The complete chloroplast genome of *Lilium amoenum* (Liliopsida: Liliaceae) from Yunnan, China

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**ABSTRACT**

*Lilium amoenum* E. H. Wilson ex Sealy is classified in Liliaceae, and it is an important ornamental plant with wonderful rose-red color and pleasant rose fragrance. In this study, we sequenced the complete chloroplast genome of *L. amoenum* by Illumina Hiseq X Ten and PacBio RS technologies. The genome size of *L. amoenum* is 152,280 bp, and displays a typical quadripartite structure: one large single-copy (LSC, 81,977 bp), one small single-copy (SSC, 17,539 bp), and a pair of inverted repeat regions (IRs, 26,382 bp). The overall GC content was 37.0%. The complete genome contained 131 genes, including 85 protein-coding genes, 38 tRNA genes, and 8 rRNA genes. Phylogenetic analysis showed that *L. amoenum* is closely related to *L. taliense* and *L. bakerianum*. The present study could afford crucial genetic information for further researches on the genus and related genera.

*Lilium* is an important ornamental flower used as a cut flower, pot plant and garden plant. However, there are some obvious problems in the existing cultivars, including variation, poor fragrance and plant size (Huang 1983). For *Lilium*, orange and white flowers are the common, but the rose-red colored flowers are rare (Huang 1983). *Lilium amoenum*, originally from the Yunnan province of China and distributed at altitudes 1900–2500 m, is a unique species with dwarf plant, wonderful rose-red color and pleasant rose fragrance (De Jong 1974). *Lilium amoenum* is classified in the section Lophophorum of the Liliaceae (De Jong 1974), and is an attractive resource for germplasm innovation and breeding varieties (Huang et al. 1990; Pan et al. 2018). Due to its confined distribution, human collection and serious ecological environment damage, the natural distribution of *L. amoenum* in the wild is becoming scarce, and may be soon endangered (Wu et al. 2010). Therefore, it is extremely urgent to develop the genetic resources to protect and use this germplasm. In the present study, we determined the chloroplast genome sequence of *L. amoenum*, and discussed the genetic relationship among various species in the Liliaceae.

The fresh leaves were collected from the *L. amoenum* planted in the germplasm nursery of Yunnan Agricultural University (104°30′30″E, 23°12′50″N). The voucher specimen was deposited at the Herbarium of Yunnan Agricultural University (No. 2020WHZ004, Shuiliian He, heshuiliian2006@163.com). Total genomic DNA was isolated from fresh leaves using a DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) according to the manufacturer’s instructions. The obtained DNA was constructed into average 350 bp paired-end (PE) library by Illumina Hiseq platform (Illumina, San Diego, CA, USA) and sequenced using an Illumina NovaSeq platform.

The output was a 4 Gb raw data of 150 bp paired-end reads. Prior to chloroplast de novo assembly, low-quality reads were filtered out and resultant clean reads were assembled using the default settings in SPAdes (Bankevich et al. 2012). The resulting clean reads were assembled using GetOrganelle pipeline (https://github.com/Kinggerm/GetOrganelle). The genome was automatically annotated using the CpgAVAS pipeline (Liu et al. 2012) and start/stop codons and intron/exon boundaries were adjusted in Geniose 8.1 (Kearse et al. 2012), and inspected by comparison against the *L. taliense* complete chloroplast genome (GenBank accession number: KY009938).

The results of whole genome sequencing showed that the size of chloroplast genome of *L. amoenum* (Genbank accession number: MT880912) was 152,280 bp with a typical tetragonal structure: one large single-copy (LSC, 81,977 bp), one small single-copy (SSC, 17,539 bp) and two reverse repeats (IRs, 26,382 bp). The total GC content was 37.0%, the GC content of large single copy (LSC) was 34.8%, and the GC content of small single copy (SSC) was 30.6%. A total of 131 genes were detected, including 85 protein coding, 38 tRNA and 8 rRNA genes. Twenty-one gene are partially or completely duplicated, including seven PCG (rpl2; rpsl23; ycf2; ndhB; rps12; rps7; ycf1), ten tRNA (two trnL-GAU, two trnA-UGC, trnL-CAA, trnL-CAU, trnR-ACG, trnV-GAC, trnN-GUU, trnH-GUG) and all four rRNA genes (4.5S, 5S, 16S & 23S rRNA). All the rRNA genes in the genome sequence were completely duplicated, including seven PCG (rpl2; rpsl23; ycf2; ndhB; rps12; rps7; ycf1), ten tRNA (two trnL-GAU, two trnA-UGC, trnL-CAA, trnL-CAU, trnR-ACG, trnV-GAC, trnN-GUU, trnH-GUG) and all four rRNA genes (4.5S, 5S, 16S & 23S rRNA).
The phylogenetic analysis included 26 complete chloroplast genomes, 22 Lilium species and four outgroups taxa. The phylogenomic relationship was inferred by the maximum likelihood (ML) method based on the general time-reversible (GTR) + Gamma substitution model in PhyML 3.0 (Larkin et al. 2007; Guindon et al. 2010), with 1000 bootstrap replicates (Letunic and Bork 2016). Phylogenetic analysis fully resolved *L. amoenum* in a clade containing *L. bakerianum* and *L. taliense* in a monophyletic Lilium. This complete cp genome provides valuable information for population genomic studies, DNA barcoding, and conservation genetics (Figure 1).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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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/](https://www.ncbi.nlm.nih.gov/) under the accession no. MT880912. The associated BioProject, SRA, and Bio-Sample numbers are SRP287370, SRX9292592, and SRS7519269, respectively.

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Figure 1. A phylogenetic tree of the *Lilium* species based on the completed chloroplast genomes of 22 species and 4 outgroup taxa. Bootstrap support values are cited at the nodes based on 1000 replicates.
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