Genetic Variability in *Ocimum* L. Germplasm: Medicinal and Economic Potential for Value Addition and Product Development

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Smita Singh, Raj Kishori Lal, and Bikarma Singh

Abstract

Plants are essential for any ecosystem and are considered as the most important source of herbal medicine. They have been used for treatment of different diseases of human beings worldwide since the beginning of human civilization. Among the plant community, one of the genus *Ocimum* L. belonging to the family Lamiaceae is of high value in terms of economic upliftment and product development. Most of the species under this genus are cultivated throughout the tropical and the subtropical agro-climatic zones for medicine and for extraction of essential oils for product development in aroma-based pharmaceutical industries. The growth form of this aromatic genus *Ocimum* can be categorized as herbs, under-shrubs or shrubs that on distillation yield essential oils of various active aroma chemicals, such as eugenol, methyl eugenol, linalool, methyl chavicol, germacrene A and D, elemicin, ß-elmene and (Z)-ocimine, and several other active constituents. These volatile compounds have a tremendous value in pharmaceutical, modern perfumery and food processing industries. Evaluation of biological activities of active ingredients of *Ocimum* indicated great medicinal properties, such as anti-biotic, anti-cancerous, anti-ageing, anti-stress, anti-pyretic, diaphoretic, diuretic, stomachic, anti-microbial and insecticidal, and

Smita Singh, Raj Kishori Lal and Bikarma Singh contributed equally with all other contributors.

S. Singh · R. K. Lal
CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India

B. Singh (✉)
Plant Sciences (Biodiversity and Applied Botany Division), CSIR-Indian Institute of Integrative Medicine, Jammu, Jammu and Kashmir, India

Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India
e-mail: drbikarma@iiim.ac.in; drbikarma@iiim.res.in

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other similar importance in herbal formulations. A survey on 40 genetic stocks (or accessions) of *Ocimum* available at the CSIR-CIMAP belonging to five species, *viz*. *Ocimum basilicum* L. (25: French basil 7, Sweet basil 6, Zanzibar basil 1, Indian basil 10 and Thai basil 1), *Ocimum tenuiflorum* L. (9: Krishna/holy basil 4 and Shyam tulsi 5), *Ocimum kilimandscharicum* Baker ex Gurke (1: Champhor tulsi), *Ocimum africanum* Lour. (2: Hoary basil) and *Ocimum gratissimum* L. (3: African basil/van tulsi/tree basil/clove basil), was undertaken for study. It has been observed that some selected lines of *Ocimum* produce high quantities of essential oils, like *Ocimum tenuiflorum* (*O. sanctum* L.) CIM-Ayu (80% eugenol) and EOH-1 (89.75% methyl chavicol). These active ingredients have high international demand for aroma-based value additions and product development from essential oils. Extension and promotion of these *Ocimum* species will add and contribute to the economic upliftment of the developing countries like India and elsewhere in the world.

Keywords

Aromatic plants · Genetic variability · *Ocimum* · Citral · Eugenol · Aroma-based product development

Abbreviations

AP	Arunachal Pradesh
BST	Bench Scale Trial
CIMAP	Central Institute of Medicinal and Aromatic Plants
CSIR	Council of Scientific and Industrial Research
J&K	Jammu and Kashmir
RBD	Randomized Block Design
UP	Uttar Pradesh
WB	West Bengal

11.1 Introduction

Ocimum L., a versatile aromatic genus of the family Lamiaceae, is represented by 66 species across the World (TPL 2013), and is well known for its medicinal properties and economically important essential oils (Kalita and Khan 2013; Singh 2019a). The genus is very variable and possesses wide range of intra- and inter-specific genetic diversity (Singh et al. 2019). The nomenclature of *Ocimum* species and its varieties is complicated and confusing, and in several instances the oil extracted from morphologically identical plants show different physico-chemical

properties. Species like *Ocimum gratissimum* L., *Ocimum africanum* Lour., *Ocimum basilicum* L., *Ocimum kilimandscharicum* Baker ex Gurke L. and *Ocimum tenuiflorum* L. are examples of well-known important species of the genus that grow in different parts of the World and are known to have curative biological functions (Lal et al. 2004; Barik et al. 2006; Singh 2019b). The plants flourish predominantly as herbs and shrubs and usually survive as annuals or perennial plants in habit. They possess glandular hairs or sessile glands secreting strongly scented volatile secondary metabolites in the form of oils. The dry leaves of *Ocimum* used as leaf herbal tea, essential oils and its chemical derivatives (eugenol, methyl eugenol, linalool, methyl chavicol, germacrene A and D, elemicin, ß-elmene, (Z)-ocimine) are exported to European and Arab countries in sizable quantity every year. The annual export of dry leaves herb, its products, essential oils and derivatives of chemical constituents of *Ocimum* is worth 5000 tons (Bhasin 2012). People know the plant as surasah in sanskrit and tulsi in hindi. Due to antioxidant and anti-ageing effects of tulsi, people use fresh leaves in panchamrut/charanamrut drink after Holi puja (Kumar et al. 2013). In India, tulsi is considered divine and is regarded not merely as a Godsent utility, as most sacred plants are viewed to be, but as an incarnation of the Goddess Herself. The classic Hindu myth, Samudramanthana, or the 'Churning of the Cosmic Ocean', explains that Vishnu spawned tulsi from the turbulent sea as a vital aid for all mankind (Anonymous 1973, Singh 2020). The tulsi leaves, when consumed, can control thirst and so is invaluable to weary travellers.

Apart from religious importance, *Ocimum* has several medicinal properties. Phytochemical investigation reveals that they are rich in carbohydrate, fibre, phosphorous, calcium, protein, iron, beta-carotene, vitamins B_1 and B_2 and in aromatic oils (Lal et al. 2004). It is effective against cold and cough, indigestion, stomach pain and diarrhoea. Nausea, ulcers, ringworm and asthma can also be effectively treated by using tulsi ingredients or extract. It is also helpful in lowering blood sugar and increasing lactation (Lal et al. 2003; Singh and Bedi 2017). The oil is used as antiperspirant as well as fly and mosquito repellent (Table 11.1). Available genetic stocks at the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP, Lucknow) include 105 genetic stocks (or accessions) belonging to five *Ocimum* species – *Ocimum kilimandscharicum*, *Ocimum africanum, Ocimum gratissimum, Ocimum tenuiflorum* and *Ocimum basilicum* – and 7 varieties, which include CIM-Ayu, CIM-Angana, CIM-Kanchan, CIM-Saumya, CIM-Surabhi, Kushmohak and Vikarsudha (Figs. 11.1 and 11.2). In future, there will be possibility to develop more varieties of herbs, increase oil yield containing specific chemicals like eugenol, methyl eugenol, germacrene A and D, linalool, elemicin, ß-elmene and (Z)-ocimine, and produce other chemical contents from other herbal products (Ismaile 2006).

Table 11.1 Medicinal uses of different *Ocimum* species

Ocimum species	Essential oil	Medicinal properties
Ocimum tenuiflorum L. (Synonym: *O. sanctum* L.)	Essential oil obtained after hydro-distillation is rich in high quality essential oil isolate, eugenol, which is of great value in flavouring and used in the synthesis of vanillin.	The plant is pungent, bitter in taste and is reported to possess antituberculosis, antiseptic, antibiotic and anti-cancerous properties. Leaves have diaphoretic, stimulant and expectorant properties. Their juice is applied in cold, cough and chest troubles.
Ocimum basilicum L.	Essential oil is pale yellow, slightly viscid with a sweet spicy herbal odour, having methyl chavicol as a main constituent. It is used in flavour and perfume industry.	The plant is stomachic, stimulant, carminative, antipyretic, diaphoretic, expectorant, diuretic and also useful in heart, brain and blood diseases, asthma, inflammations and enlarged spleen.
Ocimum gratissimum L.	Essential oil is pale yellow with high percentage of eugenol. It is used in flavouring of food products.	The plant has bitter sharp taste and is useful in diseases of brain, heart, liver and spleen, strengths the gums and removes foul breath. It is diaphoretic, stomachic and is good for the treatment of fever.
Ocimum kilimandscharicum Guerk.	Essential oil is light yellow with strong odour of camphor. The oil is widely used in perfume, flavour and pharmaceutical industry.	The plant has carminative, stimulant, antipyretic, antifungal and antibacterial properties.
Ocimum africanum Sims.	Essential oil is light yellow and is a rich source of citral. It is used in perfume, flavour and cosmetic industries. The oil has been reported to possess potent antibacterial and antifungal activities.	The plant is used to cure fever, dysentery and haemorrhages from nose. Leaves and seeds are used in migraine.

11.2 Methodology

In total, 180 collections were assembled from different States of India including few exotic ones from 6 countries (Tanzania, Thailand, Singapore, Slovak Republic, the USA and South Africa). After removing of duplicates, 40 genetic stocks were examined (Table 11.2) for high herbage content and essential oil yield with type of quality during an initial evaluation trial in field (design RBD, rep-2) (Lal 2012). The fresh 100 gm aerial parts of *Ocimum* spices were collected from plants from the field of CSIR-Central Institute of Medicinal and Aromatic Plants and processed by

Fig. 11.1 *Ocimum* germplasm for genetic improvement and development of value-added products: (**a**) *O. tenuiflorum* (synonym: *O. sanctum*, CIM-Ayu), (**b**) *O. tenuiflorum* (CIM-Kanchan), (**c**) O. *tenuiflorum* (synonym: *O. sanctum*, CIM-Angana), (**d**) *O. kilimandscharicum,* (**e**) *O. basilicum* (French basil), (**f**) O. *basilicum* (Indian basil)

hydro-distillation for 3–4 h in a clevenger apparatus to obtain the crude essential oils. Identification of the essential oil composition was done by gas chromatography (GC; Clevenger 1928).

11.3 Results and Discussion

The morphological and essential oil yields were observed in 40 accessions (or lines of five *Ocimum* species: *Ocimum tenuiflorum*, *O. kilimandscharicum*, *O. africanum*, *O. gratissimum* and *O. basilicum*) and 7 varieties, viz. CIM-Ayu, CIM-Angana, CIM-Kanchan**,** CIM-Saumya, CIM-Surabhi, Kushmohak and Vikarsudha. Essential oil yield, oil content and herb yield were found to vary from 98.98 to 465.00, 0.30 to 1.20 and 28.18 to 53.08, respectively, in different populations of *O. basilicum* (Lal et al. 2008; Verma et al. 2011).

Fig. 11.2 *Ocimum* germplasm for genetic improvement and development of value-added products: (**a**) O. *basilicum* (Sweet basil), (**b**) *O. gratissimum*, (**c**) *O. africanum* (**d**) African basil

The percentage of methyl chavicol is 89% and linalool is 1.01% in population 1 (EOH-1) chemotype (Table 11.4). Essential oil yield, oil content and herb yield were found to vary from 48.0 to 183.67, 0.29 to 0.54, and 15.83 to 36.17, respectively, in *O. africanum.* The maximum oil yield found in population 1 had high oil content (0.54%), with high citral 76.62% (geranial 46.59% + neral 30.03%) genotype (OC-1) identified (Table 11.4). Literature surveys revealed that the essential oil of *Ocimum basilicum* has been investigated in detail. Chemotypes described so far for this species are methyl chavicol, linalool and β-ocimene (Ozcan and Chalchat 2002). Essential oil compositions of *Ocimum africanum* are citral (geranial + neral) and β-ocimene. The occurrence of huge chemical variations among *Ocimum* populations collected from diverse localities seems to be due to the divergent climatological and geographical conditions as well as different genetic factors (Ojo et al. 2012). The major chemical constituents found in *Ocimum* having industrial importance are given in Fig. 11.3.

Table 11.2 Total representation of *Ocimum* accessions used in the study

Code	Genotypes/cultivar	Botanical name	Origin
G1	French basil	*Ocimum basilicum*	Chennai, AP (India)
G2	Vikarsudha	*Ocimum basilicum*	CSIR-CIMAP, Lucknow, UP (India)
G3	Sweet basil	*Ocimum basilicum*	Gandhi Nagar, Gujarat (India)
G4	French basil	*Ocimum basilicum*	Bangalore, Karnataka (India)
G5	French basil	*Ocimum basilicum*	Mangalore, Karnataka (India)
G6	French basil	*Ocimum basilicum*	Chandigarh
G7	Shyam tulsi (CIM-Angana)	*Ocimum tenuiflorum*	CSIR-CIMAP, Lucknow, UP (India)
G8	Sweet basil	*Ocimum basilicum*	Singapore
G9	Sweet basil	*Ocimum basilicum*	Singapore
G10	Sweet basil (Kushmohak)	*Ocimum basilicum*	CSIR-CIMAP, Lucknow, UP (India)
G11	Sweet basil	*Ocimum basilicum*	Košice, Slovak Republic
G12	Krishna tulsi (CIM-Ayu)	*Ocimum tenuiflorum*	CSIR-CIMAP, Lucknow, UP (India)
G13	French basil	*Ocimum basilicum*	Mangalore, Karnataka (India)
G14	Indian basil	*Ocimum basilicum*	Muzaffarpur, Bihar (India)
G15	Indian basil (CIM-Saumya)	*Ocimum basilicum*	CSIR-CIMAP, Lucknow, UP (India)
G16	Holi Basil	*Ocimum tenuiflorum*	Udaipur, Rajasthan (India)
G17	Zanzibar basil	*Ocimum basilicum*	Tanzania
G18	Indian basil	*Ocimum basilicum*	Bareilly, Uttaranchal (India)
G19	Scare Basil (CIM-Kanchan)	*Ocimum tenuiflorum*	CSIR-CIMAP, Lucknow, UP (India)
G20	Indian basil	*Ocimum basilicum*	Lucknow, UP (India)
G21	Indian basil	*Ocimum basilicum*	Lakhimpur (Kheri), UP (India)
G22	Kapoor/camphor tulsi	*Ocimum kilimandscharicum*	CSIR-CIMAP, Lucknow, UP (India)
G23	Shyam tulsi	*Ocimum tenuiflorum*	Nasik, Maharashtra (India)
G24	Holi Basil	*Ocimum tenuiflorum*	Lucknow, UP (India)
G25	Indian basil (sel-2)	*Ocimum basilicum*	CSIR-CIMAP, Lucknow, UP (India)
G26	Hoary basil (Selection-1)	*Ocimum africanum*	CSIR-CIMAP, Lucknow, UP (India)
G27	Sweet basil	*Ocimum basilicum*	Trivandrum, Kerala (India)
G28	Shyam tulsi	*Ocimum tenuiflorum*	Lucknow, UP (India)
G29	Hoary basil	*Ocimum africanum*	Allahabad, UP (India)
G30	French basil	*Ocimum basilicum*	Haridwar, Uttaranchal (India)

(continued)

Table 11.2 (continued)

Code	Genotypes/cultivar	Botanical name	Origin
G31	African basil	*Ocimum gratissimum*	CSIR-CIMAP, Lucknow, UP (India)
G32	Thai basil	*Ocimum basilicum* var. *thyrsiflora*	Thailand
G33	Shyam tulsi	*Ocimum tenuiflorum*	Puralia, WB (India)
G34	Hybrid	*O. basilicum* × *O. kilimandscharicum*	CSIR-CIMAP, Lucknow, UP (India)
G35	Tree/van basil	*Ocimum gratissimum*	Jammu, J&K (India)
G36	Indian basil	*Ocimum basilicum*	Phagwara, Punjab (India)
G37	Shyam tulsi	*Ocimum tenuiflorum*	Barabanki, UP (India)
G38	Clove basil	*Ocimum gratissimum*	Shillong, Meghalaya (India)
G39	Indian basil	*Ocimum basilicum*	Razaganj, UP (India)
G40	Indian basil	*Ocimum basilicum*	Rishikesh, Uttaranchal (India)

Table 11.3 BST trial of methyl chavicol type *Ocimum* (entries 13, design RBD, replications 3, plot size: 12.25 m^2)

Entries	Herb yield/ plot (kg)	Oil contents (%)	Oil yield (g/plot)	Methyl chavicol (%)	Linalool content (%)
EOH-1	53.08	1.20	465.00	89.75	1.01
EOH-2	38.59	0.63	240.90	81.95	0.735
EOH-3	33.50	0.39	130.63	39.32	41.12
EOH-4	36.17	0.30	108.51	55.31	28.80
EOH-5	40.59	0.31	125.84	46.89	27.27
EOH-6	28.18	0.35	98.98	61.97	19.63
EOH-7	32.60	0.43	138.65	34.12	35.23
EOH-8	37.85	0.43	160.95	67.89	4.44
EOH-9	31.29	0.50	156.45	60.83	28.54
EOH-10	35.77	0.65	232.09	51.87	23.91
EOH-11	31.00	0.68	209.00	68.30	25.45
EOH-12	35.00	0.67	232.83	17.78	43.50
CIM-Saumya	32.25	0.63	201.75	73.36	19.93
Mean ($\bar{x}$)	35.80	0.52	192.43	–	–
Range	28.18–53.08	0.30–0.88	98.98–465.00	–	–
CD $_{(5\%)}$	2.80	0.069	38.29	–	–
CD $_{(1\%)}$	3.92	0.097	53.66	–	–
F value	46.81**	59.89**	59.02**	–	–

BST Bench Scale Trial, *RBD* Randomized Block Design
**F test indicating analyzing of variance at significant level

Table 11.4 BST trial of citral type *Ocimum* (entries 12, design RBD, replications 3, plot size: 12.50 m^2)

Entries	Herb yield/plot (kg)	Oil content (%)	Oil yield (g/plot)	Neral (%) 1	Geranial (%) 2
OC-1	34.17	0.54	183.67	56.39	28.37
OC-2	23.17	0.32	70.00	30.03	46.59
OC-3	20.27	0.36	73.33	25.84	36.22
OC-4	26.10	0.28	80.33	26.42	35.84
OC-5	25.67	0.40	102.67	18.47	31.45
OC-6	15.97	0.30	48.00	–	–
OC-7	25.73	0.39	100.67	–	–
OC-8	17.93	0.33	59.00	–	–
OC-9	24.27	0.29	69.33	–	–
OC-10	15.83	0.30	49.00	–	–
OC-11	18.40	0.33	60.00	–	–
Local OC (check)	20.28	0.32	64.00	18.47	37.75
Mean ($\bar{x}$)	22.23	0.35	80.00	–	–
Range	15.83–34.17	0.28–0.54	49.00–183.67	–	–
CD $_{5\%}$	3.36	0.053	18.53	–	–
CD $1_{\%}$	4.56	0.071	25.18	–	–
F value	21.28**	15.77**	34.23**	–	–

**F test indicating analyzing of variance at significant level

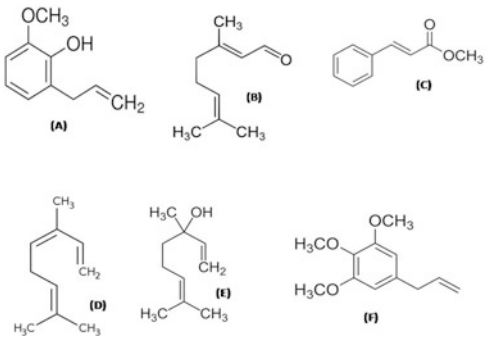

Fig. 11.3 The major chemical constituents in the genus *Ocimum*: (**a**) Eugenol, (**b**) Citral, (**c**) Methyl chavicol, (**d**) β-Ocimene, (**e**) Linalool, (**f**) Elemicin

11.4 Conclusion

In recent few decades, there has been a resurgence of interest in investigating the health-promoting uses of *Ocimum* species across the globe. The nutritional and pharmacological properties of *O. tenuiflorum*, *O. kilimandscharicum*, *O. africanum*, *O. gratissimum* and *O. basilicum*, including several varieties, viz. CIM-Ayu, CIM-Angana, CIM-Kanchan, CIM-Saumya, CIM-Surabhi, Kushmohak

and Vikarsudha in its natural form, have been traditionally used since ancient times, and have provided a new platform for researchers for synergistic interactions of several different active phytochemicals. However, because of its inherent genotypic variations and biochemical complexity, *Ocimum* standardization has eluded modern science. *Ocimum* ingredients are general vitalizers that increase physical endurance in humans, the reason being it contains no caffeine or other stimulant. Chemical characterization of two new species of *Ocimum* was observed in this study. In *O. basilicum* and *O. africanum* population, EOH-1 and OC-1, respectively, obtained maximum essential oil yield. *Ocimum* is traditionally used as a cure-all in many parts of the world, including India. The essential oil compositions of available species of the genus is very much helpful in the pharmaceutical industry and in formulation of drug principles. Preclinical studies in animal models have demonstrated several therapeutic applications of *Ocimum* and recognized them as useful active constituents having anti-diabetic, wound healing, anti-oxidant, anti-microbial, gastroprotective, immunomodulatory, anti-inflammatory, anti-cancerous and several other biological properties, which provide leads for industries in the development of new medicines and drug formulations.

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