

Complete chloroplast genome of *Hevea benthamiana*, a SALB-resistant relative wild species of rubber tree

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ABSTRACT

Hevea benthamiana is a SALB-resistant wild species of *H. brasiliensis*, the only source of mass production of high quality natural rubber. This study sequenced and analyzed the chloroplast genome of *H. benthamiana*. The chloroplast genome of *H. benthamiana* contains 161,124 bp and consists of 51,495 bp of A (31.96%), 52,022 bp of T (32.29%), 28,915 bp of G (17.95%), and 28,692 bp of C (17.81%). The ring-shaped genome includes four regions: a large single-copy region (LSC, 89,110 bp), a small single copy (SSC, 18,376 bp) region, and two inverted repeat regions (IRs, 26,819 bp). A total of 134 genes were annotated, of which 86 encode proteins; four are pseudogenes; 36 are tRNA genes, and eight are rRNA genes. Phylogenetic analyses showed that *H. benthamiana* is very closely related to *H. Brasiliensis*, this result indicates that *H. benthamiana* is highly valuable for the breeding of SALB-resistant varieties of rubber trees.

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Hevea benthamiana is a wild species of the rubber tree (*Hevea brasiliensis*), the only source of high quality natural rubber that can be produced on a large scale. The genus *Hevea* consists of 11 species (Gonçalves et al. 1999; Priyadarshan & Gonçalves 2002), but *H. benthamiana* can not only resist South American leaf blight (SALB), which is the most severe disease of rubber trees (Le Guen et al. 2008) caused by *Microcyclus ulei* (Garcia et al. 2011) and responsible for the limited development of rubber plantations in Latin America (Rocha et al. 2011) but it can also hybridize with rubber trees to produce disease-resistant varieties, such as IAN717 (Chee 1976; Lieberei 1986). This is particularly important because the use of resistant cultivars appears to be the most effective way to increase rubber tree plantings in South and Central America, as well as to anticipate an accidental introduction of SALB in Africa or Southeast Asia (Le Guen et al. 2011). *Hevea benthamiana* has been the subject of intensive efforts by rubber tree breeders since it is an important SALB-resistant parent.

Each plant contains at least three sets of relatively independent genetic information, including the nuclear, chloroplast and mitochondrial genomes. The chloroplast genome contains rich genetic information. Therefore, sequencing, assembling and annotating the chloroplast genome is highly important to clarify the genetic background and effective use of species resources. Currently, the nuclear genome of *H. brasiliensis* has been perfectly assembled (Rahman et al. 2013;

Lau et al. 2016; Tang et al. 2016; Pootakham et al. 2017; Liu et al. 2020), and the chloroplast genome of *H. brasiliensis* (Tangphatsornruang et al. 2011) and *H. camargoana* (Niu et al. 2020) have also been reported. As a relatively wild species of *H. brasiliensis*, the chloroplast genome of *H. benthamiana* has not yet been reported.

In this study, the chloroplast genome of *H. benthamiana* was sequenced, assembled, and annotated. Bronze-colored young leaves of *H. benthamiana* were collected from The Rubber Tree Germplasm Resource Nursery of the Chinese Academy of Tropical Agriculture Science (N 19°34'31.53" and E 109°31'17.97"), frozen in liquid nitrogen, and the genomic DNA was extracted using a Rapid Plant Genomic DNA Isolation Kit (Sangon Biotech Shanghai Co. Ltd., China) and stored in an ultra-low temperature specimen library at the Yunnan Institute of Tropical Crops (specimen accession number: YITC-2019-FZ-E-105). The genomic DNA of *H. benthamiana* was sequenced using the Illumina Hi-Seq 2000 platform (<http://www.illumina.com>, San Diego, CA, USA), and the chloroplast genome was assembled and annotated using CLC Genomics Workbench v3.6 (<http://www.clcbio.com>) and DOGMA (Wyman et al. 2004). The complete chloroplast sequence of *H. benthamiana* was submitted to GenBank with the accession number of MT333859.

The chloroplast genome of *H. benthamiana* contains 161,124 bp and consists of 51,495 bp of A (31.96%), 52,022 bp of T (32.29%), 28,915 bp of G (17.95%), and 28,692 bp of C

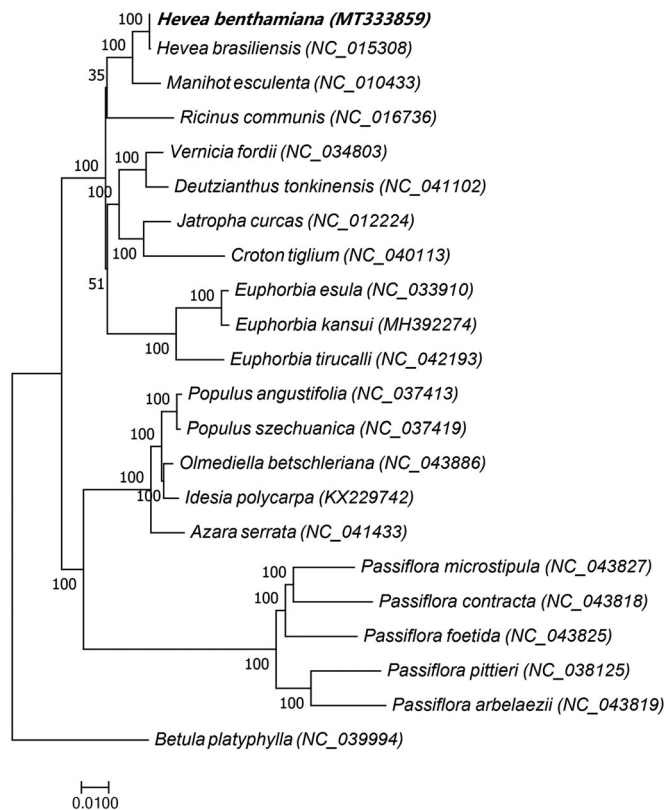


Figure 1. Maximum-likelihood phylogenetic tree based on chloroplast genome sequences of 21 Malpighiales order species, *Betula platyphylla*, which belongs to the Fagales order, was used as an outgroup. The bootstrap value was set to 1000. The species and chloroplast genome accession numbers for tree construction are *Hevea benthamiana* (MT333859), *Hevea brasiliensis* (NC_015308), *Manihot esculenta* (NC_010433), *Ricinus communis* (NC_016736), *Vernicia fordii* (NC_034803), *Deutzianthus tonkinensis* (NC_041102), *Jatropha curcas* (NC_012224), *Croton tiglium* (NC_040113), *Euphorbia esula* (NC_033910), *Euphorbia kansui* (MH392274), *Euphorbia tirucalli* (NC_042193), *Populus angustifolia* (NC_037413), *Populus szechuanica* (NC_037419), *Olmediella betschleriana* (NC_043886), *Idesia polycarpa* (KX229742), *Azara serrata* (NC_041433), *Passiflora microstipula* (NC_043827), *Passiflora contracta* (NC_043818), *Passiflora foetida* (NC_043825), *Passiflora pittieri* (NC_038125), *Passiflora arbelaezii* (NC_043819), and *Betula platyphylla* (NC_039994).

(17.81%). Like other plant species, the ring-shaped genome includes four regions: a large single-copy region (LSC, 89,110 bp), a small single copy (SSC, 18,376 bp) region, and two inverted repeat regions (IRs, 26,819 bp). A total of 134 genes were annotated, of which 86 encode proteins; four are pseudogenes, 36 are tRNA genes, and eight are rRNA genes.

The chloroplast genome sequences of 21 species of the Malpighiales order, including *H. benthamiana*, were used for phylogenetic analyses, and *Betula platyphylla*, which belongs to the Fagales order, was used as an outgroup (Figure 1). A phylogenetic tree was constructed using RAXML8.1.5 (<https://sco.h-its.org/exelixis/web/software/raxml/index.html>) (Alexandros 2006), with a bootstrap value of 1000. Phylogenetic analyses showed that *H. benthamiana* is very closely related to *H. brasiliensis*. This result indicates that *H. benthamiana* is highly valuable for the breeding of SALB-resistant varieties of rubber trees.

Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/>, reference number MT333859.

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