and deoxyribonucleic acid (DNA) were deposited with an accession identifier of RBRC_PB_017 in the Radiation Breeding Research Center affiliated with the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongeup, Republic of Korea).

Sequencing, assembly, and annotation of the chloroplast genome

Total genomic DNA from *A. japonica* plant leaves was isolated using the DNeasy Plant Mini Kit (Qiagen, Germany). The Illumina MiSeq system was used for paired-end sequencing of the genomic DNA with a 300 bp read length. A total of 2.23 Gb raw reads were generated, and 1.68 Gb high-quality reads were *de novo* assembled using CLC Assembly Cell version 4.2.1 (Qiagen, Denmark), in accordance with the methods described by Kim et al. (2015). The chloroplast genome-derived contigs were gathered and combined into a draft genome sequence, and manual correction and gap filling based on paired-end read mapping were performed to establish the complete chloroplast genome. GeSeq was used to annotate the final sequence (Tillich et al., 2017), which was then manually curated by Artemis with BLASTN searches against the NCBI database (https://www.ncbi.nlm.nih.gov/) (Rutherford et al., 2000). The chloroplast genome map was generated using OGDRAW (Greiner et al., 2019). All genome sequence data produced here are freely accessible in GenBank of NCBI under the accession number OK077520. The accession numbers of BioProject, Bio-Sample, and SRA related to this study are PRJNA761881, SAMN21365973, and SRR15827491, respectively.

Phylogenetic analysis

A total of 13 chloroplast genomes previously reported in *Atractylodes* and a genome for the outgroup species *Tugarinovia mongolica* were retrieved from NCBI GenBank. MAFFT was used to perform multiple alignments of concatenated sequences of 77 protein-coding genes and whole chloroplast genomes (Katoh and Standley 2013). Finally, maximum likelihood phylogenetic trees based on each alignment were constructed under the general time-reversible (GTR) model with gamma distribution plus invariant sites (G+I) and 1000 bootstrap replicates using MEGA 11 (Tamura et al., 2021).

Results and discussion

The total length of the chloroplast genome sequence of *A. japonica* was 153,207 bp, comprising a large single-copy section of 84,256 bp, a small single-copy section of 18,657 bp, and a pair of 25,147-bp inverted repeats (Figure 1). Gene prediction resulted in a total of 114 unique genes in the genome, which included 80 protein-coding genes, 30 transfer ribonucleic acid (tRNA) genes, and 4 ribosomal RNA (rRNA)

genes. Among the predicted genes, 15 genes, including 6 tRNA genes, *trnA-UGC* (tRNA-Ala), *trnG-UCC* (tRNA-Gly), *trnI-GAU* (tRNA-Ile), *trnK-UUU* (tRNA-Lys), *trnL-UAA* (tRNA-Leu), and *trnV-UAC* (tRNA-Val), and 9 protein-coding genes, *atpF* (ATP synthase CF0 subunit I), *ndhA* (NADH-plastoquinone oxidoreductase subunit 1), *ndhB* (NADH-plastoquinone oxidoreductase subunit 2), *petB* (cytochrome b6), *petD* (cytochrome b6/f complex subunit IV), *rpl2* (ribosomal protein L2), *rpl16* (ribosomal protein L16), *rpoC1* (RNA polymerase beta' subunit), and *rps16* (ribosomal protein S16), had 1 intron, while 3 genes, *clpP* (clp protease proteolytic subunit), *rps12* (ribosomal protein S12), and *ycf3* (photosystem I assembly protein ycf3), had 2 introns. The *rps12* gene, which had second and third exons on the inverted repeats, also had a trans-splicing function. The GC content of the entire chloroplast genome was 37.70%.

To resolve intra- and interspecific phylogenetic relationships, two phylogenetic trees based on the 77 shared protein-coding genes and whole chloroplast genomes were constructed (Figure 2). Both trees showed a similar topology: (i) *A. carlinoides* located in a basal position, (ii) four *A. macrocephala* genomes formed a monophyletic group, and (iii) nine genomes from four species, *A. chinensis*, *A. japonica*, *A. koreana*, and *A. lancea*, formed a clade with intermingled clustering of different species (Figure 2A and B). Although the genus *Atractylodes* has been established since 1838, species classification is still debated (Peng et al., 2012; Wang et al., 2020; Wang et al., 2021). *A. carlinoides* and *A. macrocephala* are clearly distinguished based on their specific morphology; however, distinguishing the other four species from each other poses a considerable challenge that leads to the misuse of *Atractylodes* rhizomes for medicinal

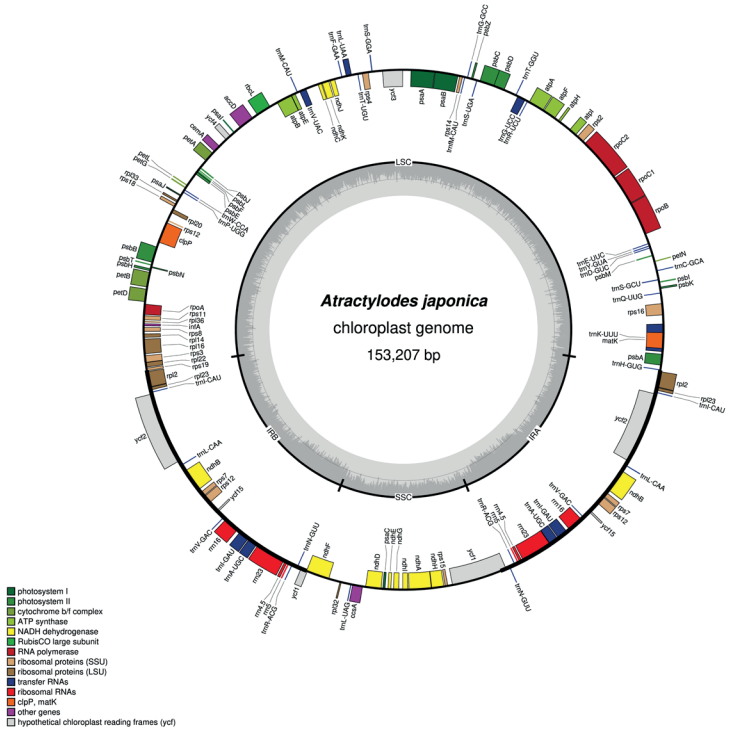

Figure 1. Chloroplast gene map of *A. japonica* native to the Korean Peninsula. Genes transcribed in a clockwise direction are depicted on the outer part of the circle, and those transcribed in a counterclockwise direction are on the inner part. The dark and light gray colors in the inner circle represent the GC and AT contents, respectively. The GC content is represented as dark gray colors in the inner circle.

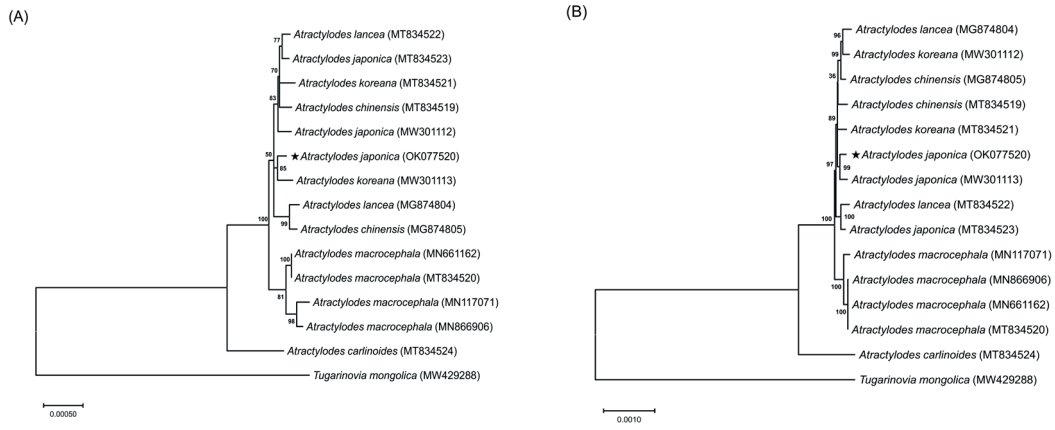

Figure 2. Phylogenetic analysis of *Atractylodes* species. (A) Phylogenetic tree based on 77 shared protein-coding sequences. (B) Phylogenetic tree based on whole chloroplast genome sequences. Each tree was constructed using the maximum likelihood method with 1000 iterations of bootstrapping. *T. mongolica* was used as an outgroup species. The NCBI GenBank accession numbers of each chloroplast genome are indicated in parentheses. The numbers on the nodes indicate bootstrap values (>50). The black star indicates *A. japonica* sequenced in this study.

purposes (Wang et al., 2020; Wang et al., 2021). The results seem to be derived from an ambiguity in the taxonomic assignment of the *Atractylodes* species rather than intraspecific and/or interspecific divergence. This implies that the criteria for the identification of the four species may differ among different countries or researchers. The findings of this study will contribute to species identification and taxonomic revision as well as a greater understanding of *Atractylodes* speciation and evolution.

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Compliance with ethical standards

The authors have no conflicts of interest to declare.

Resumen

H.I. Choi, G. Lee, J. Ryu, J.W. Park, y S.H. Kim. 2023. Genoma completo del cloroplasto de *Atractylodes japonica* nativa de la península de Corea y problemas en la identificación de especies de *Atractylodes*. Int. J. Agric. Nat. Resour. 111-115. *Atractylodes japonica* Koidz. ex. Kitam. (Asteraceae) es una planta perenne, cuyo rizoma seco se ha utilizado como medicina herbal tradicional en Asia Oriental. Aquí hemos completado el genoma del cloroplasto de *A. japonica* nativa de Corea. El genoma circular tenía una longitud de 153,207 pb, compuesto por una región de copia única grande (84,256 pb), una región de copia única pequeña (18,657 pb) y un par de regiones de repetición invertida (25,147 pb cada una). La anotación y curación manual de los genes predijeron un total de 114 genes, de los cuales 80 fueron clasificados como

genes codificantes de proteínas, 30 como genes de ácido ribonucleico de transferencia (ARNt) y 4 como genes de ARN ribosómico (ARNr). El análisis filogenético utilizando 13 genomas de 6 especies de *Atractylodes* mostró una agrupación entremezclada de 9 genomas de 4 especies, *A. chinensis*, *A. japonica*, *A. koreana* y *A. lancea*, lo que indica ambigüedad en su identificación taxonómica.

Palabras clave: Asteraceae, filogenia, plastidio, problema taxonómico.

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