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PLASTOME REPORT

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Chloroplast genome of *Ocimum basilicum* var. *purpurascens* Bentham 1830 (Lamiaceae)

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ABSTRACT

Ocimum basilicum var. *purpurascens* Bentham 1830 (Red Rubin Basil) is an aromatic herb belonging to the family Lamiaceae and is known for its medicinal uses. It is commonly used in traditional medicine to treat cardiovascular diseases and obesity. It possesses anti-inflammatory, antioxidant, antifungal, and anti-spasmodic properties. In our recent study, we assembled the chloroplast genome sequence of *O. basilicum* var. *purpurascens* using Illumina paired-end sequencing technology. The assembled chloroplast genome was 152,407 base pairs (bp), inclusive of a large single-copy (LSC) region accounting for 83,409 bp and a small single-copy (SSC) region spanning 17,604 bp. Two inverted repeats (IRs) interspersed these regions, each 25,697 bp long. The chloroplast genome harbored 132 genes, comprising 88 protein-coding genes, 36 transfer RNA (tRNA), and eight rRNA genes. Among these, nine genes encompassed a single intron, two presented with two introns, with the remaining devoid of any introns. The overall GC content of the chloroplast genome was determined to be 38%. The GC content in the LSC, SSC, and IR regions was 35.9%, 31.6%, and 43.1%, respectively. Our phylogenetic exploration of the chloroplast genomes elucidated that *O. basilicum* var. *purpurascens* exhibits close genetic affinity with *O. basilicum* var. *basilicum* and other constituents of the *Ocimum* genus within the Lamiaceae family.

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Introduction

Ocimum basilicum var. *purpurascens* Bentham 1830, commonly known as Red Rubin Basil, has a history of use in traditional medicine for the treatment of cardiovascular diseases, obesity, and the management of metabolic disorders (Gerardi et al. 2015). This variety exhibits various beneficial properties, including anti-inflammatory, antioxidant, antifungal, and anti-spasmodic effects (Carocho et al. 2016; Bhatti et al. 2017). Notably, it is prized for its rich anthocyanin content, primarily found in its leaves. These attributes make it also a valuable source of natural food colorants for the food industry (Fernandes et al. 2019). Within the *O. basilicum* species, six distinct varieties have been identified, with comprehensive investigations into their morphological differences (Rawat et al. 2016). In the case of the variety *Purpurascens*, distinctive features include purple or pink-colored bracts, calyces, and corolla. The leaves are convex-shaped, and the inflorescence is laxly branched. The stem and lamina exhibit a striking purple hue. Recently, the chloroplast genomes of various *Ocimum* species and varieties or subtypes have been sequenced. These include *O. americanum* (Vineesh et al. 2023), *O. basilicum* var. *basilicum* (Kirankumar et al. 2023), *O. gratissimum* (Balaji et al. 2021), *O. kilimandscharicum* (Renald et al. 2021), *O. tenuiflorum* subtype Rama (Harini et al. 2021), and *O. tenuiflorum* subtype Krishna (Kavya et al. 2021). However, the chloroplast genome of *O. basilicum* var. *purpurascens* remains unexplored. In this study, we sequenced the chloroplast genome of this variety, which contributes valuable data for future taxonomic classification and DNA barcoding studies within the *Ocimum* genus.

Materials and methods

Plant material, DNA extraction, and sequencing

Fresh plant material of *O. basilicum* var. *purpurascens* was collected from Hanumantha Nagar, Hosur, Hosur District, Tamil Nadu, India (Figure 1; GPS coordinates: 12°45′18.0″N 77°48′53.1″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 in the Herbarium of the SRM Institute of Science and Technology (voucher number: SRMH000147). Total genomic DNA from the fresh

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Figure 1. Reference image of *O. basilicum* var. *purpurascens* used in this study. (A) Collected samples and habitat and (B) voucher specimen.

leaves of *O. basilicum* var. *purpurascens* was extracted using the CTAB method (Doyle and Doyle 1987), with minor modifications (Balaji and Parani 2022). From the genomic DNA, a DNA library (660 bp average insert size) 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, San Diego, CA) with a paired-end sequencing length 150 bp.

Chloroplast genome assembly and annotation

The chloroplast genome of *O. basilicum* var. *purpurascens* was assembled using NovoPlasty software (Dierckxsens et al. 2017) and the *rbcL* gene of *O. gratissimum* (MW348919.1) as the seed sequence. The assembled chloroplast genome of *O. basilicum* var. *purpurascens* was annotated with GeSeq (Tillich et al. 2017). The predicted transfer RNAs (tRNAs) were identified by tRNAscan-SE (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. SAM file was generated using BWA aligner and converted into BAM file by SAMtools (Li and Durbin 2009). The depth of coverage was assessed using the Python scripts described by Ni et al. (2023).

Phylogenetic analysis

To confirm the phylogenetic position of *O. basilicum* var. *purpurascens*, the chloroplast genomes of 19 species from Lamiaceae were used. *Avicennia marina* from the family Acanthaceae of Lamiales was used as an outgroup. The chloroplast genome sequences from 20 species were aligned using MAFFT v. 7.4 (Katoh and Standley 2013). A phylogenetic tree was constructed using the maximum-likelihood method with best fit model of T92 + G and implemented in MEGA version 11.0.13 (Tamura et al. 2021). The bootstrap analysis was performed with 1000 replicates.

Results

General features of the chloroplast genome

We used 18,481× quality-filtered data (2.8 GB) and assembled the entire chloroplast genome without any gap. After the assembly, the average depth of coverage of individual bases was 158× (Figure S3). The assembled chloroplast genome of *O. basilicum* var. *purpurascens* was a typical circular tetrameric structure of 152,407 bp in length (Figure 2), consisting of a large single-copy (LSC) region (83,409 bp), a small single-copy (SSC) region (17,604 bp) and a pair of inverted repeats (IRs) (25,697 bp). The percentage of the four bases in the whole chloroplast genome of *O. basilicum* var. *purpurascens* was 31.43% A, 31.43% T, 18.83% G, and 18.30% C. The chloroplast genome encoded 132 unique genes, including 88 protein-coding genes, 36 tRNA genes, and eight rRNA genes. Nine protein-coding genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps16*) were single-intron genes, and two genes (*pafI*, *clpP1*) had two introns (Figures S1 and S2). The chloroplast genome had a total GC content of 38%. The GC content of the IR region (43.1%) was significantly higher than that of the LSC region (35.9%) and SSC region (31.6%). The complete chloroplast genome of *O. basilicum* var. *purpurascens* with supportive annotations was submitted to GenBank under the accession number OR478166.1. The raw reads were deposited

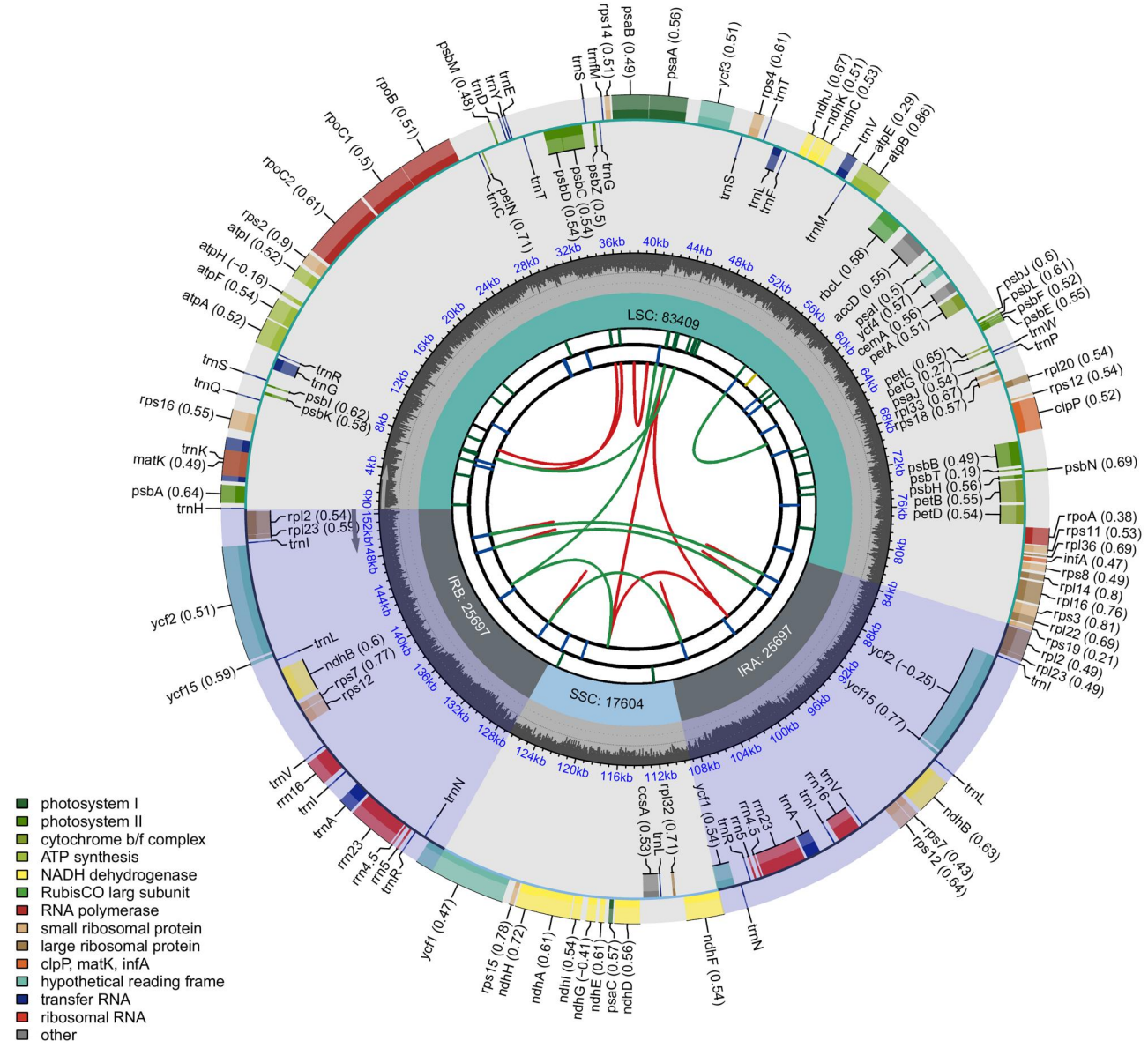

Figure 2. The complete chloroplast genome map of *O. basilicum* var. *purpurascens*. Genes on the inside of the circle are transcribed in a clockwise direction, and genes on the outside of the circle are transcribed in a counter-clockwise direction.

in the GenBank Sequence Read Archive (accession no. SRR25760192).

Phylogenetic analysis

Phylogenetic analysis was performed to understand the phylogenetic relationship of *O. basilicum* var. *purpurascens* with other species. The maximum-likelihood tree was constructed using MEGA with the best-fit model of T92 + G (T92: Tamura 3 parameter). The phylogenetic analysis showed high bootstrap values for most of the nodes in the phylogenetic tree (Figure 3). Our analysis clearly showed that *O. basilicum* var. *purpurascens* was more closely related to *O. basilicum* var. *basilicum* and other species of *Ocimum* within the Lamiaceae family. The complete chloroplast genome of *O. basilicum* var. *purpurascens* described in detail in this study can be subsequently used for phylogenetic analysis, DNA barcoding, and molecular marker studies for the differentiation at the species and variety level.

Discussion

Compared with nuclear and mitochondrial genomes, chloroplast genomes are highly conserved and widely used in phylogenetic and evolutionary studies. With the development of high-throughput sequencing technology, chloroplast genome sequences play an essential role in phylogenetic research (Li et al. 2021). This study's phylogenetic relationship based on the complete chloroplast sequences showed high bootstrap support. The phylogenetic tree was in agreement with the previous studies (Christina and Annamalai 2014; Jürges et al. 2018), which showed that *O. basilicum* var. *purpurascens* was more closely related to *O. basilicum* var. *basilicum*, and other *Ocimum* species level. This study addresses a knowledge gap by sequencing the chloroplast genome of

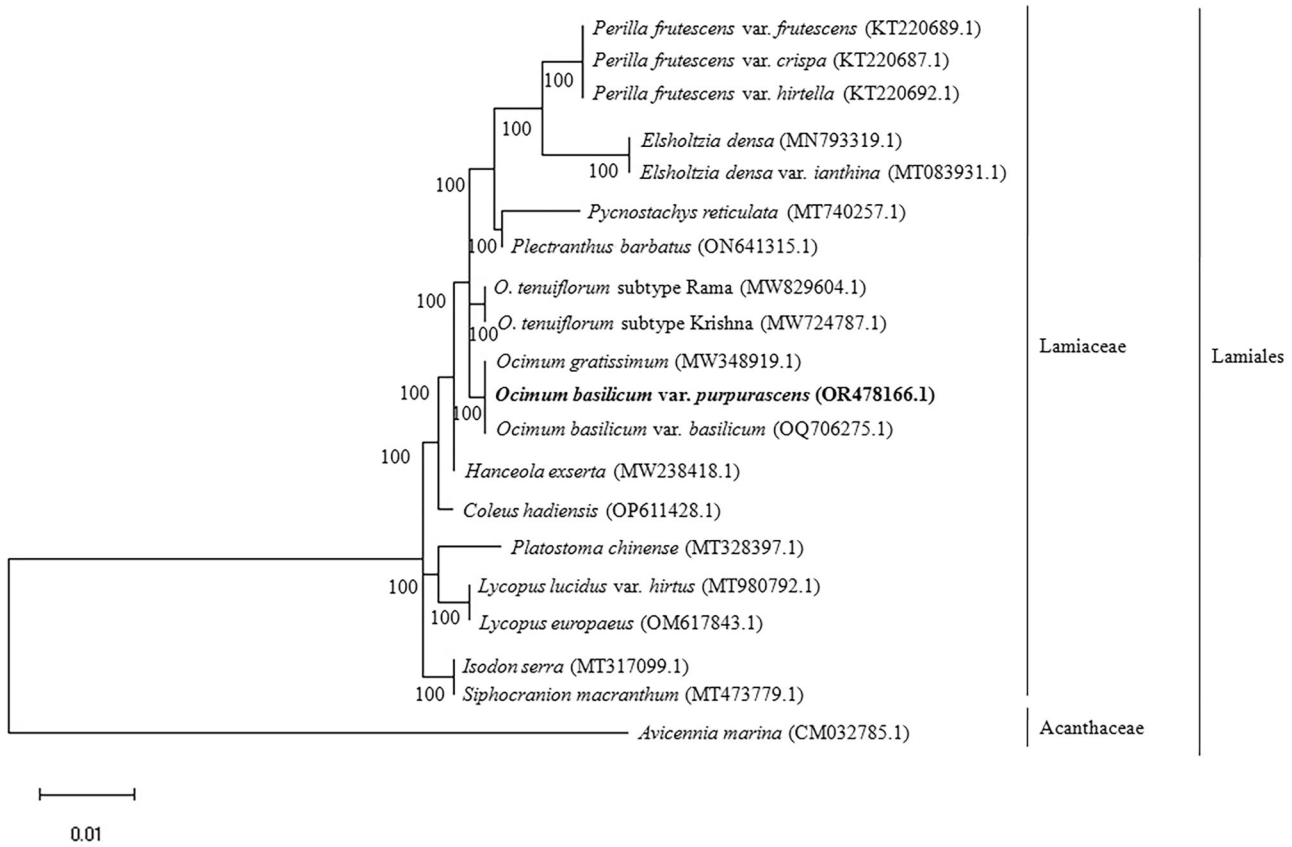

Figure 3. Phylogenetic tree constructed by maximum-likelihood (ML) analysis based on complete chloroplast genome sequences, including *O. basilicum* var. *purpurascens* (OR478166.1) * sequenced in this study. The numbers on the nodes indicate bootstrap values with 1000 replicates. The sequences used for tree construction are as follows: *O. basilicum* var. *purpurascens* (OR478166.1; this study), *O. basilicum* var. *basilicum* (OQ706275.1; Kirankumar et al. 2023), *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), *L. europaeus* (OM617843.1), *Elsholtzia densa* (MW793319.1; Fu et al. 2020), *Elsholtzia densa* var. *ianthina* (MT083931.1; Yan-Ci 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).

O. basilicum var. *purpurascens*. This contributes vital data for *Ocimum* genus taxonomy and DNA barcoding. Significantly, it enhances the understanding of the phylogenetic relationships within the Lamiaceae family, and helps to develop species-specific molecular markers, crucial for authentication of the *Ocimum* species in herbal trade.

Conclusions

In this study, the chloroplast genome sequence of *O. basilicum* var. *purpurascens* was sequenced, *de novo* assembled, and annotated. In addition, the phylogenetic tree strongly supported the phylogenetic position of *O. basilicum* var. *purpurascens*, showing that *O. basilicum* var. *purpurascens* was closer to *O. basilicum* var. *basilicum* and other *Ocimum* species. Thus, the chloroplast genome of *O. basilicum* var. *purpurascens* not only enriches the genomic information of *Ocimum* but also lays the foundation for understanding the evolution of Lamiaceae species. This study provides valuable chloroplast genome resources of the genus *Ocimum*, which will help to develop species-specific markers to authenticate *O. basilicum* herbal drug at the variety level.

Ethical approval

No permission was required to collect *O. basilicum* var. *purpurascens* because it is widely distributed in the wastelands and roadsides in tropical regions. The plant species was collected from Hanumantha Nagar, Hosur, Hosur District, Tamil Nadu, India (GPS coordinates: 12°45′18.0″N 77°48′53.1″E).

Author contributions

A Venkatesan, 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. K. Devanathan for helping to collect the plant material and Dr. Senthilkumar Umapathy for authentication.

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 NCBI (https://www.ncbi.nlm.nih.gov/). The complete chloroplast genome of *O. basilicum* var. *purpurascens* was deposited in GenBank under the accession OR478166 (https://www.ncbi.nlm.nih.gov/nuccore/OR478166). The associated NGS sequencing data files are available from the BioProject, Bio-Sample, and SRA submission under the accession numbers PRJNA1008863, SAMN37131994, and SRR25760192, respectively.

References

Balaji R, Parani M. 2022. DNA barcoding of the market samples of single-drug herbal powders reveals adulteration with taxonomically unrelated plant species. Diversity. 14(6):495. doi:10.3390/d14060495.

Balaji R, Ravichandiran K, Tanuja , Parani M. 2021. The complete chloroplast genome of *Ocimum gratissimum* from India – a medicinal plant in the Lamiaceae. Mitochondrial DNA B Resour. 6(3):948–950. doi:10.1080/23802359.2021.1889413.

Bhatti HA, Tehseen Y, Maryam K, Uroos M, Siddiqui BS, Hameed A, Iqbal J. 2017. Identification of new potent inhibitor of aldose reductase from *Ocimum basilicum*. Bioorg Chem. 75:62–70. doi:10.1016/j.bioorg.2017.08.011.

Carocho M, Barros L, Barreira JCM, Calhelha RC, Soković M, Fernández-Ruiz V, Buelga C, Morales O, Ferreira ICFR. 2016. Basil as functional and preserving ingredient in "Serra da Estrela" cheese. Food Chem. 207:51–59. doi:10.1016/j.foodchem.2016.03.085.

Christina VLP, Annamalai A. 2014. Nucleotide based validation of *Ocimum* species by evaluating three candidate barcodes of the chloroplast region. Mol Ecol Resour. 14(1):60–68. doi:10.1111/1755-0998.12167.

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45(4):e18. doi:10.1093/nar/gkw955.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19:11–15.

Fernandes F, Pereira E, Círić A, Soković M, Calhelha RC, Barros L, Ferreira ICFR. 2019. *Ocimum basilicum* var. *purpurascens* leaves (Red Rubin Basil): a source of bioactive compounds and natural pigments for the food industry. Food Funct. 10(6):3161–3171. doi:10.1039/c9fo00578a.

Fu G, Liu J, Li J. 2020. The complete chloroplast genome sequence of *Elsholtzia densa*, a herb with volatile aroma component. Mitochondrial DNA B Resour. 5(1):595–596. doi:10.1080/23802359.2019.1710597.

Gerardi C, Tommasi N, Albano C, Blando F, Rescio L, Pinthus E, Mita G. 2015. *Prunus mahaleb* L. fruit extracts: a novel source for natural food pigments. Eur Food Res Technol. 241(5):683–695. doi:10.1007/s00217-015-2495-x.

Harini P, Balaji R, Parani M. 2021. The complete chloroplast genome of *Ocimum tenuiflorum* L. subtype Rama Tulsi and its phylogenetic analysis. Mitochondrial DNA B Resour. 6(8):2224–2226. doi:10.1080/23802359.2021.1944381.

Jürges G, Sahi V, Rios Rodriguez D, Reich E, Bhamra S, Howard C, Slater A, Nick P. 2018. Product authenticity versus globalization—the Tulsi case. PLoS One. 13(11):e0207763. doi:10.1371/journal.pone.0207763.

Katoh K, Standley DM. 2013. MAFFT Multiple sequence Alignment Software Version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780. doi:10.1093/molbev/mst010.

Kavya NM, Balaji R, Tanuja, Parani M, Senthilkumar P. 2021. The complete chloroplast genome of *Ocimum tenuiflorum* L. subtype Krishna Tulsi and its phylogenetic analysis. Mitochondrial DNA B Resour. 6(8):2358–2360. doi:10.1080/23802359.2021.1951133.

Kirankumar SI, Balaji R, Tanuja, Parani M. 2023. The complete chloroplast genome of *Ocimum basilicum* L. var. *basilicum* (Lamiaceae) and its phylogenetic analysis. Mitochondrial DNA B Resour. 8(11):1169–1173. doi:10.1080/23802359.2023.2275835.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 25(14):1754–1760. doi:10.1093/bioinformatics/btp324.

Li J, Tang J, Zeng S, Han F, Yuan J, Yu J. 2021. Comparative plastid genomics of four *Pilea* (Urticaceae) species: insight into inter specific plastid genome diversity in *Pilea*. BMC Plant Biol. 21(1):25. doi:10.1186/s12870-020-02793-7.

Liu S, Ni Y, Li J, Zhang X, Yang H, Chen H, Liu C. 2023. CPGView: a package for visualizing detailed chloroplast genome structures. Mol Ecol Resour. 23(3):694–704. doi:10.1111/1755-0998.13729.

Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 44(W1):W54–W57. doi:10.1093/nar/gkw413.

Natarajan P, Murugesan AK, Govindan G, Gopalakrishnan A, Kumar R, Duraisamy P, Balaji R, Tanuja, Shyamli PS, Parida AK, et al. 2021. A reference-grade genome identifies salt-tolerance genes from the salt-secreting mangrove species *Avicennia marina*. Commun Biol. 4(1):851. doi:10.1038/s42003-021-02384-8.

Ni Y, Li J, Zhang C, Liu C. 2023. Generating sequencing depth and coverage map for organelle genomes. protocols.io. doi:10.17504/protocols.io.4r3l27jkxg1y/v1.

Rawat R, Negi KS, Mehta PS, Tiwari V, Verma SK, Bisht IS. 2016. Study of six varieties of sweet basil (*Ocimum basilicum* L.) and their morphological variations. J Non-Timber For Prod. 23:1–6.

Renald SY, Balaji R, Tanuja, Parani M. 2021. The complete chloroplast genome and phylogenetic analysis of *Ocimum kilimandscharicum* Gurke (Camphor Basil) from India. Mitochondrial DNA Part B. 6(8):2164–2165.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 38(7):3022–3027. doi:10.1093/molbev/msab120.

Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45(W1):W6–W11. doi:10.1093/nar/gkx391.

Vineesh S, Balaji R, Tanuja, Parani M. 2023. The complete chloroplast genome of *Ocimum americanum* Linnaeus 1755 and phylogenetic analysis among the Lamiaceae family. Mitochondrial DNA B Resour. 8(10):1077–1081. doi:10.1080/23802359.2023.2264545.

Wang Y, Wang H, Zhou B, Yue Z. 2021. The complete chloroplast genomes of *Lycopus lucidus* and *Agastache rugosa*, two herbal species in tribe Mentheae of Lamiaceae family. Mitochondrial DNA B Resour. 6(1):89–90. doi:10.1080/23802359.2020.1847617.

Wu P, Xu C, Chen H, Yang J, Zhang X, Zhou S. 2021. NOVOWrap: an automated solution for plastid genome assembly and structure standardization. Mol Ecol Resour. 21(6):2177–2186. doi:10.1111/1755-0998.13410.

Yan-Ci Y, Yan-Ling Z, Yong-Jun F. 2020. The complete chloroplast genome of *Elsholtzia densa* var. *ianthina* (Lamiaceae). Mitochondrial DNA B Resour. 5(2):1671–1672.

Zhang HY, Ma WZ, Yan HF, Wang DQ. 2020. Characterization of the complete plastid genome of Chinese medicinal plant *Isodon serra* (Lamiaceae). Mitochondrial DNA B Resour. 5(3):2111–2112. doi:10.1080/23802359.2020.1765429.

Zhao F, Chen YP, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksby M, Wang Q, et al. 2021. An updated tribal classification of Lamiaceae based on plastome phylogenomics. BMC Biol. 19(1):2. doi:10.1186/s12915-020-00931-z.

Zhu Y, Zhang X, Yan S, Feng C, Wang D, Yang W, Daud MK, Xiang J, Mei L. 2023. SSR identification and phylogenetic analysis in four plant species based on complete chloroplast genome sequences. Plasmid. 125:102670. doi:10.1016/j.plasmid.2023.102670.