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PLASTOME ANNOUNCEMENT

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The complete chloroplast genome of *Ocimum basilicum* L. var. *basilicum* (Lamiaceae) and its phylogenetic analysis

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ABSTRACT

Ocimum basilicum L. var. *basilicum* (Sweet Basil) is an aromatic herb belonging to the family Lamiaceae and is known for its medicinal uses. It is commonly used in traditional medicine for its therapeutic value, including anti-allergic, anti-inflammatory, antioxidant, antitumor, and antimicrobial properties. In this study, we generated the complete chloroplast genome sequence of *O. basilicum* var. *basilicum* using Illumina paired-end sequencing data. The chloroplast genome was 152,407 bp in length, containing a large single-copy (LSC) region of 83,409 bp and a small single-copy region (SSC) of 17,604 bp, separated by a pair of inverted repeats (IRs) of 25,697 bp. The genome contained 134 genes, including 89 protein-coding, 37 tRNA, and eight rRNA genes. Nine genes had one intron, two genes had two introns, and others did not have any intron. Overall GC content of the chloroplast genome was 38%, while that of LSC, SSC, and IR regions was 35.9%, 31.6%, and 43.1%, respectively. Phylogenetic analysis of the chloroplast genomes revealed that *O. basilicum* var. *basilicum* was closely related to *Ocimum basilicum* from the *Ocimum* species.

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Introduction

Ocimum basilicum L. is used in traditional medicine for treating lung and respiratory disorders (Aminian et al. 2021). The leaves and flowers of sweet basil are used to treat insect stings, snake bites, and skin illnesses (Rudayni et al. 2021). The roots and seeds were used to treat bleeding piles, respiratory disorders, hemorrhage, and fever. The estimated consumption of the dried *O. basilicum* plants in herbal medicine was 1000–2000 MT. It is possibly used as a substitute or adulterant in *Ocimum americanum* because of their common trade names, 'Kali tulsi' and 'Ban tulsi' (Ravikumar et al. 2018). *O. basilicum* is abundant in phytochemicals compounds such as alkaloids, tannins, flavonoids, and saponins, which possess anti-inflammatory, antioxidant, antiviral, and antimicrobial activities (Shahrajabian et al. 2020; Kurnia et al. 2023). Six varieties were described in *O. basilicum*, and the morphological variations among the varieties of *O. basilicum* were well-studied (Rawat et al. 2016). In this study, we describe the chloroplast genome of one of the varieties, *O. basilicum* var. *basilicum*. It is a shrub that grows up to 97.5 cm in height. Leaves are elliptic to egg-shaped and are up to 8 cm long. The stem and lamina are purple to green in color (Rawat et al. 2016). The pubescence on the stem and lamina is medium. The color of the flowers is pinkish-white. In recent years, the complete chloroplast genomes of *Ocimum* species have been reported (Balaji et al. 2021; Harini et al. 2021; Kavya et al. 2021). However, the complete chloroplast genome of *Ocimum basilicum* variety *basilicum* remains to be studied. In this study, we sequenced the chloroplast genome of *O. basilicum* var. *basilicum*, which provides information for further taxonomic and DNA barcoding studies among the *Ocimum* species.

Materials and methods

Plant material of *O. basilicum* var. *basilicum* (Figure 1) was collected from Pallavaram, Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°58′01.4″N 80°08′42.3″E). The herbarium specimen was prepared and authenticated by the taxonomist (Dr. Senthilkumar Umapathy, Department of Botany, Madras Christian College, Tambaram, Tamil Nadu, India; email: sensonsam85@gmail.com). The voucher specimen was deposited under the accession number MH178193 in the Madras Herbarium (MH) of the Botanical Survey of India, Southern Regional Centre, T.N.A.U. Campus, Coimbatore, Tamil Nadu, India (https://bsi.gov.in/regional-centres/en?rcu=133, Dr. M. U. Sharief, Scientist-F and Head of Office, email: sc@bsi.gov.in). Total genomic DNA from *O. basilicum* var. *basilicum* was extracted using the CTAB method (Doyle and

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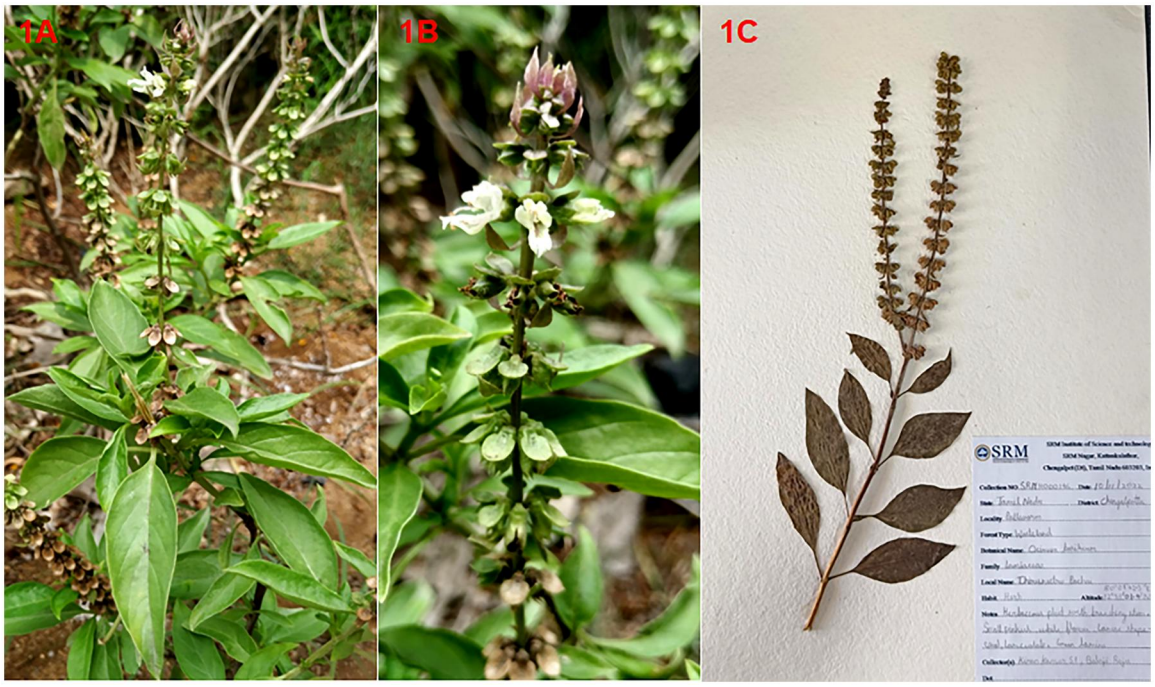

Figure 1. The picture of the collected *Ocimum basilicum* var. *basilicum* sample. This image shows the whole plant (A), inflorescence (B), and herbarium specimen (C), which were recorded by the authors (Kirankumar S.I. and Raju Balaji).

Doyle 1987), with minor modifications (Balaji and Parani 2022). From the genomic DNA, a paired-end DNA library was constructed using the Nextera XT Library Prep Kit (Cat. No. FC-131-1024), according to the manufacturer's protocol. The library was sequenced on the Illumina Novoseq 6000 platform (Illumina Inc., San Diego, CA) with a paired-end sequencing length of 150 bp. We generated 3.15 Gb of data with more than 86% q30 bases. The complete chloroplast genome of *O. basilicum* var. *basilicum* was assembled using the program GetOrganelle v1.7.5 (Jin et al. 2020), with *O. basilicum* chloroplast genome (NC_035143.1) as a reference. The assembled chloroplast genome of *Ocimum basilicum* var. *basilicum* 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 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 to obtain the coverage map (Fernando et al. 2012). The phylogenetic analysis was performed based on the complete chloroplast genome sequences of 20 species from the Lamiales order. A maximum-likelihood tree was generated using 1000 bootstrap replicates, and the best substitution model of Timura-3 parameter, G + I, in MEGA version 11.0.13 (Tamura et al. 2021) from the alignments created using the MAFFT program (Katoh and Standley 2013).

Results and discussion

The chloroplast genome sequence of *O. basilicum* var. *basilicum* was 152,407 bp with a mean coverage of 446× (Figure 2). It showed a typical quadripartite structure, including a large single-copy (LSC) region of 83,409 bp, a small single-copy (SSC) region of 17,604 bp, and a pair of inverted repeats (IRs) of 25,697 bp. The chloroplast genome contained 89 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Nine protein-coding genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, and *rps16*) were single-intron genes, and two genes (*ycf3*, *clpP1*) had two introns (Supplementary Figures 1 and 2). The overall GC content was 38%, while LSC, SSC, and IR regions were 35.9%, 31.6%, and 43.1%, respectively. The average depth of assembled chloroplast genome is 775.90× (Supplementary Figure 3). The complete chloroplast genome of *O. basilicum* var. *basilicum* with supportive annotations was submitted to GenBank under the accession number OQ706275.1. The raw reads were deposited in the GenBank Sequence Read Archive (accession no. SRR23991168).

The complete chloroplast genome sequences of 19 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 alignments using the MAFFT tool (Katoh and Standley 2013). The phylogenetic tree was constructed using MEGA software (Tamura et al. 2021). The tree included the chloroplast genomes of 20 species within the Lamiales order, including *O. basilicum* var. *basilicum* from this study, 18 species from the Lamiaceae family, and *Avicennia marina* from the Acanthaceae as an outgroup. The complete chloroplast genome sequences were utilized to understand the phylogenetic relationship among the species (Figure 3). All the species of *Ocimum* formed a clade, and this group was closely related to *Hanceola exserta*. Chloroplast genome-based analysis has yielded a more robust species resolution within the Lamiaceae family, surpassing the resolution achieved through morphological characters (Sobti and Pushpangadan 1979) and RAPD markers (Singh et al. 2004). Within the *Ocimum* genus, our

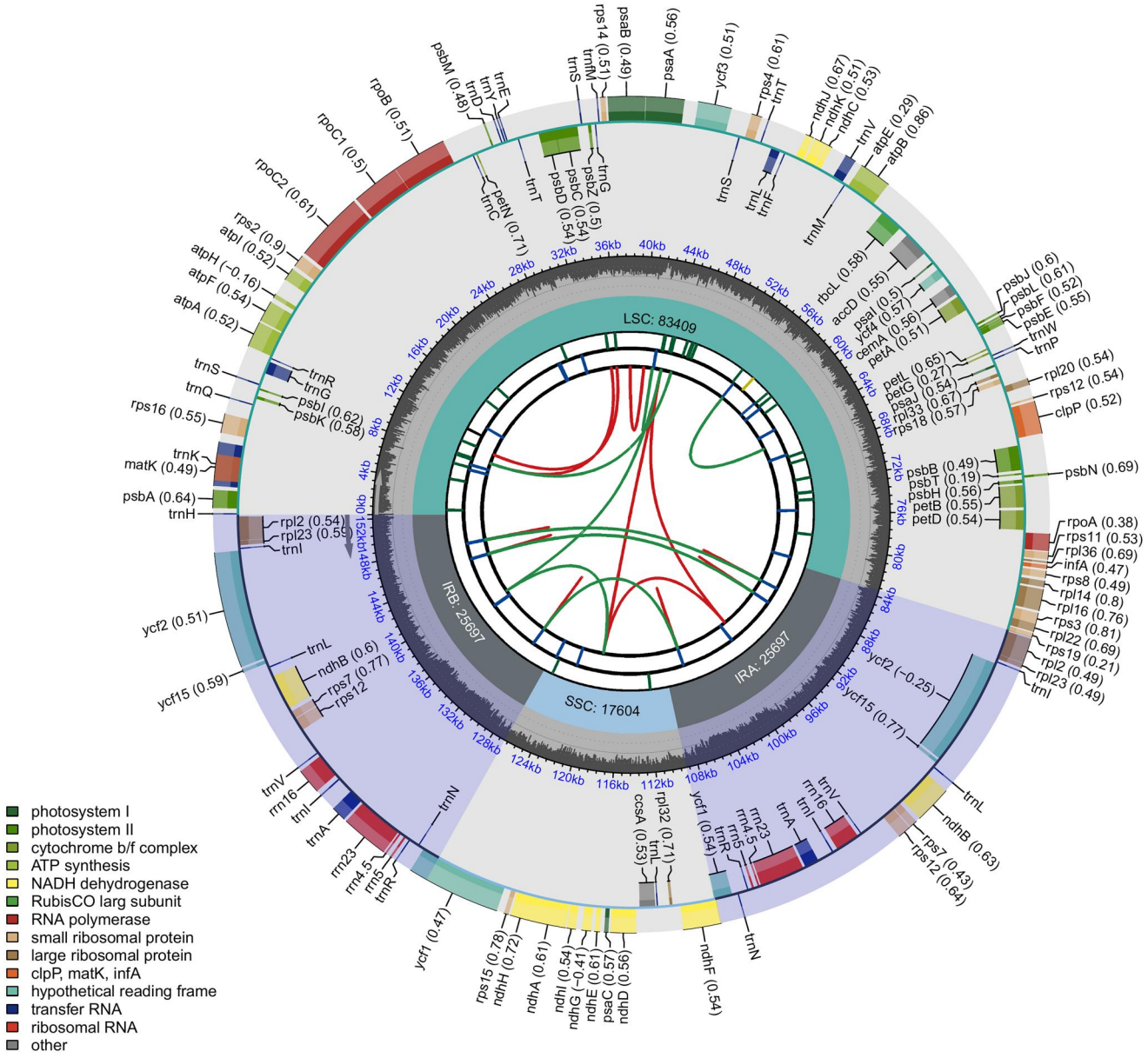

Figure 2. Circular map of the chloroplast genome of *O. basilicum* var. *basilicum*. From the center going outward, the first circle shows the distribution of the repeats connected with red (the forward direction) and green (the reverse direction) arcs. The second circle displays the tandem repeats marked with short bars. The third circle shows the LSC, SSC, IRa, and IRb regions. The fourth circle shows the percent 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.

findings indicate a close relationship between *O. basilicum* var. *basilicum* and *O. gratissimum*, as well as *O. tenuiflorum*, which aligns with prior research using chloroplast DNA barcoding markers such as *rbc*L, *mat*K, and *psb*A-*trn*H (Christina and Annamalai 2014). The complete chloroplast genome of *O. basilicum* var. *basilicum* described in detail in this study can be subsequently used for phylogenetic analysis, DNA barcoding, and molecular marker studies for the differentiation at the *O. basilicum* at variety level.

Conclusions

In this study, the chloroplast genome sequence of *O. basilicum* var. *basilicum* was sequenced and annotated. The phylogenetic analysis showed that the chloroplast genome of *O. basilicum* var. *basilicum* is closely related to *O. gratissimum* and other *Ocimum* species within the Lamiaceae family. This study provides valuable chloroplast genome resources of the genus *Ocimum*, which lay the foundation for the study of phylogenetic analysis and molecular markers to confirm the authenticity of sweet basil (*O. basilicum*) and its herbal products.

Author contributions

S.I. Kirankumar, 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 providing his

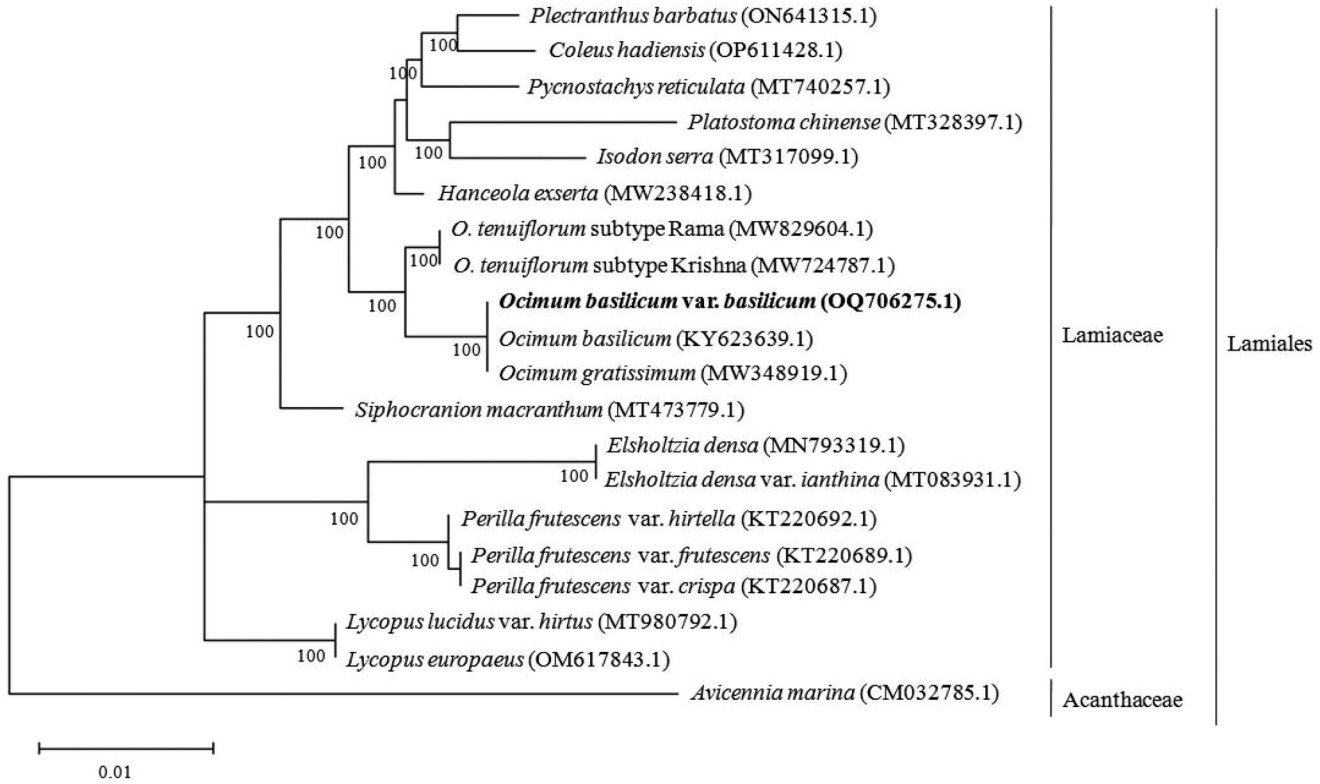

Figure 3. Phylogenetic tree constructed by maximum-likelihood (ML) analysis based on complete chloroplast genome sequences, including *O. basilicum* var. *basilicum* (OQ706275.1) sequenced in this study. The numbers on the nodes indicate bootstrap values with 1000 replicates. The sequences used for tree construction are as follows: *Ocimum basilicum* (KY623639.1; Rabah et al. 2017), *O. gratissimum* (MW348919.1; Balaji et al. 2021), *O. tenuiflorum* subtype Rama (MW829604.1; Harini et al. 2021), *O. tenuiflorum* subtype Krishna (MW724787.1; Kavya et al. 2021), *Platostoma chinense* (MT328397.1), *Pycnostachys reticulata* (MT740257.1; Wu et al. 2021), *Plectranthus barbatus* (ON641315.1), *Coleus hadiensis* (OP611428.1), *Hanceola exserta* (MW238418.1; Zhu et al. 2023), *Isodon serra* (MT317099.1; Zhang et al. 2020), *Siphocranion macranthum* (MT473779.1; Zhao et al. 2021), *Lycopus lucidus* var. *hirtus* (MT980792.1; Wang et al. 2021), *Lycopus europaeus* (OM617843.1), *Elsholtzia densa* (MN793319.1; Fu et al. 2020), *Elsholtzia densa* var. *ianthina* (MT083931.1; Yang et al. 2020), *Perilla frutescens* var. *hirtella* (KT220692.1), *P. frutescens* var. *frutescens* (KT220689.1), *P. frutescens* var. *crispa* (KT220687.1), and *Avicennia marina* (CM032785.1; Natarajan et al. 2021).

comments and suggestions on the final manuscript and Dr. Senthilkumar Umapathy for his help in the authentication of plant material.

Ethical approval

No permissions were required for the sample collection of *O. basilicum* var. *basilicum*, because it is widely distributed in the wastelands and roadsides in the tropical regions. The plant species was collected from Pallavaram, Chengalpattu District, Tamil Nadu, India (GPS coordinates: 12°58′01.4″N 80°08′42.3″E).

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings of this study are openly available in NCBI (https://www.ncbi.nlm.nih.gov/). The complete chloroplast genome of *Ocimum basilicum* var. *basilicum* was deposited in GenBank under the accession OQ706275 (https://www.ncbi.nlm.nih.gov/nuccore/OQ706275). The associated NGS sequencing data files are available from the BioProject, Bio-Sample, and SRA submission under the accession numbers PRJNA949446, SAMN33944295, and SRR23991168, respectively.

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