

The complete chloroplast genome of *Ocimum americanum* Linnaeus 1755 and phylogenetic analysis among the Lamiaceae family

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ABSTRACT

Ocimum americanum Linnaeus 1755 (Lemon basil) is an essential medicinal species in the *Ocimum* genus. Its leaf decoction is traditionally used to treat diabetes, constipation, diarrhea, piles, and dysentery. The essential oils from this species have intense fungicidal activity. The complete chloroplast genome sequence of *O. americanum* was assembled from Illumina paired-end sequencing data. The *O. americanum* chloroplast genome was 152,460 bp in length, containing a large single copy (LSC) region of 83,459 bp and a small single copy (SSC) region of 17,607 bp, separated by a pair of inverted repeats (IRs) of 25,697 bp. The genome contained 134 unique genes, including 89 protein-coding, 37 tRNA, and eight rRNA genes. Among them, nine genes had a single intron, and two genes contained two introns. The overall GC content of the chloroplast genome was 38%, while the corresponding values of LSC, SSC, and IR regions were 35.8%, 31.7%, and 43.1%, respectively. In the phylogenetic analysis, all the *Ocimum* species formed a group closely related to *Plectranthus barbatus*. *O. americanum* was more closely related to *O. gratissimum* and *O. basilicum* than the other species of *Ocimum* included in this study.

ARTICLE HISTORY

Received 10 May 2023
Accepted 24 September 2023

KEYWORDS



Chloroplast genome;
Lamiaceae; lemon basil;
Ocimum americanum;
phylogenetic analysis


Introduction

Ocimum americanum Linnaeus 1755 (Lemon Basil) is a potential traditional medicinal plant species. The decoction from the leaves of this species is commonly used for treating diabetes, constipation, diarrhea, piles, and dysentery (Bassole et al. 2005). The pharmacological activities of *O. americanum* are antioxidant, antifungal, antimicrobial, insecticidal, larvicidal, and gastric cytoprotective (Javanmardi et al. 2003, Vidhya et al. 2020). The estimated consumption of *O. americanum* as herbal medicine was 500–1000 MT (in dried condition). It is possibly used as a substitute or adulterant instead of *Ocimum tenuiflorum* (Ravikumar et al. 2018). The genus *Ocimum* contains around 65 species, and the key species used in herbal medicine are *O. americanum*, *O. basilicum*, *O. tenuiflorum*, and *O. gratissimum*. Classification of *Ocimum* species is challenging due to their significant morphological similarities and lack of genetic diversity. Species relationships were studied using morphological characters (Sobti and Pushpangadan 1979), pollen morphology (Harley et al. 1992), and DNA markers (Singh et al. 2004, Chen et al. 2013, Kumar et al. 2016). Singh et al. (2004) used Random Amplified Polymorphic DNA (RAPD) markers and classified five *Ocimum* species into two groups: one with *O. tenuiflorum* and *O. gratissimum*, and the other with *O. basilicum*, *O. kilimandscharicum*, and *O. americanum*. However, Chen



Figure 1. Reference image of the *Ocimum americanum* plant used in this study. This image was taken by the authors (Suresh Vineesh and Raju Balaji).

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 Supplemental data for this article can be accessed online <https://doi.org/10.1080/23802359.2023.2264545>.

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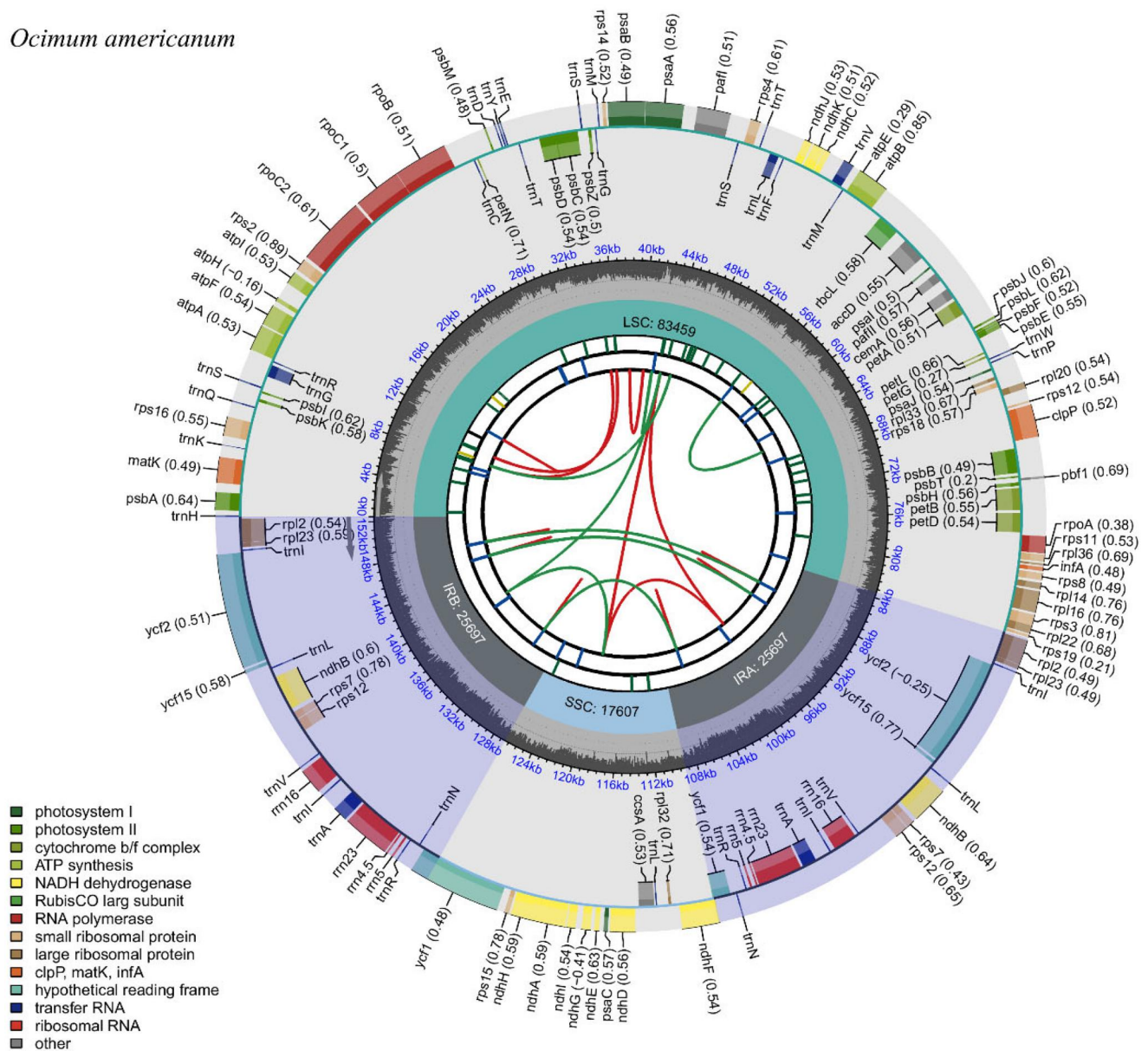
Ocimum americanum

Figure 2. Circular map of the chloroplast genome of *Ocimum americanum*. From the center going outward, the first circle shows the distribution of the repeats connected with red (the forward direction) and green (the reverse direction) arcs. The second circle displays the tandem repeats marked with short bars. The third circle shows the LSC, SSC, IRa, and IRb regions. The fourth circle shows the percentage of GC content. The next circle shows the genes having different colors based on the functional groups. The functional classification is shown at the bottom left. Genes inside the circle are transcribed in a clockwise direction, and those outside are in a counter-clockwise direction.

et al. (2013) reported that *O. gratissimum* was closely related to *O. americanum* and *O. basilicum*. Based on the analysis of the *psbA-trnH* sequence, Kumar et al. (2016) classified the *Ocimum* species into *Gratissimum* group with *O. gratissimum* and *O. americanum* and *Sanctum* group with *O. tenuiflorum* and *O. adscendens*. In this study, we sequenced and assembled the complete chloroplast genome of *O. americanum* using Illumina paired-end sequencing data to contribute to the systematics, DNA barcoding, molecular markers, and phylogenetic analysis within the *Ocimum* genus.

Materials and methods

Fresh plant material of *O. americanum* (Figure 1) was collected from the wasteland in Ozhavetti village, Madhurandhagam,

Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°29'49.4"N 79°52'32.9"E). The voucher specimen (MH178052) was deposited in the Madras Herbarium, Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, Tamil Nadu, India (<https://bsi.gov.in/regional-centres/en?rcu=133>, Dr. M. U. Sharief, Scientist-E and Head of Office, email: sc@bsi.ov.in). *O. americanum* is a small and erect plant with lanceolate or ovate-lanceolate leaves, Apex of the leaf is acute, the margin is entire or sparsely serrate, and leaves are pubescent beneath with long hairs on midrib and lateral veins. Stems are quadrangular in shape and purple to brown in color, and the flowers are white (Suddee et al. 2004). Total genomic DNA from *O. americanum* was extracted following the Cetyltrimethyl ammonium bromide method (Doyle and Doyle 1987), with minor modifications (Nithaniyal et al. 2014).

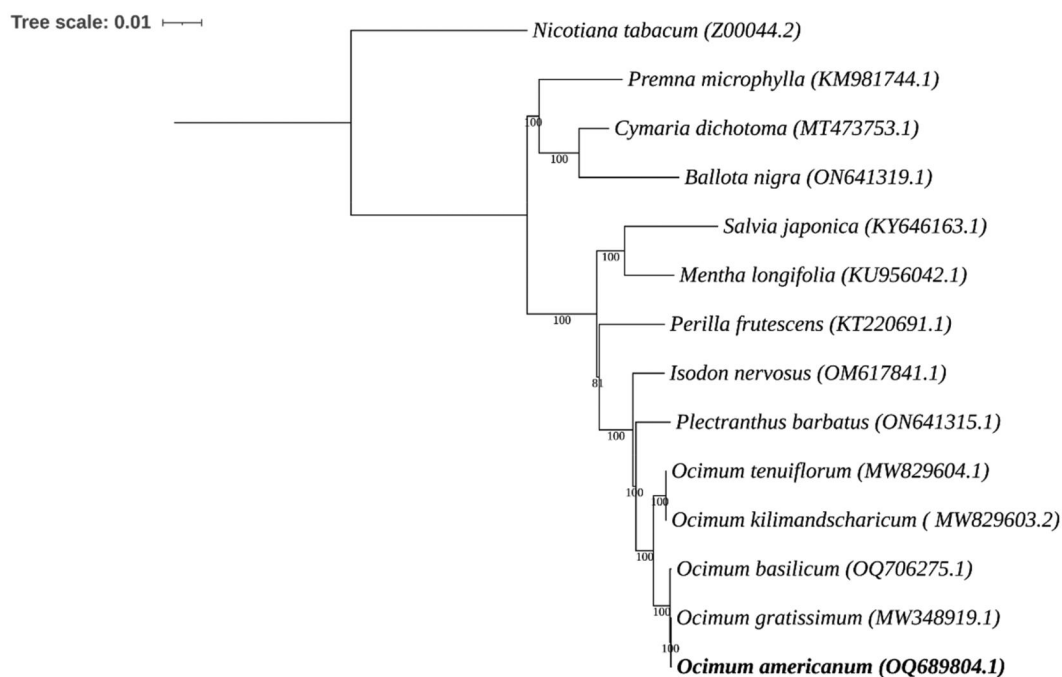


Figure 3. Phylogenetic tree based on the whole chloroplast genome sequences of 13 species from Lamiaceae family, including the chloroplast genome of *O. americanum* (OQ689804) from this study. *Nicotiana tabacum* (Solanaceae) was used as an outgroup. The bootstrap support values are shown on the nodes. The following sequences were used: *O. americanum* OQ689804.1 (this study), *O. gratissimum* MW348919.1 (Balaji et al. 2021), *O. basilicum* OQ706275.1 (Rabah et al. 2017), *O. tenuiflorum* MW829604.1 (Harini et al. 2021), *O. kilimandscharicum* MW829603.2 (Renald et al. 2021), *Plectranthus barbatus* ON641315.1, *Isodon nervosus* OM617841.1, *Perilla frutescens* KT220691.1, *Mentha longifolia* KU956042.1 (Vining et al. 2017), *Salvia japonica* KY646163.1 (He et al. 2017), *Premna microphylla* KM981744.1 (Yang and Kong 2016), *Ballota nigra* ON641319.1, *Cymaria dichotoma* MT473753.1 (Zhao et al. 2021), and *Nicotiana tabacum* Z00044.2 (Shinozaki et al. 1986).

According to the manufacturer's procedure, a paired-end DNA library was constructed using the Nextera XT Library Prep Kit (Cat. No. FC-131-1024). The library was sequenced on the Illumina Novoseq 6000 platform (Illumina Inc., San Diego, CA) with a paired-end sequencing length of 150 bp. Paired-end sequencing generated 2.3 Gb data with q30 bases of more than 87%. The chloroplast genome of *O. americanum* was assembled using NovoPlasty (k-mer 31) with *O. gratissimum* L. (Balaji et al. 2021) as a reference seed sequence (Dierckxsens et al. 2017). The assembled complete chloroplast genome of *O. americanum* was annotated with GeSeq (Tillich et al. 2017). The predicted transfer RNAs (tRNAs) were identified by tRNAscan-SE 2.0 (Lowe and Chan 2016). In addition, the CPGView (www.1kmpg.cn/cpgview/; Liu et al. 2023) was applied to structures to visualize the intron-containing genes. The sequencing depth of the assembled chloroplast genome was done by aligning to the raw reads using a BWA aligner (Li and Durbin 2009). The bam file was viewed using the software Qualimap for the coverage map (Fernando et al. 2012). A phylogenetic tree was constructed with 1000 bootstrap replicates using RAxML (Random Axelerated Maximum Likelihood) version 8 (Stamatakis 2014) from the alignments created using the MAFFT program (Katoh and Standley 2013).

Results and discussion

The assembled complete chloroplast genome sequence of *O. americanum* was 152,460 bp with a mean coverage of 128 \times . The circular map of the chloroplast genome is shown in Figure 2. It showed a typical quadripartite structure, including an LSC (large single copy) region of 83,459 bp, an SSC (small

single copy) region of 17,607 bp, and a pair of inverted repeats (IRs) of 25,697 bp. The overall GC content was 38%, while LSC, SSC, and IR regions were 35.8%, 31.7%, and 43.1%, respectively. The chloroplast genome contained 89 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Nine genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl16*, *rpl2*, *rpoC1*, *rps16*) were with a single intron, and two genes (*pafl* and *clpP*) contained two introns (Supplementary Figures 1 and 2). The minimum, maximum, and average depth of coverage of the assembled chloroplast genome was 10 \times , 697 \times , and 109 \times , respectively (Supplementary Figure 3). The complete chloroplast genome of *O. americanum* with annotations was submitted to GenBank (Accession No. OQ689804). The raw reads were deposited to NCBI under the Sequence Read Archive database (Accession No. SRR23955912).

The complete chloroplast genome sequences of 13 species were retrieved from the National Center for Biotechnology Information (NCBI) to conduct the phylogenetic analysis. The complete chloroplast genome sequences were subjected to multiple sequence alignment using the MAFFT tool. The phylogenetic tree was constructed using RAxML (Random Axelerated Maximum Likelihood; Stamatakis 2014). The tree included the chloroplast genomes of 14 species, including *O. americanum* from this study, 13 from the Lamiaceae family, and *N. tabacum* from Solanaceae as an outgroup. The complete chloroplast genome sequences were utilized to understand the phylogenetic relationships among species (Figure 3). All the species of *Ocimum* formed a clade, and this group was closely related to *Plectranthus barbatus*. *O. americanum* was closely related to *O. gratissimum* and *O. basilicum* within *Ocimum*. This relationship was consistent

with a previous report based on RAPD and ISSR markers (Chen et al. 2013). The application of chloroplast genome-based analysis demonstrated a more robust species resolution within the Lamiaceae family compared to the resolutions observed using morphological characters (Sobti and Pushpangadan 1979) and RAPD markers (Singh et al. 2004). This complete chloroplast genome of *O. americanum* can be subsequently used for phylogenetic analysis, DNA barcoding, and authentication of lemon basil.

Conclusion

In this study, the chloroplast genome sequence of *Ocimum americanum* was assembled and annotated for the first time. The phylogenetic analysis showed that the chloroplast genome of *O. americanum* is closely related to *O. gratissimum* and *O. basilicum* within the Lamiaceae family. This study provides valuable chloroplast genome resources of the genus *Ocimum*, which lay the foundation for the study of phylogenetic relationships within the Lamiaceae family and the development of species-specific molecular markers for plant authentication of herbal drugs.

Author contributions

S. Vineesh, R. Balaji, and Tanuja collected the specimen material, conducted the experiment, analyzed the sequence data, and drafted the paper. M. Parani contributed to the conception and design of this work. All the authors carefully read, revised, and approved the final manuscript to be published. We thank Dr. D. Narasimhan for his help in the authentication of plant material and for providing comments and suggestions on the final manuscript.

Ethical approval

No permissions were required for the sample collection of *Ocimum americanum* L. because it is widely distributed in the wastelands and roadsides in tropical regions. The plant species was collected from Ozhavetti village, Madhurandhagam, Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°29'49.4"N 79°52'32.9"E).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We acknowledge the funding from the SRM-DBT Partnership Platform for Contemporary Research Services and Skill Development in Advanced Life Sciences Technologies [Grant No. BT/PR12987/INF/22/205/2015] and SRM Institute of Science and Technology for providing research and computation facilities.

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Data availability statement

The data supporting this study's findings are publicly available in the NCBI database. The complete chloroplast genome of *O. americanum* was deposited in GenBank under the accession number OQ689804 (<https://www.ncbi.nlm.nih.gov/nuccore/OQ689804.1>). The next-generation sequencing data files are available from the SRA, Bio-Sample, and BioProject ID under the accession numbers PRJNA948343, SAMN33896533, and SRR23955912, respectively.

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