

DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF *Ocimum* L. (Lamiaceae) NATURALLY OCCURRING IN ASSAM, INDIA

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ABSTRACT

The inflorescence, bracts, and floral architecture are key characteristics for distinguishing species of *Ocimum* L.; however, extensive morphological variability often complicates species delimitation within the genus. This study documents the diversity, diagnostic characters, and phylogenetic relationships of naturally occurring *Ocimum* taxa in Assam, India, including three new regional distribution records for northeastern India. Morphological characterization was complemented with sequence analysis of the nuclear ribosomal DNA internal transcribed spacer 2 (nrDNA-ITS2) region to assess species boundaries and relationships. Phylogenetic analysis based on ITS2 sequences successfully resolved most accessions at the species level and broadly supported the classical grouping of *Ocimum* into herbaceous and shrubby lineages. However, limited resolution was observed at the infraspecific level, particularly in *Ocimum tenuiflorum*, where Krishna and Radha types were not genetically differentiated. Additionally, certain accessions exhibited weak or ambiguous clustering patterns, reflecting potential complexities such as hybridization or incomplete lineage sorting. These findings indicate that while ITS2 is a useful and practical marker for species-level identification in *Ocimum*, it has limitations in resolving closely related or infraspecific taxa. The study highlights the importance of integrating morphological and molecular approaches and provides a baseline for future multi-locus and genomic investigations aimed at resolving complex evolutionary relationships within the genus.

Keywords: Basil, natural populations, species diversity, nrDNA-ITS2, phylogeny.

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INTRODUCTION

Ocimum L., commonly known as basil, is a focal point of modern research in phytochemistry, molecular biology, and medicinal studies due to its significant therapeutic properties. *Ocimum* species provide essential oils, medicines, and food products (Nascimento et al., 2023). Basil oil is renowned for its antimicrobial (Cowan, 1999), antioxidant (Cook & Samman, 1996), anticancer (Kathirvel & Ravi, 2012; Zarlaha et al., 2014; Behbahani, 2014; Perna et al., 2022; Abdoul-Latif et al., 2022), anti-inflammatory (Saran et al., 2022), and antiviral properties. Basil seeds are rich in protein (11.4–22.5 g per 100 g), dietary fiber, essential amino acids, and lipids containing linolenic and linoleic fatty acids, as well as calcium, magnesium, potassium, and phenols such as rosmarinic acid, orientin, and vicenin (Bravo et al., 2021). The hydrocolloidal properties of *Ocimum basilicum* seeds are beneficial in food and pharmaceutical applications for their emulsifying, fat-replacement, foam-stabilizing, gelling, and thickening properties (Razavi & Naji-Tabasi, 2022). The essential oil of *Ocimum* is commercially valuable in dental products, hair care, perfumes, and soaps (Tucker & De Baggio, 2000). Basil oil imparts a green, herbal character to fragrance types such as aldehydic, chypre, eau de cologne, and fougère (Srivastava et al., 2018). The Asian native species *Ocimum tenuiflorum* is globally recognized for its cosmetic, cultural, and medicinal applications (Bhamra et al., 2022). It can also serve as an alternative crop for economically disadvantaged rural communities, offering double the gross profit within two years (Shah et al., 2019).

Ocimum L. is primarily an East Indian genus (Bentham, 1832). In Assam, Gammie (1895) first reported this genus from Sadiya in the Lakhimpur district. Subsequently, Carter & Carter (1921) identified *O. basilicum* in regions of Dibrugarh, Sadiya, and Khamti Basti, and *Ocimum sanctum* in Sadiya. Kanjilal (1939) documented three species: *O. sanctum* L., *Ocimum canum* Sims, and *Ocimum basilicum* L. var. *purpurascens* as cultivated in Assam. In India, seven *Ocimum* species have been reported

(Kumar et al., 2018), with four species (*Ocimum americanum* L., *Ocimum basilicum* L., *Ocimum gratissimum* L., and *Ocimum tenuiflorum* L.) naturally distributed in northeastern India, excluding *Ocimum africanum* Lour., *Ocimum filamentosum* Forssk., and *Ocimum kilimandscharicum* Gürke (Powo, 2023).

The genus is highly complex, and species are often misidentified due to polyploidy (Tucker, 1986). Based on comparative morphology and chromosome number, Khosla (1995) classified *Ocimum* into two groups: Basilicum (annuals) and Sanctum (perennials and woody). He suggested that *Ocimum* species evolved from perennial to annual habits through both abrupt and gradual speciation. Speciation in *Ocimum* likely occurred through ecological or geographical races, leading to eco-geographically differentiated subspecies.

According to Paton (1992), ICBN rules have often been ignored in the naming of *Ocimum* species, resulting in multiple names for the same species. The genus is further complicated by inter- and intra-specific hybridization (De Masi et al., 2006). Vieira et al. (2001) argued that morphological traits alone are unreliable for species identification when characters overlap. The chemotaxonomy of *Ocimum* is also complicated due to the existence of chemical races or chemotypes within species (Javanmardi et al., 2002). Correct identification is crucial for commercialization and quality maintenance in relevant industries.

Considering the immense economic and medicinal significance of the genus *Ocimum*, retrieving DNA marker sequences from Type specimens is critical to facilitate accurate species identification. Unfortunately, DNA sequences for many *Ocimum* Type specimens are not available in public databases, which pose challenges for molecular comparison, phylogenetic analysis, and taxonomic resolution. This gap likely stems from the early discovery of *Ocimum* species, mostly during the 18th and 19th centuries, which is long before the advent of DNA-based taxonomy in the late 20th century. Nevertheless, the availability of reference sequences from Type specimens would provide unequivocal validation for *Ocimum* identity in

studies related to agronomy, breeding, medicine, phytochemistry, and commercial applications. Misidentification and adulteration, which are common due to morphological complexity within *Ocimum*, can be significantly reduced once the marker sequences are available for all Type specimens. Therefore, taxonomic studies of *Ocimum* require further exploration of molecular markers to provide greater insight and clarity. Molecular data, combined with morphological investigations, can offer greater authenticity regarding species identification, characterization, phylogeny, and classification based on shared traits.

At present, the use of multi-locus markers has become standard in phylogenetic analyses to minimize biases associated with single-locus datasets. The nuclear ribosomal DNA internal transcribed spacer (nrDNA-ITS) region is widely employed, often in combination with chloroplast and mitochondrial markers, for species-level identification and phylogenetic studies in plants and fungi. Although the complete ITS region (ITS1–5.8S–ITS2) is commonly used in plant systematics, the ITS2 subregion alone has also gained prominence due to its high discriminatory power and ease of amplification and alignment (Alvarez & Wendel, 2003; Zhu et al., 2018; Sokołowska et al., 2022). In plant taxa characterized by varying ploidy levels and frequent hybridization, maternally inherited plastid markers such as *matK* and *rbcL* are particularly valuable for tracing maternal lineages, as plastid genomes are typically uniparentally inherited and largely non-recombinant. In contrast, nuclear markers, despite their higher variability, may yield complex or conflicting phylogenetic signals due to biparental inheritance and reticulate evolution (Soltis & Soltis, 1998; Shaw et al., 2005; Hollingsworth et al., 2011; Dong et al., 2012). In this context, the present study aims to document the morphological diversity and infer ITS2-based phylogenetic relationships among naturally occurring *Ocimum* species of Assam.

MATERIALS AND METHODS

Sample Collection: *Ocimum* plant specimens were collected from 20 different

districts of Assam, including Baksa, Barpeta, Biswanath, Cachar, Dima Hasao, Dhemaji, Goalpara, Golaghat, Hojai, Jorhat, Kamrup, Karbi Anglong, Karimganj, Kokrajhar, Morigaon, Nagaon, Sivasagar, Sonitpur, Tinsukia, and Udalguri, during 2019–2022. A total of 65 samples were collected during their flowering and fruiting phases from various geographic locations across Assam. A distribution map of the collected samples was created using Quantum Geographic Information System (QGIS) software (version 3.4.0), incorporating altitude information from Assam's Digital Elevation Model (DEM) (Fig. 1). After careful verification, 36 voucher specimens representing six different *Ocimum* species and one *Ocimum* accession (voucher number KJOT03; GenBank accession number OR546435) that could not be assigned to a specific species were selected from the 65 natural *Ocimum* specimens for phylogenetic analysis, along with 17 reference sequences from the NCBI database. The list of *Ocimum* accessions, including species names, voucher numbers, locations, collection dates, and GenBank accession numbers, is provided in Table 1.

Preparation of Voucher Specimens: Voucher specimens were prepared following the standard field and herbarium techniques of Jain & Rao (1997), including plant specimen drying, pressing, poisoning, mounting, and labelling on herbarium sheets. The voucher specimens were submitted to GUBH (accredited by the New York Botanical Garden).

Morphological Characterization: Micro-morphological features were investigated using a stereo-zoom binocular microscope (CZM4, Labomed) and compared with voucher specimens accessed from regional and national herbaria, including ARUN, ASSAM, CAL, and GUBH, for species identification. Additionally, digital type specimens from online herbaria databases, namely BSI–IVH, G, JSTOR, KEW, LINN, MNHN, MO, and NY, were consulted for accurate identification. A comprehensive characterization was conducted by examining all possible morphological features (vegetative and reproductive). Eleven morphological traits

were included in the study: habit (H), stem (S), leaves (L), inflorescence (I), flower (F), bract (B), calyx (K), corolla (C), androecium (A), gynoecium (G), ovary (O), and nutlets (N). Common and vernacular names of the species are also provided where available.

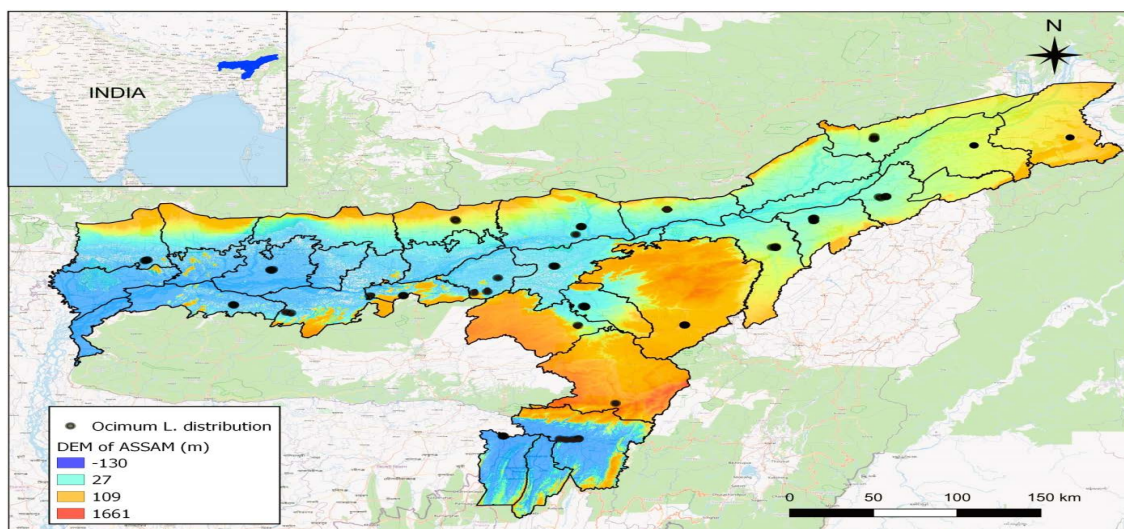


Figure 1. Digital Elevation Model map showing the distribution and locations of naturally occurring *Ocimum* L. species in Assam, India. The data was obtained based on field surveys and investigations. Map prepared through Quantum Geographic Information System software version 3.4.0

Table 1. List of *Ocimum* spp. considered in the present study with their species name, location and GenBank accession number

(A) Samples collected from Assam				
Sl. No.	Species name	GenBank accession number	Location with Geo-coordinates	Voucher No.
1	<i>Ocimum africanum</i> Lour.	OR546425	Hawaipur, Hojai, Assam 25.9470486°N, 92.8217206°E	HJOAf06
2	<i>Ocimum africanum</i> Lour.	OR546444	Rajapara, Jobepara, Goalpara, Assam 25.943020°N, 91.125926°E	GPOAf03
3	<i>Ocimum africanum</i> Lour.	OR546445	Kallapara Rani, Kamrup, Assam 26.0568257°N, 91.6095214°E	KMOAf01
4	<i>Ocimum africanum</i> Lour.	OR546446	Boro village, Dima Hasao, Assam 25.152019°N, 93.025092°E	DHOAf05
5	<i>Ocimum americanum</i> L.	OR546418	Bongaon, Sivasagar, Assam 26.963614°N, 94.579233°E	SSOA01
6	<i>Ocimum americanum</i> L.	OR546426	Kachugaon, Golaghat, Assam 26.535300°N, 93.984486°E	GGOA05
7	<i>Ocimum americanum</i> L.	OR546427	Near Dhing road, Nagaon, Assam 26.354608°N, 92.672065°E	NGOA03
8	<i>Ocimum americanum</i> L.	OR546428	Bamunkuchi, Patacharkuchi, Barpeta, Assam 26.509024°N, 91.216154°E	BPOA04

9	<i>Ocimum americanum</i> L.	OR546429	Rongpur, Silchar, Cachar, Assam 24.837364°N, 92.814094°E	CCOA06
10	<i>Ocimum americanum</i> L.	OR546436	Tegheria, Morigaon, Assam 26.125152°N, 92.224861°E	MGOA01
11	<i>Ocimum americanum</i> L.	OR546437	Nagakhelia, Dhemaji, Assam 27.496987°N, 94.568214°E	DJOA04
12	<i>Ocimum americanum</i> L.	OR546438	Near Haflong lake, Dima Hasao, Assam 25.165653°N, 93.016702°E	DHOA02
13	<i>Ocimum americanum</i> L.	OR546440	Behali, Biswanath, Assam 26.834406°N, 93.359094°E	BNOA01
14	<i>Ocimum americanum</i> L.	OR546441	Teok, Jorhat, Assam 26.835472°N, 94.425015°E	JHOA03
15	<i>Ocimum basilicum</i> L.	OR546430	Jhapirbond, Cachar, Assam 24.924937°N, 92.862283°E	CCOB07
16	<i>Ocimum basilicum</i> L.	OR546431	Sechajan, Dhemaji, Assam 27.521842°N, 94.584608°E	DJOB04
17	<i>Ocimum basilicum</i> L.	OR546442	Kataligaon, Baksa, Assam 26.658109°N, 91.364520°E	BKOB01
18	<i>Ocimum basilicum</i> L.	OR546443	Haflong East, Dima Hasao, Assam 25.163500°N, 93.022108°E	DHOB03
19	<i>Ocimum basilicum</i> L.	OR546447	Noti Khal, Karimganj, Assam 24.866962°N, 92.364067°E	KGOB04
20	<i>Ocimum basilicum</i> L.	OR546451	Lathabori, Morigaon, Assam 26.264054°N, 92.338285°E	MGOB04
21	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i>	OR546420	Jyotigaon, near Manas WLS, Baksa, Assam 26.659148°N, 91.014115°E	BPOG01
22	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i>	OR546421	Tangla, Udalguri, Assam 26.637284°N, 91.911887°E	UGOG02
23	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i>	OR546422	Rampur, Hojai, Assam 25.997222°N, 92.852250°E	HJOG02
24	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i>	OR546423	Haibargaon, Nagaon, Assam 26.355250°N, 92.677572°E	NGOG02
25	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i>	OR546424	Basistha, Kamrup, Assam 26.096961°N, 91.786225°E	KMOG03
26	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i>	OR546439	Dakuapara, Boko, Kamrup, Assam 25.983057°N, 91.245144°E	KPOG01
27	<i>Ocimum gratissimum</i> L. ssp. <i>gratissimum</i> var. <i>macrophyllum</i> Briq.	OR546419	Near Hoollongapar Gibbon sanctuary, Jorhat, Assam 26.6785654°N, 94.3555723°E	JHOG02

28	<i>Ocimum kilimandscharicum</i> G.	OR546448	Jatinga riverside area, Cachar, Assam 25.037152°N, 92.779682°E	CCOK05
29	<i>Ocimum kilimandscharicum</i> G.	OR546449	Near Haflong station, Dima Hasao, Assam 25.145260°N, 93.028762°E	DHOK04
30	<i>Ocimum kilimandscharicum</i> G.	OR546450	Near Dehing Patkai WLS, Tinsukia, Assam 27.381458°N, 95.587070°E	TSOK01
31	<i>Ocimum tenuiflorum</i> L.	OR546416	Near archaeological park, Hojai, Assam 25.998056°N, 92.852050°E	HJOT05
32	<i>Ocimum tenuiflorum</i> L.	OR546417	Satra Baradi, Barpeta, Assam 26.321256°N, 91.006125°E	BPOT02
33	<i>Ocimum tenuiflorum</i> L.	OR546432	Diphu, Karbi Anglong, Assam 25.837525°N, 93.437886°E	KAOT05
34	<i>Ocimum tenuiflorum</i> L.	OR546433	Dolabari Tezpur, Sonitpur, Assam 26.658498°N, 92.799805°E	SPOT04
35	<i>Ocimum tenuiflorum</i> L.	OR546434	Near Haticherra rubber plantation, Cachar, Assam 24.927739°N, 92.835332°E	CCOT05
36	<i>Ocimum</i> sp.	OR546435	Tengapara, Kokrajhar, Assam 26.407734°N, 90.282329°E	KJOT03
(B) Reference nrDNA-ITS sequences taken from NCBI Database				
Sl. No.	Species name	GenBank accession number	Location	Reference
1	<i>Ocimum africanum</i> Lour.	MF468220.1	Bangalore, Karnataka, India	Kumar et al., 2017
2	<i>Ocimum americanum</i> L.	KX096056.1	Malappuram, Kerala, India	Sumitha et al., 2016
3	<i>Ocimum basilicum</i> L.	MZ190985.1	Urumqi, Xinjiang, China	Huixia & Kejian 2021
4	<i>Ocimum basilicum</i> L.	ON685479.1	Portugal	Unpublished data retrieved from NCBI
5	<i>Ocimum basilicum</i> L.	ON685500.1	Portugal	-do-
6	<i>Ocimum filamentosum</i> Forssk.	MF468206.1	Bangalore, Karnataka, India	Kumar et al., 2017
7	<i>Ocimum gratissimum</i> L.	FJ593402.1	Kunming, Yunnan, China	Zhong et al., 2008
8	<i>Ocimum gratissimum</i> L.	MF468218.1	Bangalore, Karnataka, India	Kumar et al., 2017
9	<i>Ocimum gratissimum</i> L.	MN257704.1	Norbyvagen, Uppsala, Sweden	Veldman et al., 2019
10	<i>Ocimum gratissimum</i> L.	PQ143346.1	Xuan Mai, Viet Nam	Unpublished data retrieved from NCBI
11	<i>Ocimum gratissimum</i> L.	PX061747.1	Khyber Pakhtunkhwa, Pakistan	-do-

12	<i>Ocimum gratissimum</i> L.	PX699296.1	Thai Nguyen, Viet Nam	-do-
13	<i>Ocimum kilimandscharicum</i> G.	MF468215.1	Bangalore, Karnataka, India	Kumar et al., 2017
14	<i>Ocimum tenuiflorum</i> L.	KT235956.1	Pune, Maharashtra, India	Lingojar et al., 2015
15	<i>Ocimum tenuiflorum</i> L.	MK087938.1	Torrance, Los Angeles, USA	Lu et al., 2018
16	<i>Ocimum tenuiflorum</i> L.	MW022508.1	Bangalore, Karnataka, India	Chalageri et al., 2020
17	<i>Ocimum tenuiflorum</i> L.	OL536579.1	Washington, Columbia, USA	Steier & Carlsen 2021

Genomic DNA Isolation: Genomic DNA from 36 representative samples was isolated for amplification of the nrDNA-ITS2 region. DNA was extracted from 500 mg of silica-dried leaves using the cetyltrimethylammonium bromide (CTAB) method (Dellaporta et al., 1983). The isolated DNA was purified via RNase treatment to obtain high-quality genomic DNA. The DNA was quantified using a UV/Vis BioSpectrometer (Eppendorf, Germany).

Amplification of the nrDNA-ITS2 Region and Sequencing: After experiencing several PCR failures while using the universal primers with *Ocimum* DNA to amplify the entire ITS region (ITS1–5.8S–ITS2) in our experiments, we considered the ITS2 as suggested by Chen et al. (2010) and successfully amplified the ITS2 region using the forward primer ITS–S2F (5′–A TGCGATACTTG GTGTGAAT–3′) and the reverse primer BEL–3 (5′–GACGCTTCTCCA GACTACAAT–3′), as previously reported (Chen et al. 2010; Chiou et al. 2007). For each sample, a 25 µL PCR mix was prepared containing 10× PCR buffer, 0.5 units of *Taq* DNA polymerase, 2.5 mM MgCl₂, 200 µM each of dNTPs (GeNei™, Bangalore, India), 10 pmol of each primer (Eurofins Scientific, Bangalore, India), and 30 ng of template DNA. PCR amplification was carried out in a Thermal Cycler (Nexus GSX1, Eppendorf) using the following program: 3 minutes of pre-denaturation at 95 °C; 35 cycles of 15 seconds denaturation at 95 °C, 40 seconds annealing at 45 °C, and 1 minute extension at 72 °C; followed by a 7-minute final extension at 72 °C.

The amplified DNA products were electrophoresed in 1% (w/v) agarose gel at 60 V for 45 minutes. A low-range DNA ruler (GeNei™, Bangalore, India) was used to confirm the nrDNA-ITS2 amplicons with a size of 400–500 bp. The distinct amplicons were excised and purified using a QIAquick Gel Extraction Kit (QIAGEN), following the manufacturer's instructions. The cleaned DNA products were sequenced using the same primers on a Sanger sequencing platform, outsourced to Eurofins Scientific (Bangalore, India).

Sequence Retrieval and Submission: The ABI files received from the service provider were viewed using MEGA 11 software (Tamura et al., 2021) to check sequence quality and correct any erroneous base calls. High-quality sequences were retrieved after trimming noisy ends. Contig sequences were generated from each forward-reverse pair using the CAP3 online tool. The ITS2 sequences were deposited in the NCBI database to obtain GenBank accession numbers for each sequence.

Phylogenetic Analysis: The phylogenetic relationships of *Ocimum* species found in Assam were analyzed along with 17 reference taxa from the NCBI database (Table 1). Sequences were aligned using the MUSCLE algorithm (Edgar, 2004a, b) in MEGA 11 software. A maximum likelihood (ML) phylogenetic tree was generated with 1000 bootstrap replicates. Tamura & Nei (1993) substitution model with a uniform rate for all sites was considered in the maximum likelihood analysis.

RESULTS

The morphological characterization of *Ocimum* samples collected from various localities in Assam revealed a range of variations both among and within species. Seven distinct taxa were identified and confirmed based on morpho-molecular studies: *Ocimum africanum* Lour., *Ocimum americanum* L., *Ocimum basilicum* L., *Ocimum gratissimum* L. ssp. *gratissimum*, *Ocimum gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq., *Ocimum kilimandscharicum* Gürke, and *Ocimum tenuiflorum* L. (Table 1, Supplementary Table S1). A taxonomic key derived from morphological comparisons among the accessions is presented in Table 2. Detailed photographic representations of *O. americanum*, *O. basilicum*, *O. gratissimum* ssp. *gratissimum*,

and *O. kilimandscharicum* are provided in Figures 2–3, respectively. For *O. africanum* Lour., *O. gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq., and *O. tenuiflorum* L., refer to previous publications by the same authors (Kalita & Devi 2023a, b; Kalita et al., 2023). The occurrence and distribution of *O. kilimandscharicum* in northeastern India, including Assam, is reported here for the first time. The authors have also recently reported new distribution records for *O. africanum* and *O. gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq. in Assam. Two subtypes of *O. tenuiflorum*, viz. Krishna type (purple plant; vouchers CCOT05 and HJOT05) and Radha type (green plant; vouchers SPOT04, BPOT02, and KAOT05) were also recorded in the present study.

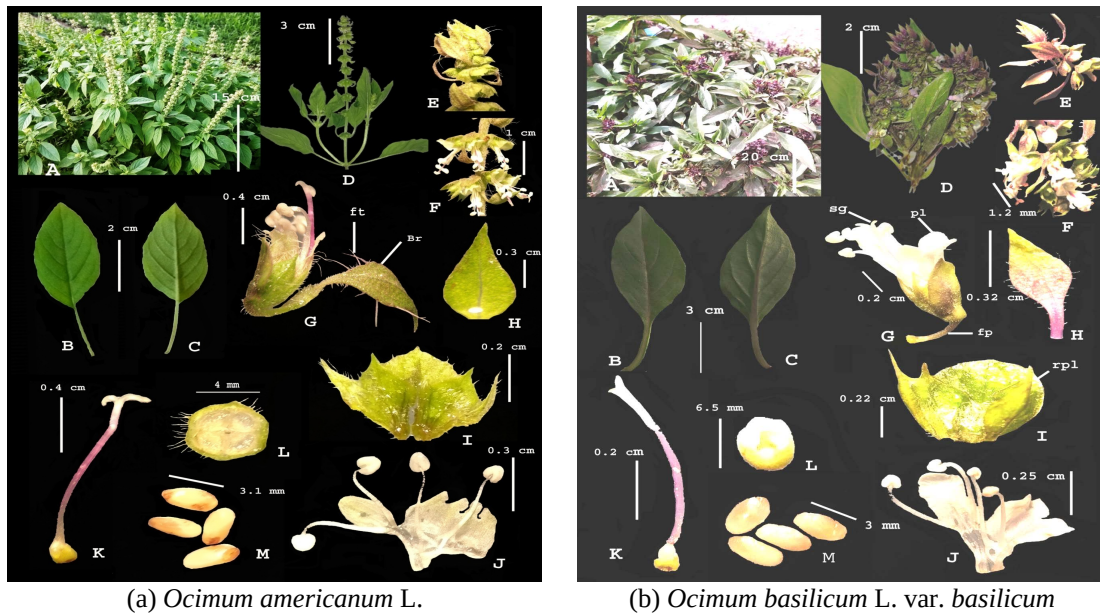


Figure 2. A, habit of plant. B, adaxial side of leaf. C, abaxial side of leaf. D, inflorescence. E, inflorescence tip. F, closure view of inflorescence showing floral arrangement. G, complete flower. H, bract. I, calyx dissected view. J, dissected corolla showing epipetalous stamen. K, gynoecium exhibiting gynobasic style and bifid stigma. L, ovary T.S. revealing ovules. M, nutlets

Morphological analysis of the accessions revealed overlapping characteristics in *Ocimum* sp. (voucher KJOT03, GenBank OR546435), exhibiting features intermediate between *O. tenuiflorum* and *O. gratissimum*. The leaves of *Ocimum* sp. (KJOT03) are sparsely serrate,

primarily in the upper half; they are glabrous on the adaxial surface with hairs restricted to the veins (Fig. 4). The inflorescence axis is densely pubescent, and the bracts are ovate with a broad base and caducous. The verticillasters are recurved and clustered, and

the calyx bears patent hairs, all of which are consistent with *O. gratissimum*. Conversely, several traits resemble *O. tenuiflorum*: the pedicel is slender and longer than the calyx; the posterior lip is cuspidate with a purple tip; the anterior lip bears two median lanceolate teeth that are curved upwards and longer than the lateral teeth; and the posterior lip possesses two obovate-oblong median lobes equal in length to the two oblong lateral lobes. Thus, the

distinguishing characters attributable to the unidentified *Ocimum* sp. KJOT03 include a densely pubescent stem; yellow-green leaves with irregular serration and hairy veins/margins; an inflorescence oriented at approximately 45° with closely spaced verticils; small, caducous bracts; a corolla with a shorter posterior lip; and globose nutlets that become mucilaginous when wet.

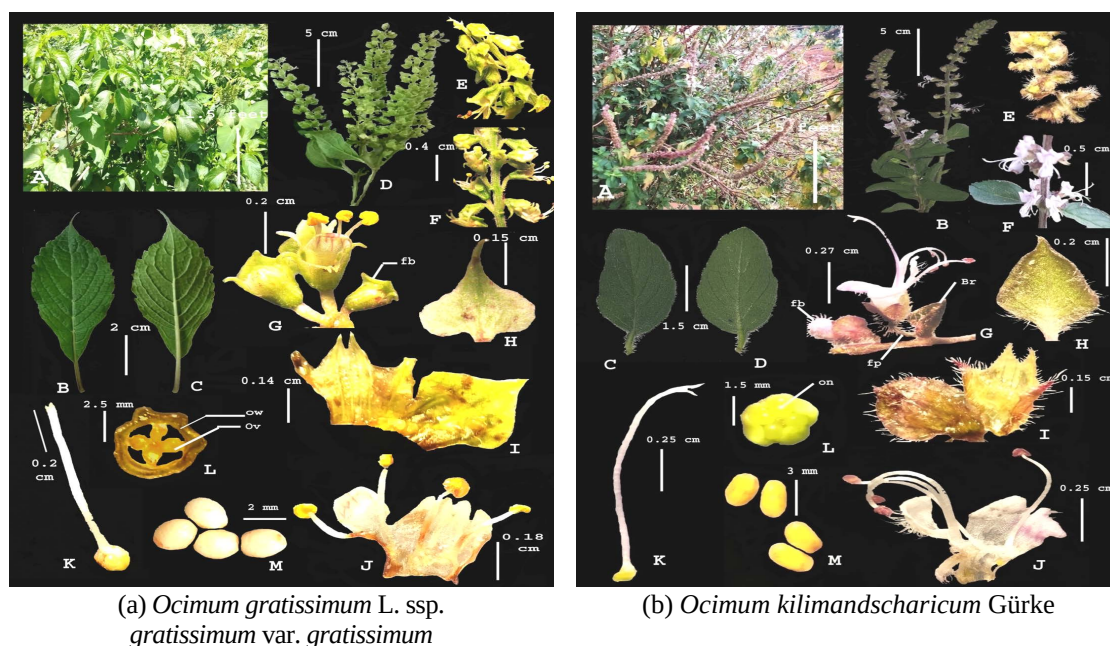


Figure 3. A, habit of plant. B, adaxial side of leaf. C, abaxial side of leaf. D, inflorescence. E, inflorescence tip. F, closure view of inflorescence showing floral arrangement. G, complete flower. H, bract. I, calyx dissected view. J, dissected corolla showing epipetalous stamen. K, gynoecium exhibiting gynobasic style and bifid stigma. L, ovary T.S. revealing ovules. M, nutlets

All accessions from Assam, along with reference sequences from NCBI GenBank, segregated into two major clusters (Cluster-A and Cluster-B) in the phylogenetic tree (Fig. 5). Cluster-A comprised *O. africanum*, *O. americanum*, *O. basilicum*, and *O. kilimandscharicum* with 84% bootstrap support, while Cluster-B comprised *O. filamentosum*, *O. gratissimum*, and *O. tenuiflorum* with 81% bootstrap support. In Cluster-A, all the accessions of *O. basilicum* clustered together, representing the subgroup Basilica with 64% bootstrap support, except

two that are from Portugal (unpublished NCBI data). Accessions of *O. africanum*, *O. americanum*, and *O. kilimandscharicum* formed distinct sub-clusters with bootstrap support of 99%, 97% and 97%, respectively, but are generally considered under the subgroup Americana. In Cluster-B, all the accessions of *O. tenuiflorum* formed a distinct sub-cluster with 97% bootstrap support, which represents the subgroup Sanctum. On the other hand, all the accessions of *O. gratissimum* clustered together forming a subgroup Gratissima. However, *O. filamentosum*

exhibited a close relationship to *Gratissima*. Thus, overall four distinct subgroups, viz. Americana (comprising *O. africanum*, *O. americanum*, and *O. kilimandscharicum*), Basilica (*O. basilicum*), Sanctum (*O. tenuiflorum*), and *Gratissima* (*O. gratissimum*) are well

segregated in the phylogenetic tree. Although the present analysis clustered all the accessions of *O. tenuiflorum* together as a distinct subgroup, Sanctum, it failed to segregate the subspecies Krishna type (CCOT05 and HJOT05) and Radha type (BPOT02, KAOT05, and SPOT04) of *O. tenuiflorum*.

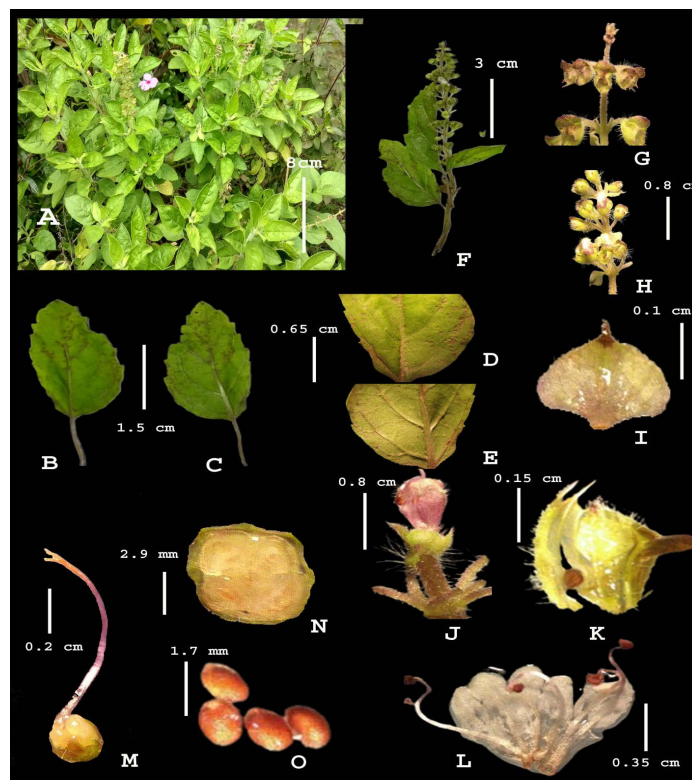


Figure 4. *Ocimum* sp. KJOT03. A, habit of plant. B, adaxial side of leaf. C, abaxial side of leaf. D, magnified view of upper leaf surface. E, magnified view of lower leaf surface. F, inflorescence. G, inflorescence tip. H, closure view of inflorescence showing floral arrangement. I, bract. J, complete flower. K, calyx dissected view. L, dissected corolla showing epipetalous stamen. M, gynoecium. N, ovary revealing seeds. O, nutlets

The unidentified *Ocimum* sp. (OR546435; voucher KJOT03) was separated in the phylogenetic tree (Fig. 5), revealing its distinctiveness, and supporting the morphological ambiguity already described. *O. filamentosum*, a non-native species to Assam, showed close affinity to Clove Basil (*O. gratissimum*) within Cluster-B. Notably, the infraspecific taxon *O. gratissimum* ssp. *gratissimum* var. *macrophyllum* (OR546419) was segregated from accessions of

O. gratissimum ssp. *gratissimum* and also from other *O. gratissimum* accessions with 90% and 67% bootstrap support, respectively, in the phylogenetic tree (Fig. 5).

DISCUSSION

The present study demonstrates the utility of the nrDNA-ITS2 marker, combined with detailed morphological characterization, for assessing species diversity and relationships within *Ocimum* in Assam. The phylogenetic

analysis largely supports species-level delimitation and aligns with classical classifications based on growth habit, separating herbaceous and shrubby groups. Specifically, herbaceous species (*O. americanum*, *O. basilicum*) formed Cluster-A, while shrubby species (*O. filamentosum*, *O.*

gratissimum, *O. tenuiflorum*) formed Cluster-B (Fig. 5). This pattern corroborates early classifications by Wallich (1831) and later groupings into Sanctum and Basilicum categories (Sobti & Pushpangadan, 1977; Khosla, 1995; Pushpangadan & Bradu, 1995), supporting the value of integrative taxonomy.

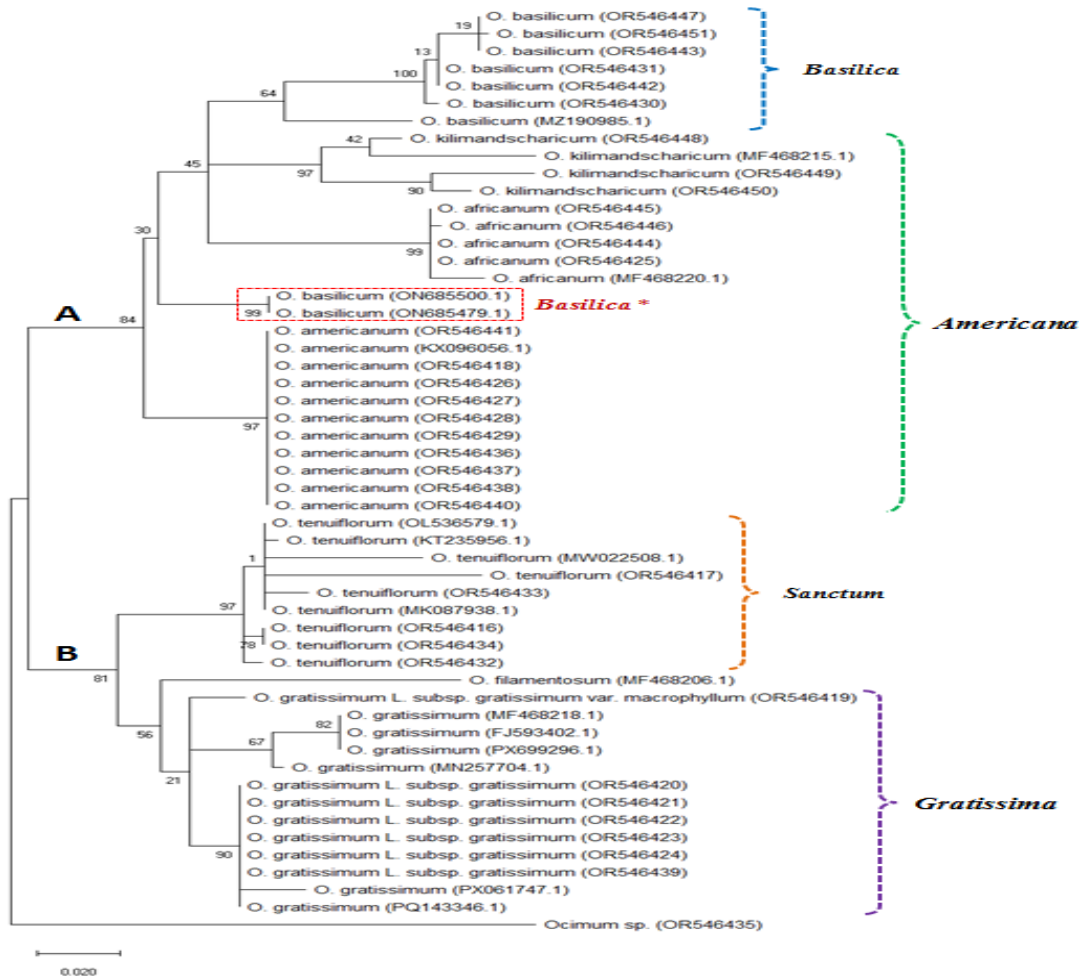


Figure 5. Maximum Likelihood phylogenetic tree of *Ocimum* spp. based on nrDNA-ITS2 sequences. The analysis included 53 sequences (36 from this study, with accession numbers beginning with OR, and 17 from NCBI), representing seven species. Bootstrap support values (%) from 1000 replicates are indicated at the nodes. *Unpublished data from Portugal

Species-level segregation was generally well resolved. Distinct sub-clustering of *O. africanum*, *O. americanum*, and *O. kilimandscharicum* was observed, despite their overlapping morphological traits (Suddee et al., 2004; Charles & Simon, 1992; Paton et

al., 2009). Although *O. basilicum* is considered closely related to *O. americanum* and *O. kilimandscharicum* (Ryding, 1994), the present analysis clearly delineates these species. Within *O. basilicum*, accessions from Assam and one from China clustered together, while

two unpublished accessions from Portugal formed a distinct subgroup within Cluster-A, indicating possible geographic structuring.

The hybrid origin of *O. africanum* from *O. americanum* and *O. basilicum* (Paton & Putievsky, 1966) contributes to its complex phylogenetic placement. Previous studies using RAPD markers (Chowdhury et al., 2017) and *rbcL* sequences (Akinyemi & Ayodele, 2020) have reported varying affinities of *O. africanum* with related species, highlighting the limitations of single-locus markers such as *rbcL*. Similarly, chloroplast genome analysis placed *O. kilimandscharicum* closer to *O. tenuiflorum* (Renald et al., 2021). In contrast, our ITS2-based analysis reveals an intermediate position of *O. africanum* between *O. americanum* and *O. basilicum* (Fig. 5), consistent with its hybrid origin. Nonetheless, further studies using mitochondrial and chloroplast markers could substantiate the present findings and provide a clearer picture of this natural hybrid origin.

Cluster-B (shrubby taxa) showed clear separation into Sanctum and Gratissima groups. All accessions of *O. tenuiflorum* clustered together with strong support (97% bootstrap), but without resolving the Krishna (purple) and Radha (green) subtypes, indicating limited infraspecific resolution of ITS2. Similar limitations of ITS regions at lower taxonomic levels have been noted previously (Li et al., 2015; Qin et al., 2017; Sokołowska et al., 2022). The close relationship between *O. tenuiflorum* and *O. gratissimum* observed here is consistent with earlier reports (Singh et al., 2004; Jürges et al., 2018).

In contrast to Molina et al. (2018), who failed to distinguish *O. tenuiflorum* and *O. basilicum* using ITS2, the present study successfully segregates these species. However, plastid genome evidence shows differing relationships; for example, Kavya et al. (2021) reported the kinship of *O. tenuiflorum* subtype Krishna with *O. basilicum*, *O. gratissimum*, and *O. tenuiflorum*. Such discrepancies further highlight the limitations of single-locus nuclear

markers and the influence of hybridization, introgression, and incomplete lineage sorting in *Ocimum*.

Unresolved or ambiguous placements also indicate underlying complexity. The genetically distinct but phylogenetically uncertain placement of *Ocimum* sp. (voucher KJOT03; GenBank OR546435), which shows intermediate morphology between *O. tenuiflorum* and *O. gratissimum*, suggests possible hybridization or ongoing speciation. This warrants further investigation using population-level sampling and cytogenetic approaches.

Overall, while ITS2 is a practical and effective marker for species-level identification in *Ocimum*, its limitations at the infraspecific level and in resolving complex evolutionary histories are evident. Therefore, future studies should incorporate multi-locus and chloroplast markers, along with genomic approaches, to achieve more robust phylogenetic resolution and to address hybrid- and subspecies-level questions in the genus.

CONCLUSION

This study provides a comprehensive assessment of the diversity and phylogenetic relationships of naturally occurring *Ocimum* taxa in Assam through an integrative morpho-molecular approach. The nrDNA-ITS2 marker proved effective for resolving most taxa at the species level and broadly supported traditional groupings within the genus. However, its limited resolution at the infraspecific level, as observed in *O. tenuiflorum*, and the presence of weakly supported or ambiguous phylogenetic relationships indicate that ITS2-based inferences should be considered preliminary. The findings highlight the need for multi-locus and genomic approaches to achieve a more robust understanding of evolutionary relationships in *Ocimum*, particularly in the context of hybridization, polyploidy, and potential cytonuclear discordance. Unresolved patterns identified in this study, including the distinct yet ambiguous placement of *Ocimum* sp. (KJOT03), should be treated as hypotheses for a new species for future investigation.

The study also underscores a broader taxonomic challenge: the lack of ITS sequence data from type specimens of many *Ocimum* species. Addressing this gap would significantly enhance the reliability of molecular identification and phylogenetic inference. Incorporating such reference data, along with expanded molecular datasets, will

be critical for improving taxonomic resolution and supporting applications in agriculture, pharmacology, and biodiversity conservation. Overall, this work establishes a foundational dataset for *Ocimum* diversity in Assam and provides a framework for future research aimed at resolving complex taxonomic and evolutionary questions within the genus.

Table 2. Key to the species of *Ocimum*

1. Pedicel shorter than calyx.....	2
1'. Pedicel longer than calyx.....	7
2. Fruiting calyx open; annual herbs; leaves 2–6 cm × 1–3 cm.....	3
2'. Fruiting calyx closed; perennial shrubs or under shrubs; leaves 5–13 cm × 3–5 cm.....	5
3. Two median anterior lips almost equal to posterior lip of calyx.....	<i>O. africanum</i>
3'. Two median anterior lips more prolonged than the posterior lip of calyx.....	4
4. Inflorescence 7–12 cm long; fruiting calyx 2.5–2.7 × 3.6–3.8 mm; bract elliptic.....	<i>O. americanum</i>
4'. Inflorescence 5–9 cm long; fruiting calyx 2.8–3.1 × 4.3–4.6 mm; bract lanceolate.....	<i>O. basilicum</i>
5. Decurved flowering and fruiting calyces; flower verticils 0.7–1.0 cm apart; strongly reflexed towards the inflorescence axis.....	<i>O. gratissimum</i> ssp. <i>iringense</i>
5'. Recurved flowering and fruiting calyces, flower verticils 1–1.5 cm apart; not strongly reflexed towards the inflorescence axis.....	6
6. Inflorescence 10–13 cm long, dense, axis softly pubescent; leaves pubescent on both sides; calyx horizontal or slightly upward.....	<i>O. gratissimum</i> ssp. <i>gratissimum</i> var. <i>gratissimum</i>
6'. Inflorescence 15–22 cm long, lax, axis glabrescent; leaves glabrous, hairs restricted to veins beneath; calyx downwards pointing.....	<i>O. gratissimum</i> ssp. <i>gratissimum</i> var. <i>macrophyllum</i>
7. Pedicel not much longer than calyx; inflorescence axis pubescent; anterior corolla lip boat shaped and villous on back.....	<i>O. kilimandscharicum</i>
7'. Pedicel twice the length of calyx; inflorescence axis puberulent; anterior corolla lip obovate oblong and glabrous.....	<i>O. tenuiflorum</i>

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Supplementary Table S1. Morphological descriptions of *Ocimum* species naturally growing in Assam, indicating their voucher number, common name, and morphological characteristics. A, androecium; B, bract; C, corolla; G, gynoecium; F, flower; H, habit; I, inflorescence; K, calyx; L, leaves; N, nutlets; O, ovary; S, stem

Species	Voucher number	Common name	Morphological characteristics
<i>O. africanum</i> Lour.	KMOAf01 GPOAf03 DHOAf05 HJOAf06	Citron basilika, Hoary/Lao/Lime/Lemon Basil, Sweet/Thai lemon Basil (English); Nimbu/ Nimboo Tulsi (Hindi); Lebu/Nemu Toolakhi (Assamese)	(H) annual herbs or sub-shrubs; 20–70 cm tall; (S) round-quadrangular, puberulent; (L) opposite decussate, ovate, 4–5 × 2–3 cm, acute, aequilateral, shallowly serrate, glabrous above, pubescent on veins beneath, petiole 3–5 cm long, slender, puberulent; (I) lax, 8–12 × 2–3 cm, verticils 1.5–3 cm apart, axis lanate; (B) ovate, 0.4–0.5 × 0.1–0.25 cm, acute, villous; (F) white or pinkish white, 0.8–1.2 × 0.4–0.7 cm, densely pubescent, pedicel recurved, shorter than calyx, pubescent; (K) campanulate, 0.4–0.6 × 0.3–0.45 cm, villous, posterior lip round, apiculate, decurrent on tube, slightly accrescent, anterior lip with two median lanceolate, acuminate teeth, longer than posterior, lateral tooth broad, acute, almost equal to posterior, villous hair at throat; (C) colorless or pinkish white, 0.4–0.8 × 0.5–1.2 cm, lobes entire, villous on back, anterior lip boat shaped, posterior lip with two median oblong lobes, two lateral obovate lobes, tube pubescent and straight; (A) stamens didynamous, 0.4–1.5 cm long, posterior villous base; (G) two equal lobes, 0.8–1.2 cm long, ovary superior, tetralocular, gynobasic style, bifid stigma; (N) narrowly oblong, black at maturity, 2.5–3 × 1–1.5 mm, minutely tuberculate, mucilaginous when wet.
<i>O. americanum</i> L.	BPOA04 MGOA01 BNOA01 JHOA03 GGOA05 CCOA06 DHOA02 SSOA01 NGOA03 DJOA04	American/Hoary/Wild Basil (English); Arni, Bovri, Dulal, Raihan, Kala, Vana Tulsi (Hindi); Bon Toolakhi (Assamese); Lopong (Karbi)	(H) annual herbs, 20–40 cm tall; (S) round quadrangular, short adpressed or retrorse hairs; (L) ovate-lanceolate, 3.2–3.8 × 1–1.5 cm, acute, cuneate, entire or sparsely serrate, glabrous or slightly pubescent above, pubescent on midrib, petiole 0.2–1.5 cm long, slender, pubescent with short antrorse and long patent hairs; (I) 7–12 × 1.2–1.5 cm, lax, verticils 0.8–1 cm apart, axis pubescent; (B) ovate, 0.5–0.7 × 0.2–0.4 cm, apex acute or acuminate, base attenuate, margin pilose; (F) pinkish or white, 0.8–1.2 × 0.3–0.5 cm, moderately hairy, pedicel recurved, 0.1–0.2 cm long, shorter than fruiting calyx; (K) campanulate, 0.3–0.38 × 0.28–0.32 cm, posterior lip rounded, decurrent on tube, anterior lip with two median lanceolate, acuminate teeth, slightly longer than posterior, lateral teeth almost equal to posterior, throat open, villous throat, glabrous base; (C) white, 0.6–0.8 × 0.4–0.6 cm, lobes entire, villous back, posterior lip two median

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			oblong lobes, two lateral ovate-oblong, tube glabrous on both sides; (A) stamens didynamous, 0.5–0.6 cm long, posterior with transverse process near base; (G) two equal lobes, 0.55–0.7 cm long, ovary superior, tetralocular, gynobasic style, bifid stigma; (N) black, narrowly oblong, 1.6–1.9 × 0.7–0.9 mm, minutely tuberculate, mucilaginous when wet.
<i>O. basilicum</i> L.	CCOB07 DHOB03 KGOB04 DJOB04 MGOB04 BKOB01	Basil, Lettuce, Basilic, Bush, Cinnamon, Common, French, Greek, Green Ruffles, Hot Basil, Indian, Mexican, Saint Joseph's Wort, Sweet, Thai Basil (English); Babui/Goolal/Nazbo/Sabja/Van Tulsi, Kamakasturi (Hindi); Ada Kuri, Bon Toolakhi (Assamese); Cheujing nyapa (Deori); Dieng tolshi (Jaintia)	(H) annual herbs, 20–50 cm tall; (S) round quadrangular, glabrous or sparsely pubescent; (L) dark green, elliptic ovate, 4–6 × 2.5–3 cm, acute, aristate, cuneate, oblique, sparsely serrate, pubescent on both sides with long hairs in midrib, petiole 1.5–3 cm long and pubescent; (I) lax or dense, 5–7 × 0.8–1.4 cm, verticils 1.2–1.5 cm apart, axis pubescent; (B) 0.6–0.75 × 0.3–0.55 cm, ovate, elliptic, elliptic lanceolate, apex acuminate, base cuneate or attenuate, margin pilose, pubescent on both sides; (F) crimson white, 1–1.5 × 0.35–0.55 cm, less pubescent, pedicel 1–2 mm long, hairy; (K) green purplish, 0.35–0.45 × 0.3–0.38 cm, campanulate, posterior lip rounded, decurrent on tube, apiculate, anterior lip with two median lanceolate, acuminate teeth, slightly longer than posterior, lateral teeth broad, acute, almost equal to posterior, throat open, villous hair at throat and glabrous base; (C) crimson white, 0.7–0.95 × 0.5–0.65 cm, lobes obscurely crenate, posterior lip with two median oblong lobes, anterior lip boat shaped, oblong in outline, villous on back, corolla tube straight and glabrous on both sides; (A) stamens didynamous, 0.6–0.75 cm long, posterior having tuft hair near base; (G) two equal lobes, 0.6–0.8 cm long, ovary superior, tetralocular, gynobasic style, bifid stigma; (N) dark brown, ovoid ellipsoid, 1.8–3 × 0.75–0.95 mm, mucilage when wet.
<i>O. gratissimum</i> L.	BPOG01 UGOG02 KPOG01 KMOG03 NGOG02 HJOG02	African/Clove/East Indian/Russian/Shrubby/Tree/Wild Basil, Scent leaf, Tea Bush (English); Ban/ Jangli/Vriadha/Mali/Ram Tulsi (Hindi); Ram Toolakhi (Assamese)	(H) perennial shrubs or undershrubs, 1–3.5 m tall; (S) woody at the base, round-quadrangular, glabrescent; (L) spreading, 7–12 × 2.8–6 cm, elliptic ovate, acute, acuminate, oblique, entire, sparsely serrate or serrate in the upper half, sometimes glabrous or puberulent above, hairs restricted to veins on abaxial side, petiole 1.0–2.5 cm long, pubescent; (I) lax or dense, 8–15 × 0.6–1.2 cm, verticils 0.8–2 cm apart, axis glabrous or sparsely or densely pubescent; (B) ovate, 0.3–0.45 × 0.38–0.45 cm, caducous, acute, base rounded, sub sessile or sessile, margin ciliate, pubescent; (F) crimson white, 0.8–1 × 0.2–0.35 cm, moderately pubescent, pedicels 2–3 mm

Species	Voucher number	Common name	Morphological characteristics
			long, pubescent, recurved; (K) greenish, purple at tips, 0.28–0.32 × 0.2–0.3 cm, posterior lip round, decurrent on tube, anterior lip shorter than posterior, two lateral teeth with two median teeth pressed against posterior lip, throat closed, tube with or without patent hairs; (C) crimson white, 0.45–0.65 × 0.38–0.55 cm, lobes obscurely crenate, hairs on back, posterior lip oblong, lobes equal, anterior lip boat shaped, sometimes similar with posterior lip, tube straight, puberulous outside, glabrous inside; (A) stamens didynamous, 0.45–0.6 cm long, posterior pair having transverse process near base, occasionally equal in length with anterior stamens; (G) two equal lobes, 0.43–0.7 cm long, ovary superior, tetralocular ovary, ovary more or less globose, gynobasic style, bifid stigma; (N) brown, sub-globose, 1.5–2 × 0.8–1 mm, minutely tuberculate, mucilaginous when wet.
<i>O. gratissimum</i> L. ssp. <i>gratissimum</i> var. <i>macrophyllum</i> Briq.	JHOG02	African/Clove/Shrubby/ Tree/Wild Basil (English); Ram Tulsi (Hindi); Ram Toolakhi (Assamese)	(H) perennial shrubs, 1.5–2.13 m tall; (S) erect, much branched, woody at the base, round-quadrangular, glabrous; (L) 6–12 × 4.5–7 cm in size, serrate, surface smooth, hairs restricted to veins beneath, apex acuminate, multicostate divergent reticulate venation; petiole 1.5–4 cm long, slender; (I) lax, 15–22 cm long, axis glabrescent, verticils 0.8–1.2 cm apart; (B) 3–4 × 1.8–2.5 mm, green, ovate with broad base, caducous, apex acute, base cordate, sub sessile or sessile, margin ciliate, pubescent on both sides; (F) crimson/ light pastel yellow, 0.5–0.8 × 0.15–0.25 cm, pedicels 3–3.5 mm long, pubescent, spreading, recurved; (K) 2–3.2 × 2–3.5 mm, slightly downwards pointing against the inflorescence axis, green, slightly purplish at tips, posterior lip rounded, wider at tip, acute apex, decurrent on tube, anterior lip shorter than posterior, two hooked lateral curved teeth (uncinate lip) slightly lower than the two median teeth, median lobes of anterior lip pressed against posterior one in fruiting calyx, throat closed, tube with patent hairs; (C) light pastel yellow, 4–5.5 × 2–3 mm, barely exceeding the calyx, lobes obscurely crenate, minute hairs at back, posterior lip oblong and comparatively shorter than anterior lip, lobes are equal in length, anterior lip boat shaped, tube straight, puberulous outside, glabrous inside; (A) stamens 4.5–5 mm long, didynamous, occasionally equal in length with anterior stamens, posterior pair having tufts of hairs at base (barbate filament base); (G)

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			two equal lobes, 6.5–8 mm long, ovary superior, more or less globose, incurved bifid stigma, gynobasic style; (N) 1.8–2.2 × 1.5–2 mm, ivory in colour, brown at maturity, sub globose, minutely tuberculate, producing mucilage when wet.
<i>O. kilimandscharicum</i> Gürke	CCOK05 DHOK04 TSOK01	African/Camphor/Heavy flowered/Hoary/ Kilimanjaro/Perennial Basil, Fever Plant (English); Kapur/Kapuri/Manjari/Patra/Pushpa/Suvasa Tulsi (Hindi); Kopur, Korpur Toolakhi (Assamese)	(H) perennial shrubs or undershrubs, 1–1.5 m tall; (S) much branched and convoluted, dense shrub, woody at base, young stems above, retrose hairs present; (L) 3–4 × 1.8–2.2 cm, small to medium, simple, opposite decussate, elliptic oblong, crenate, acute, pubescent on both side denser on veins, margin serrate, petiole 1–2 cm long; (I) 9–15 × 2–2.5 cm, raceme hairy, verticils 0.3–0.5 cm apart, densely pubescent, many flowered whorls, pedicel 1.5–1.8 cm long; (B) green, purplish at edges, 0.38–0.48 × 0.3–0.5 cm, acute, persistent, pubescent, longer than calyx; (F) pinkish or white, 0.9–1.2 × 0.25–0.38 cm, bilabiate, hypogynous, pedicel 0.2–0.4 cm long, longer than calyx, pubescent; (K) purplish, 0.3–0.35 × 0.28–0.35 cm, persistent, two lipped, anterior lip with two median lanceolate teeth curved upwards, bigger than campanulate one, two lateral teeth below the median tooth, throat open, more hairy on margins and less in inner side; (C) pinkish white, 0.5–0.7 × 0.35–0.45 cm, 2–3 times longer than calyx, anterior lip boat shaped, villous on back, posterior lip have four almost equal sized lobes, tube pubescent and straight; (A) stamens didynamous, 0.55–0.8 cm long, twice of corolla, didynamous, exerted, floccose filament base; (G) two equal lobes, ovary superior, tetralocular, style slender 0.7–0.85 cm long, simple, gynobasic, stigma bifid; (N) brown, oblong, 1.8–2.5 × 0.7–0.8 mm, unchanged when wet (non mucilaginous).
<i>O. tenuiflorum</i> L.	BPOT02 SPOT04 CCOT05 HJOT05 KAOT05	Common/Holy/ Mint/Sacred Basil, Brush-leaf-tea (English); Ajaka, Krishna/Kala/Lakshmi/Radha/ Rama/Shyama/Tulas/Tulsi, Varanda, Vishnupriya (Hindi);	(H) annual herb or perennial shrubs, 30–120 cm tall; (S) round–quadrangular, hirsute, short to long hair; (L) spreading, 4.5–5.5 × 2–2.5 cm, ovate oblong, green or purplish green, completely serrate or only upper half serrate, undulate margin, glabrous or puberulous, petiole 0.8–2.5 cm long, hirsute or glabrous, thick at base; (I) lax, 5–9 × 1.5–2 cm, verticils 0.8–1.2 cm apart, arranged at right angle to inflorescence axis; (B) ovate or obovate, 0.25–0.35 × 0.4–0.55 cm, obtuse, acuminate, cuspidate cuneate, sessile or sub–sessile, margin ciliate, pubescent; (F) pinkish or purplish white, 0.75–0.82 × 0.22–0.35 cm, pedicel 0.35–0.5 cm long, slender,

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		Toolakhi, Kola/Koliya Toolakhi (Assamese); Dhulungshi (Bodo); Lapane (Dimasa); Runhmui (Lushai); Shyamsata (Riang); Tulosi (Khamtis)	pubescent, longer than calyx; (K) 0.3–0.35 × 0.27–0.36 cm, posterior lip round or cuspidate, green or purple in tip, anterior lip with two median lanceolate teeth curved upwards, longer than two lateral teeth and posterior lip, throat open, villous; (C) pinkish, purplish white, 0.6–0.75 × 0.32–0.5 cm, lobes pubescent on back, posterior lip with two obovate oblong median lobes slightly longer or equal than two oblong lateral lobes, anterior lip obovate oblong; (A) stamens didynamous, 0.5–0.75 cm long, posterior pair having small tuft of hairs at base; (G) two sub equal lobes, 0.72–0.8 cm long, ovary superior, tetralocular; (N) brown, oblong, 1.5–2 × 0.6–0.7 mm, smooth, unchanged when wet (non mucilaginous).
<i>Ocimum</i> sp.	KJOT03	Common/Holy/Sacred Basil (English); Toolakhi (Assamese)	(H) annual herb or perennial shrubs, 30–50 cm tall; (S) round–quadrangular, densely pubescent; (L) spreading, yellow-green, 3.8–4.2 × 1.2–1.8 cm, oblique, completely serrate or only upper half serrate, irregular serration, glabrous, veins and leaf margins hirsute, petiole 0.9–1.2 cm long, hairy, thin and flexible; (I) lax, 6–8 × 0.8–1 cm, verticils 0.5–0.6 cm apart, arranged at 45° to inflorescence axis; (B) ovate, 0.18–0.25 × 0.3–0.45 cm, caducous, acute, base rounded, sub sessile or sessile, acuminate, cuspidate cuneate, sessile or sub–sessile, margin pubescent; (F) pinkish or pinkish white, 0.8–1 × 0.2–0.4 cm, pedicel 0.15–0.25 cm long, slender, pubescent, longer than calyx; (K) 0.3–0.32 × 0.24–0.32 cm, posterior lip round or cuspidate, green, anterior lip with two median lanceolate teeth curved upwards, longer than two lateral teeth and posterior lip, throat open, villous; (C) white or pinkish white, 0.55–0.62 × 0.3–0.4 cm, pubescent, lobes obscurely crenate, posterior lip oblong and comparatively shorter than anterior lip, lobes are equal in length, anterior lip obovate oblong, tube straight, puberulous; (A) stamens didynamous, 0.48–0.7 cm long, having small tuft of hairs at base; (G) two sub equal lobes, 0.8–0.9 cm long, ovary superior, tetralocular; (N) brown, globose, 1.2–1.8 × 0.6–0.65 mm, minutely tuberculate, mucilaginous when wet.