A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil

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Abstract *Ocimum* species represent commercially important medicinal and aromatic plants. The essential oil biosynthesized by *Ocimum* species is enriched with specialized metabolites specifically, terpenoids and phenylpropanoids. Interestingly, various *Ocimum* species are known to exhibit diverse chemical profiles, and this chemical diversity has been at the center of many studies to identify commercially important chemotypes. Here, we present various chemotypes from the *Ocimum* species and emphasize trends, implications, and strategies for the quality and yield improvement of essential oil. Globally, many *Ocimum* species have been analyzed for their essential oil composition in over 50 countries. Asia represents the highest number of chemotypes, followed by Africa, South America, and Europe. *Ocimum basilicum* L. has been the most widespread and well-studied species, followed by *O. gratissimum* L., *O. tenuiflorum* L., *O. canum* Sims, *O. americanum* and *O. kilimandscharicum* Gürke. Moreover, various molecular reasons, benefits, adverse health effects and mechanisms behind this vast chemodiversity have been discussed. Different strategies of plant breeding, metabolic engineering, transgenic, and tissue-culture, along with anatomical modifications, are surveyed to enhance specific chemotypic profiles and essential oil yield in numerous *Ocimum* species. Consequently, chemical characterization of the essential oil obtained from *Ocimum* species has become indispensable for its proper utilization. The present chemodiversity knowledge from *Ocimum* species will help to exploit various applications in the industrial, agriculture, biopharmaceutical, and food sectors.

Keywords Chemodiversity · Chemotype · Essential oil · Metabolic engineering · *Ocimum* · Specialized metabolites

Abbreviations

4CL 4-Coumarate-CoA ligase
CRISPR Clustered regularly interspaced short palindromic repeats
CVOMT Chavicol-O-methyltransferase
DMAPP Dimethylallyl pyrophosphate
Introduction

Among the diverse specialized metabolites biosynthesized in the plant kingdom, volatile organic compounds (VOCs) constitute plant-derived essential oils. They are secreted and stored in different specialized structures, such as intra-cytoplasmic oil bodies, ducts and cavities, glandular trichomes, and osmophores (Jacobowitz and Weng 2020; Rehman et al. 2016). Ocimum genus, which belongs to the Lamiaceae family, includes highly aromatic and essential oil-bearing plants with a pantropical distribution (Li et al. 2016; Suddee et al. 2005). According to World Flora Online, 66 Ocimum species have been reported until now (http://www.worldfloraonline.org). However, only a few species, such as Ocimum basilicum Linnæus (L.), O. gratissimum L., O. tenuiflorum L., O. canum Sims, O. americanum L. and O. kilimand-scharicum Gürze have been predominantly valued for their phytopharmaceuticals, aroma and flavors. These Ocimum species are endowed with enormous phytochemical diversity. The essential oil of Ocimum species is a complex mixture of odoriferous VOCs. It has extensive applications in the culinary, cosmetics, medicinal, flavor, fragrance, perfumery, nutraceutical, and toiletry industries (Pandey et al. 2014; Singh et al. 2015).

Different tissues of Ocimum species are utilized in fresh, dried, frozen form or distilled essential oil. The French, Greek, Italian, and Mexican cuisines include mainly fresh leaves of Ocimum species due to their unique aroma. Such fresh aromatic leaves are also suited as flavorings or spices in sauces, stews, salads, and decorations. It can be applied in other food preparations, such as meat, fish, butter, cheese, and beverages (Bown 2001; Meyers 2003; Piva et al. 2021), while essential oil is employed as a food preservative and flavoring agent (Li and Chang 2016). The nanocomposite film prepared from O. basilicum seed mucilage can be used for food packaging (Rohini et al. 2020). Further, the essential oil of O. basilicum has been applied to prepare edible coating and food packaging system to increase food shelf-life (Amor et al. 2021; Mohammadi et al. 2021). The various fragrant compounds from Ocimum species essential oil have found utility in personal care products like soaps, mouthwashes, perfumes, hair care, and dental products (Tucker and DeBaggio 2000). Over the years, Ocimum species have been traditionally exploited to treat various ailments in Indian Ayurveda and traditional African, Chinese and European medicine. Several species of the Ocimum genus possess multiple pharmacological properties, e.g., in vitro antimicrobial, antiviral, antimalarial activities and in vivo analgesic, anti-inflammatory, anti-diarrhoeal, antidiabetic, anticancer, radiation protective, anti-hyperlipidemic activities, etc. (Ali et al. 2021; Pandey et al. 2014; Purushothaman et al. 2018; Santos et al. 2021; Singh and Chaudhuri 2018; Singh et al. 2015, 2016), whereas essential oil is valued in aromatherapy (Li and Chang 2016). On the other hand, silver and copper nanoparticles synthesized using aqueous leaf extract of O. americanum have shown therapeutic properties, including in vitro antibacterial, anticancer and catalytic properties, which can be used for photocatalytic dye degradation (Manikandan et al. 2021a, b). Recently, a molecular docking study showed that apigenin, oleanolic acid and ursolic acid from O. basilicum are potential inhibitors of chymotrypsin-like protease of severe acute respiratory syndrome coronavirus (SARS-CoV2) and could be effective in the treatment of coronavirus disease (COVID-19) (Matondo et al. 2021). Moreover, the hydrogel obtained from O. basilicum seeds paves the way in the biomedical field for targeted drug delivery and sustained drug release (Lodhi et al. 2020). Apart from this, O. basilicum leaf extract has been utilized for preparing mosquito repellent fabrics (Kantheti...
et al. 2020). Additionally, pesticidal activities like fungicidal, nematicidal, larvicidal, insecticidal, trypanocidal, etc., are exhibited by Ocimum species essential oil and their organic or aqueous extracts (Bhavya et al. 2021; Chowdhary et al. 2018; Singh et al. 2014). Furthermore, several Ocimum species have phytoremediation potential for the removal of toxic compounds, such as pesticides (Ramírez-Sandoval et al. 2011), organic dyes (Dada et al. 2020), crude oil (Choden et al. 2021) and heavy metals from soil (Lakshmanraj et al. 2009). Also, bioremediation of heavy metals like copper and chromium is facilitated by O. basilicum seeds (Gupte et al. 2012; Melo and D’Souza 2004). Ocimum basilicum seeds have been used as an effective coagulant for the treatment of textile and paper recycling waste water (Mosaddeghi et al. 2020; Shamsnejati et al. 2015).

The promotion of organic, natural, and green consumerism has led to an increased demand for plant-based products. Meanwhile, natural plant products have globally maintained their place in the market under competition from synthetic compounds. Subsequently, plant-derived essential oils are gaining ground despite the availability of synthetic substitutes of essential oils (Khan 2018). It is estimated that the market of Ocimum species essential oil will grow by 186.5 million USD from 2019 to 2023, with an 8% compound annual growth rate, and Europe will account for the largest market share (https://www.technavio.com/report/global-basil-essential-oil-market-industry-analysis). Overall, with such a huge market potential, essential oil of Ocimum species is of great economic importance for the developing countries in terms of foreign exchange revenue.

Based on the occurrence of one or more major chemical compounds above a fixed threshold level of relative concentration in the essential oil, several chemotypes have been identified from Ocimum species (Kumar et al. 2019; Simon et al. 1990; Varga et al. 2017). Previously, Grayer et al. (1996) had proposed to describe the chemotype(s) based on all the major compounds constituting greater than 20% of the total essential oil, while many researchers have now considered compounds above 10% (Varga et al. 2017). Subsequently, Holm and Hiltunen (1999) have summarized the data on Ocimum species chemotypes until 1999. To the best of our knowledge, no such attempt was made summarizing all available chemotype data from Ocimum species till now. Hence, we explore the existing chemodiversity from essential oil of Ocimum species with potential causes, mechanisms, and the role behind such vast diversity. Additionally, various biotechnological approaches are discussed that can be employed for the chemotypic improvement with better essential oil yield and composition in Ocimum species.

### Essential oil and chemical composition of Ocimum species

The essential oil of Ocimum species, also commonly known as basil oil, is biosynthesized and stored in a specific structure called glandular trichomes present on leaf, stem, and flower (Maurya et al. 2019; Werker et al. 1993). It is made up of secretory cell(s) containing the enzymatic machinery for essential oil biosynthesis and an oil sac for storage. There are two types of glandular trichomes, viz, capitate and peltate, which can be distinguished based on their size and number of the secretory cells (Werker et al. 1993). The essential oil can be obtained from the fresh, semi-dry, or dry aerial plant tissues at the flowering stage by steam distillation or hydro-distillation. The supercritical fluid extraction method is also used to avoid the loss of top notes from the essential oil during the distillation (Occhipinti et al. 2013). Interestingly, in several cases, the essential oil distilled solely from the flowers is superior in chemical composition and thus, of high market value. The essential oil content in leaves of Ocimum species generally varies from 0.5 to 1.4%. However, the composition of essential oil, its yield, and the content varies according to many factors, such as the variety, developmental stage, harvesting season, distillation method, geographical region, and climatic conditions of the plant used (Verma et al. 2013). The specialized metabolites like monoterpenoids, sesquiterpenoids, and phenylpropanoids majorly constitute the essential oil (Pandey et al. 2014). Their analysis and characterization from essential oil is conventionally carried out by gas chromatography–mass spectrometry and recently with advanced liquid chromatography–mass spectrometry.

From a biosynthetic point of view, terpenoids are biosynthesized by the condensation of two isoprene precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These are derived from the mevalonic acid (MVA) pathway localized in the cytosol or 2-C-methyl-d-erythritol-4-
Anand et al. 2016; Iijima et al. 2004). Later, coniferyl is in a few steps converted to the 4-coumaroyl-CoA are derived from the amino acid phenylalanine, which applications (Tables 1 and 2). The phenylpropanoids Till now, more than 100 monoterpenoids and over 135 sesquiterpenoids are bisabolene, bergamotene, caryophyllene, cadinol, farnesene, and germacrene. Similarly, the major sesquiterpenoids are the essential oil of several Ocimum species with diverse (Rana and Blazquez 2015), methyl eugenol/borneol/caryophyllene, caryophyllene, cadinol, farnesene, and germacrene. Till now, more than 100 monoterpenoids and over 135 sesquiterpenoids have been identified from the essential oil of several Ocimum species are derived from the amino acid phenylalanine, which is in a few steps converted to the 4-coumaroyl-CoA (Anand et al. 2016; Iijima et al. 2004). Later, coniferyl and coumaryl alcohols derived from 4-coumaroyl-CoA act as precursors for biosynthesis of various phenylpropanoid derivatives (Gang et al. 2001; Lavhale et al. 2018, 2021; Liu et al. 2015). About 10 phenylpropanoids have been reported from essential oil of Ocimum species, and among them, eugenol, methyl cinnamate, methyl chavicol, and methyl eugenol occur predominantly (Table 3). The structures of the main terpenoids and phenylpropanoids present in the essential oil from various Ocimum species are depicted in Fig. 1. These metabolites have culinary, Industrial, consumer and therapeutic applications (Tables 1, 2, and 3).

**Chemotypes reported from various Ocimum species and their hybrids**

The chemistry of O. basilicum is most studied because of its worldwide distribution. Among the published reports on the chemical diversity in Ocimum species, most of the reports (43%) are available on O. basilicum, followed by O. gratissimum (16%), while minimum reports (0.3%) are available on O. adscendens Willdenow (Willd.), O. urticifolium Roth, and O. suave Willd. (Fig. 2). Globally, 16 Ocimum species have been analyzed for their essential oil composition across 55 countries. Subsequently, nine to ten Ocimum species cover the most chemotypes from Asia, followed by Africa, South America, and Europe (Fig. 3). Looking at the countrywide distribution pattern, India has 75 different chemotypes from eight Ocimum species, while 56 different chemotypes are reported from Brazil from eight Ocimum species. Ocimum basilicum has been the most widespread and studied species, followed by O. gratissimum, O. tenuiflorum, O. canum, O. americanum, and O. kilimandscharicum. Overall, a total of 76 chemotypes have been identified from O. basilicum, indicating the enormous chemodiversity, while others like O. gratissimum and O. tenuiflorum have 30 and 24 chemotypes, respectively (Fig. S1). The species O. basilicum, O. americanum, O. gratissimum, O. kilimandscharicum, O. minimum, O. suave, and O. campechianum Miller (Mill.) have shown a combination of monoterpenoid- and phenylpropanoid-rich chemotypes. The phenylpropanoid-rich chemotypes are exclusively reported from O. tenuiflorum, O. selloi Bentham (Benth.), O. micranthum Willd., O. adscendens, O. uraltoil Roth, and O. citriatum Hornemann (Hornem.) species, while O. canum has monoterpenoid-rich chemotypes (Fig. S2). Furthermore, the species-wise predominant occurrence of specialized metabolites in the essential oil of different Ocimum species is shown in Fig. 4. Additionally, species-wise chemotype details are highlighted in the following section.

Various chemotypes from O. basilicum cover its many subspecies and varieties that can be distinguished based on phenotype and chemical composition. The predominantly occurring chemotypes of O. basilicum essential oil are citral (Padalia et al. 2017), methyl chavicol (Olugbade et al. 2017), linalool, methyl cinnamate/linalool (Raina and Gupta 2018), linalool/1,8-cineole (da Costa et al. 2016), linalool/methyl chavicol (Olugbade et al. 2017), linalool, methyl chavicol/linalool (Maurya and Sangwan 2019), etc. (Table S1). Some of the uncommon chemotypes reported for O. basilicum essential oil are citral/spathulenol (Vieira and Simon 2006), limonene/borneol (Ademiluyi et al. 2016), linalool/epi-bicycloesquiphellandrene (Antić et al. 2019), borneol/β-ocimene (Farhang et al. 2014), menthone/methyl chavicol (Hassanpouraghdam et al. 2010), α-muurolol/γ-muurolene (Amaral-Baroli et al. 2016), etc. (Table S1). The major essential oil chemotypes from O. tenuiflorum are characterized by a large amount of eugenol (23–77%) and methyl eugenol (36–93%) (Raina et al. 2013; Raina and Misra 2018). Moreover, eugenol/β-caryophyllene, eugenol/methyl eugenol (Rana and Blazquez 2015), methyl eugenol/β-caryophyllene (Piras et al. 2018), etc., occur as major
Table 1  Monoterpenoid constituents in essential oil from different Ocimum species with their various applications

| Compound* | Culinary | Industrial | Consumer | Therapeutic |
|-----------|----------|------------|----------|-------------|
| Monoterpenoid hydrocarbons |          |            |          |             |
| 3-Carene; δ-3-Carene | Flavoring agent | Absorbent, adsorbent, polish, paper manufacturing, pesticide, adhesive, paint, printing | Personal care products, cleaning and washing products, laundry products, air care products | – |
| δ-2-Carene | – | Fragrance ingredient, raw material in manufacturing | Cleaning and washing products, personal care products, cosmetics | – |
| Camphene | Flavoring agent | Fragrance ingredient, adhesive, manufacturing chemical, paint, pesticide for non-agricultural use, polish, solvent | Air fresheners, cleaning and washing products, disinfectant, personal care products | – |
| Camphene hydrate | Flavoring agent | – | – | – |
| p-Cymene | Flavoring agent | Fragrance ingredient, fluid property modulator, manufacturing chemical, furniture, paint, soaps, pesticide for non-agricultural use, polish, process regulator, solvent, surface treatment for metals | Air fresheners, cleaning and washing products, personal care products, cosmetics | – |
| o-Cymene | – | Fragrance ingredient | Air fresheners, cleaning and washing products, personal care products, cosmetics | – |
| Fenchene | – | – | Cleaning and washing products | – |
| Limonene; d-Limonene | Flavoring agent, coloring agent | Fragrance ingredient, Insecticides, absorbent, adsorbent, adhesive, colorant, drugs, fluid property modulator, impregnation of leather, paper, textile, wood, lubricant, manufacturing chemical, detergents, metal, paper, paint, plastic, pesticide, solvent | Air fresheners, auto air freshener, cleaning and washing products, personal care products, hand body lotion, hair conditioner, cosmetics | Antimicrobial |
| Myrcene; β-Myrcene | Flavoring agent | Fragrance ingredient, manufacturing detergent, chemical, furniture, fluid property modulator, solvent | Air cleaners and anti-odor agents, cleaning and washing products, personal care products, cosmetics | – |
| Ocimene; β-Ocimene | Flavoring agent | Fragrance ingredient, manufacturing chemical | Cleaning and washing products | – |
| α-Ocimene | Flavoring agent | – | – | – |
| cis-β-Ocimene, (Z)-Ocimene | Flavoring agent | Fragrance ingredient | Personal care products, cosmetics | – |
| (E)-Ocimene, trans-β-Ocimene | Flavoring agent | – | Cleaning and washing products | – |
| allo-Ocimene, trans-allo-Ocimene | Flavoring agent | Fragrance ingredient | – | – |
| neo-allo-Ocimene | Flavoring agent | – | – | – |
| Compound* | Culinary | Industrial | Consumer | Therapeutic |
|-----------|----------|------------|----------|-------------|
| *cis*-allo-Ocimene | Flavoring agent | – | – | – |
| α-Phellandrene | Flavoring agent | Fragrance ingredient | Cleaning and washing products | – |
| β-Phellandrene | Flavoring agent | Fragrance ingredient, fluid property modulator | Air fresheners, cleaning and washing products | – |
| α-Pinene | Flavoring agent | Fragrance ingredient, absorbent, adsorbent, detergent, fluid property modulator, lubricant, manufacturing chemical, drug, furniture, oils, paper, paint, pesticide, solvent | Air fresheners, automotive care products, cleaning and washing products | – |
| β-Pinene | Flavoring agent | Fragrance ingredient, absorbent, adsorbent, detergent, drug, plastic, paint, pesticide, solvent | Air fresheners, automotive care products, cleaning and washing products, personal care products, cosmetics | – |
| Pinocarvone | Flavoring agent | – | Personal care products, cosmetics | – |
| Sabinene | Flavoring agent | – | – | – |
| α-Terpinene | Flavoring agent | Adhesive, binding agent, fluid property modulator, manufacturing furniture, chemical, solvent, paint | Air fresheners, cleaning and washing products, personal care products | – |
| γ-Terpinene | Flavoring agent | Adhesive, binding agent, detergent, fluid property modulator, manufacturing plastic, rubber, paint, polish, surface treatment | Air fresheners, automotive care products, cleaning and washing products, personal care products | – |
| Terpinolene; α-Terpinolene | Flavoring agent | Fragrance, absorbent, adsorbent, adhesive, air treatment, binding agent, detergent, fluid property modulator, impregnation of leather, paper, textile, lubricant, manufacturing chemical, water treatment, paint remover, solvent, rubber, plastic, polish | Automotive care products, cleaning and washing products, personal care products, cosmetics | – |
| α-Thujene | Flavoring agent | Fragrance | Air fresheners | – |
| Oxygenated monoterpenoids | | | | |
| Borneol | Flavoring agent | Fragrance ingredients, manufacture of its esters | Air care products, cleaning and furnishing care products, laundry and dishwashing products, personal care products | Antibacterial, anticoagulant activity, relief of minor aches |
| Bornyl acetate | Flavoring agent | Fragrance ingredients | Air care products, cleaning and furnishing care products, laundry and dishwashing products, personal care products | – |
| Compound                  | Culinary          | Industrial                                                                 | Consumer                                                                 | Therapeutic                          |
|---------------------------|-------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------|
| Camphor                   | Flavoring agent   | Fragrance ingredients, absorbent, adsorbent, air treatment, anti-freezing,   | Air fresheners, cleaning and washing products, personal care products,    | Antibiotic, anti-inflammatory        |
|                           |                   | colorant, explosives, fluid property modulator, lubricant, manufacturing    | cosmetics                                                                |                                      |
|                           |                   | chemical, pesticide                                                         |                                                                          |                                      |
| 1,8-Cineole               | Flavoring agent   | Fragrance ingredients, manufacturing chemical, polish, pesticide             | Air fresheners, cleaning and washing products, personal care products     | Antibiotic, anti-inflammatory        |
| 2,3-Dehydro-1,8-Cineole   | Flavoring agent   | –                                                                           | –                                                                        |                                      |
| *exo*-2-Hydroxycineole    | Flavoring agent   | –                                                                           | –                                                                        |                                      |
| acetate                   |                   |                                                                              |                                                                          |                                      |
| Citronellal               | Flavoring agent   | Fragrance ingredients, herbicide, absorbent, adsorbent, adhesive, air       | Air fresheners, automotive care products, cleaning and washing products,  | –                                    |
|                           |                   | treatment, fluid property modulator, manufacturing chemical, polish, water  | personal care products, cosmetics                                         |                                      |
|                           |                   | treatment                                                                    |                                                                          |                                      |
| Citronellol               | Flavoring agent   | Fragrance ingredients                                                        | –                                                                        |                                      |
| Citronellyl acetate       | Flavoring agent   | Absorbent, adsorbent, Air treatment, fluid property modulator, manufacturing | Air fresheners, automotive care products, cleaning and washing products,  | –                                    |
|                           |                   | chemical                                                                      | personal care products, cosmetics                                         |                                      |
|                           |                   |                                                                              |                                                                          |                                      |
| *p*-Cymene-8-ol           | Flavoring agent   | Fragrance ingredients                                                        | Cleaning and washing products                                             | –                                    |
| Carvacrol                 | Flavoring agent   | Pesticide                                                                     | –                                                                        |                                      |
| Fenchone                  | Flavoring agent   | Fragrance ingredients                                                        | Personal care products                                                     | –                                    |
|                           |                   |                                                                              |                                                                          |                                      |
| Fenchol; Fenchyl alcohol  | Flavoring agent   | Fragrance ingredients, polish, fluid property modulator, manufacturing      | Cleaning and washing products                                             | –                                    |
|                           |                   | furniture, chemical, pesticide                                               |                                                                          |                                      |
|                           |                   |                                                                              |                                                                          |                                      |
| *β*-Fenchyl alcohol       | –                 | Pesticide                                                                     | –                                                                        |                                      |
| Fenchyl acetate           | Flavoring agent   | Fragrance ingredients                                                        | Cleaning and washing products                                             | –                                    |
| Geranial                  | Flavoring agent   | Fragrance ingredients, manufacturing chemical, soaps                          | Cleaning and washing products                                             | –                                    |
| Geraniol; *β*-Geraniol    | Flavoring agent   | Fragrance ingredients, absorbent, adsorbent, fungicides, drug, fluid        | Air fresheners, automotive care products, cleaning and washing products,  | –                                    |
|                           |                   | property modulator, impregnation agent, lubricant, manufacturing drug,      | personal care products                                                     |                                      |
|                           |                   | chemical, paint, softener, repellent, water treatment                        |                                                                          |                                      |
| Geranyl acetate           | Flavoring agent   | Fragrance ingredients, absorbent, adsorbent, adhesive, air treatment, fluid | Air fresheners, automotive care products, cleaning and washing products,  | –                                    |
|                           |                   | property modulator, water treatment, pesticide                               | personal care products, cosmetics                                          |                                      |
| Compound       | Culinary            | Industrial                                                                 | Consumer                                                                 | Therapeutic                      |
|----------------|---------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------|
| Geranyl formate| Flavoring agent     | Fragrance ingredients, absorbent, adsorbent, fluid property modulator, paint, pesticide, polish | Cleaning and washing products, personal care products                      |                                  |
| Hotrienol      | Flavoring agent     | –                                                                          | –                                                                         | –                                |
| Isoborny acetate| Flavoring agent     | Fragrance ingredients                                                     | Air care products, cleaning and furnishing care products, laundry and dishwashing products, personal care products | –                                |
| Isopulegol     | Flavoring agent     | –                                                                          | –                                                                         | –                                |
| Linalool; β-Linalool| Flavoring agent | Fragrance ingredients, absorbent, absorbent, adhesive, air treatment, bleaching, fragrance, impregnation agent, lubricant, manufacturing beverages, chemical, furniture, paint, paper, solvent, surface treatment, water treatment, softener, polish, repellent, plastic, pesticide, synthesis of linalool esters and vitamin E | Air fresheners, automotive care products, cleaning and washing products, personal care products | –                                |
| Linalool oxide | Flavoring agent     | –                                                                          | –                                                                         | –                                |
| trans-Linalool oxide| Flavoring agent | –                                                                          | –                                                                         | –                                |
| Linalyl acetate| Flavoring agent     | Fragrance ingredients, absorbent, adsorbent, air treatment, detergent, fluid property modulator, lubricant, manufacturing automotive care products, chemical, metal, machine, plastic, paint, pesticide, surface treatment, repellent, water treatment | Air fresheners, automotive care products, cleaning and washing products, personal care products | –                                |
| Menthol        | Flavoring agent     | Fragrance ingredients, absorbent, adsorbent, fluid property modulator, manufacturing drug, chemical, beverages, paint, polish, pesticide, repellent, water treatment | Air fresheners, cleaning and washing products, personal care products | Antimicrobial, allergy, asthma, anti-inflammatory, antipruritic |
| Menthone       | Flavoring agent     | Fragrance ingredients                                                     | –                                                                         | –                                |
| Myrtenal       | Flavoring agent     | –                                                                          | –                                                                         | –                                |
| Myrtenol       | Flavoring agent     | Fragrance ingredients                                                     | –                                                                         | –                                |
| Myrtenyl formate| Flavoring agent     | –                                                                          | Cleaning and washing products                                            | –                                |
| Neral          | Flavoring agent     | Fragrance ingredients, fluid property modulator, manufacturing chemical   | Cleaning and washing products                                            | –                                |
| Compound          | Culinary             | Industrial                                                                 | Consumer                                                                 | Therapeutic          |
|-------------------|----------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------|
| Nerol             | Flavoring agent      | Fragrance ingredients, absorbent, adsorbent, air treatment, fluid property modulator, manufacturing chemical, furniture, polish, paint, pesticide, solvent, surface treatment, repellent | Automotive care products, cleaning and washing products, personal care products | –                    |
| Nerol acetate; Neryl acetate | Flavoring agent      | Fragrance ingredients, absorbent, adsorbent, air treatment, fluid property modulator, manufacturing chemical, polish | Automotive care products, cleaning and washing products, personal care products | –                    |
| Perilla alcohol   | Flavoring agent      | Fragrance ingredients                                                      | Cleaning and washing products                                            | –                    |
| Perilla aldehyde  | Flavoring agent      | Fragrance ingredients                                                      | Personal care products                                                   | –                    |
| Pinol             | Flavoring agent      | Fragrance ingredients                                                      | –                                                                        | –                    |
| Piperitone        | Flavoring agent      | Fragrance ingredients                                                      | –                                                                        | –                    |
| \(\alpha\)-Terpineol | Flavoring agent      | Fragrance ingredients, absorbent, adsorbent, air treatment, fluid property modulator, casting agent, colorant, conductive material, foaming agent, lubricant, manufacturing drug, chemical, furniture, paint, polish, sewage treatment, solvent, surface treatment, welding soldering | Automotive care products, cleaning and washing products, personal care products | –                    |
| \(\text{trans-}\beta\)-Terpineol | Flavoring agent      | Fragrance ingredients                                                      | –                                                                        | –                    |
| Terpinene-4-ol    | Flavoring agent      | Fluid property modulator, paint, manufacturing chemical, drug, furniture, polish. | Automotive care products, cleaning and washing products                  | –                    |
| \(\alpha\)-Terpinyl acetate | Flavoring agent | Fragrance ingredients, fluid property modulator, sewage treatment, repellent | Automotive care, cleaning and washing products, personal care products | –                    |
| \(\beta\)-Thujone; \(\text{trans-}\)Thujone | Flavoring agent | –                                                                          | –                                                                        | –                    |
| 4-Thujanol        | Flavoring agent      | –                                                                          | –                                                                        | –                    |
| Verbenone         | Flavoring agent      | –                                                                          | –                                                                        | –                    |
| Methyl thymol     | Flavoring agent      | Fragrance ingredients                                                      | Cleaning and washing products                                            | –                    |
| Thymol            | Flavoring agent      | Fragrance ingredients, fungicide, fluid property modulator, manufacturing drug, chemical, paint, polish, pesticide | Cosmetics, cleaning and washing products, personal care products, automotive care products | Antimicrobial, antifungal |

*Monoterpenoid compounds which are present in *Ocimum* species but their use is not yet reported; \(\beta\)-Thujene; Thuj-\(\text{\textgreek{g}}\)-2,4(10)-diene; Verbenene; Bornyl chloride; Camphol; Isopinocamphole; endo-Fenchyl acetate; \(\alpha\)-Fenchyl acetate; iso-Isopulegol; cis-Linalool oxide; Linalool formate; Epoxylinanol; Mentha-1,5-dien-8-ol; \(\text{trans}\)-Ocimene oxide; \(\text{\textgreek{f}}\)-Pino-carveol; Terpendiol \(\text{\textgreek{f}}\); \(\delta\)-Terpineol; \(\alpha\)-Thujone; \(\text{trans-}\)4-Thujanol; \(\text{cis}\)-Tagetone; \(\text{trans}\)-Tagetone; Dihydratagetone; \(\text{cis}\)-Sabinene hydrate; \(\text{trans}\)-Sabinene hydrate; \(\text{cis}\)-Verbenol
chemotypes, while exceptional chemotypes β-elemene/β-caryophyllene (Kitchlu et al. 2013), 1,8-cineole/β-bisabolene (Carović-Stanko et al. 2011), eugenol/α-cubebe, eugenol/α-humulene (Saran et al. 2017), etc., have also been detected in *O. tenuiflorum* (Table S2). *O. americanum* essential oil can be represented by camphor (Raina and Misra 2018), camphor/limonene (Verma et al. 2016), citral (Vieira and Simon 2006), methyl cinnamate (Vieira and Simon 2006), methyl chavicol/linalool (Singh et al. 2013), etc., as main chemotypes (Table S3). However, anisole, β-bisabolene/1,8-cineole (Vieira and Simon 2013), eugelol/camphor (Raina and Misra 2011), eugenol/camphor (Rao et al. 2011), etc., are the uncommon chemotypes (Table S4). Apart from this, *O. americanum* essential oil is enriched with different VOCs in hybrids that may not be present in the parental species (da Costa et al. 2014). For instance, linalool/methyl chavicol chemotype has been identified from the intraspecific hybrids between various *O. basilicum* cultivars. Further, hybridization also favored the generation of camphor, neral, geranial, β-selinene, bicyclogermacrene, (E)-caryophyllene, and methyl chavicol (da Costa et al. 2016). Similarly, *O. citriodorum* Visiani (Vis.) is an interspecific hybrid between *O. basilicum* and *O. canum* with a strong lemony scent. The essential oil from this hybrid is incredibly rich in citral, comprising a mixture of geranial (31–46%) and neral (21–35%) (Carović-Stanko et al. 2010b; Raina and Gupta 2018) (Table S9). Recently, natural hybridization between terpenoid-rich *O. kilimandscharicum* and phenylpropanoid-rich *O. basilicum* has led to two novel hybrids with the chemotype of methyl chavicol/β-linalool (Gurav et al. 2020) (Table S9).

**Potential causes of the vast chemodiversity in *Ocimum* species**

The chemical composition at any developmental stage of the plant is determined by its genotype along with differential expression and regulation of genes involved in the biosynthetic pathways (Gonçalves and Romano 2013; Singh et al. 2015) (Fig. 5). For instance, *O. gratissimum* and *O. tenuiflorum* both are phenylpropanoid-rich species; however, higher expression of EUGENOL-O-METHYLTRANSFERASE (EOMT) in *O. tenuiflorum* leads to more methyl eugenol. As expected, lower EOMT expression results in eugenol-rich chemotype in *O. gratissimum* (Anand et al. 2016). Further, the genetic regulation of specialized metabolic pathways at the post-transcriptional and post-translational levels is considerably responsible for chemodiversity (Fig. 5). For example, a higher transcript and protein level of CHAVICOL-O-
| Compound* | Culinary | Industrial | Consumer | Therapeutic |
|-----------|----------|------------|----------|-------------|
| **Sesquiterpenoid hydrocarbons** | | | | |
| α-Bisabolene; (E)-α-Bisabolene | Flavoring agent | – | – | – |
| β-Bisabolene | Flavoring agent | – | – | – |
| cis-α-Bisabolene | Flavoring agent | – | – | – |
| γ-Bisabolene | Flavoring agent | – | Air care products | – |
| Bourbonene; β-Bourbonene | Flavoring agent | – | – | – |
| δ-Cadinene | Flavoring agent | – | – | – |
| (–)-Calamenene; trans-Calamenene | Flavoring agent | – | – | – |
| β-Caryophyllene; trans-β-Caryophyllene | Flavoring agent | Fragrance ingredients, absorbent, adsorbent, air treatment, polish | Cleaning and washing products | – |
| trans-Caryophyllene | Flavoring agent | – | Air care products | – |
| β-Cedrene | – | Fragrance ingredients | – | – |
| Copaene; α-Copaene | Flavoring agent | Fragrance ingredients | – | – |
| β-Elemene | – | – | Cleaning and washing products | – |
| δ-Elemene | Flavoring agent | Fragrance ingredients | – | – |
| Farnesene; α-Farnesene | Flavoring agent | Fragrance ingredients, fuel additive | – | – |
| (E)-β-Farnesene | Flavoring agent | Fragrance ingredients, intermediates, lubricants and greases | Personal care products | – |
| Germacrene D | Flavoring agent | – | Cleaning and washing products | – |
| β-Guaiene | Flavoring agent | Fragrance ingredients | Cleaning and washing | – |
| Humulene; α-Humulene | Flavoring agent | Fragrance ingredients | Cleaning washing products | – |
| α-Muurolene | Flavoring agent | – | – | – |
| Valencene | Flavoring agent | Fragrance ingredients | Cleaning washing products | – |
| **Oxygenated sesquiterpenoids** | | | | |
| α-Bisabolol | Flavoring agent | – | – | – |
| epi-α-Bisabolol | Flavoring agent | – | Cosmetics | – |
| Cubenol | Flavoring agent | – | – | – |
| Caryophyllene oxide | Flavoring agent | – | Cleaning and washing products | – |
| β-Caryophyllene oxide | Flavoring agent | – | – | – |
| Cedrol | Flavoring agent | Fragrance ingredients, manufacturing chemical | Cleaning and washing products, personal care products, cosmetics | Traditional medicine |
| Elemol | Flavoring agent | Fragrance ingredients, absorbent, adsorbent, air treatment | – | – |
| **Compound** | **Culinary** | **Industrial** | **Consumer** | **Therapeutic** |
|--------------|--------------|----------------|-------------|----------------|
| -Eudesmol    | Flavoring agent | – | – | – |
| Farnesol     | Flavoring agent | Fragrance ingredients | Pesticide | Cosmetics |
| Guaiol       | Flavoring agent | – | – | – |
| T-muurolol   | – | – | – | – |
| Nerolidol; \((E)-\)Nerolidol | – | – | – | – |
| – | – | – | – | – |

| **Compound** | **Culinary** | **Industrial** | **Consumer** | **Therapeutic** |
|--------------|--------------|----------------|-------------|----------------|
| – | – | – | – | – |

**METHYLTRANSFERASE (CVOMT)** was observed in *O. basilicum* (line SD). However, ubiquitination of CVOMT has led to a reduction in enzyme activity, and thus, it decreased methyl chavicol content in *O. basilicum* (line SD). Alternatively, in the absence of this ubiquitination, *O. basilicum* (line EMX-1) showed higher CVOMT activity with high methyl chavicol content (Xie et al. 2008). The vast array of terpenoids in the *Ocimum* species is biosynthesized by different terpene synthases (TPSs) that have an exclusive ability to catalyze multiple product formation using a single substrate (Iijima et al. 2004) (Fig. 5). Additionally, both molecular and environmental factors affect chemical composition (Verma et al. 2013).

The existence of different chemotypes in *Ocimum* species could also be attributed to cross-pollination leading to intra- and inter-specific hybridization, resulting in higher variation in the chemical profiles (Gurav et al. 2020; Varga et al. 2017). Other factors, such as plant habit, can also influence specialized metabolism. For instance, the popular sanctum group of *Ocimum* species (*O. tenuiflorum, O. gratissimum, O. viride* Willd., *O. suave* and *O. carnosum*) prominently harbors phenylpropanoid biosynthesis because of perennial woody habit. The possible explanation for this is phenylpropanoid biosynthetic pathway generates monolignol alcohols required for lignin biosynthesis. In contrast, the basilicum group of *Ocimum* species (*O. canum, O. basilicum, O. americanum,* and *O. kilimandscharicum*) harbors terpenoid biosynthesis because of the annual herbaceous habit (Khosla 1995). Also, natural evolutionary events, polyploidy, and selective breeding can play a significant role in chemical diversification as observed in different *Ocimum* species (Carović-Stanko et al. 2010a; Iijima et al. 2004). Similarly, the occurrence of either phenylpropanoid- or terpenoid-rich *Ocimum* species may be attributed to the diversification of pathways during the evolution (Singh et al. 2015). Thus, looking at such a massive chemodiversity, the next obvious point arises about why plant generates them?

**Multiple benefits of chemodiversity to *Ocimum* species**

Albeit humans have explored plant-derived aromatic compounds for their benefits, plants do not produce...
| Compound\(^a\)          | Culinary | Industrial                                                                 | Consumer                                                                 | Therapeutic               |
|-------------------------|----------|----------------------------------------------------------------------------|--------------------------------------------------------------------------|---------------------------|
| *cis*-Anethole          | –        | Flavoring agent                                                            | Fragrance ingredients, manufacturing drug, chemical                       | –                         |
| *trans*-Anethole        | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, air treatment, manufacturing drug, metal, radio, paint, fluid property modulator, surface treatment, metals, water treatment | Air care products, cleaning and furnishing care products, laundry and dishwashing products, cleaning and washing products, personal care products |
| Anisaldehyde            | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, air treatment, manufacturing drug, metal, radio, paint, fluid property modulator, surface treatment, metals, water treatment | Cleaning and washing products, automotive care products, personal care products |
| Chavicol                | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | –                         |
| Chavibetol              | –        | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | –                         |
| Cinnamyl acetate        | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | Air care product          |
| Eugenol                 | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | Cosmetics, cleaning and washing products, personal care products, automotive care products |
| Eugenol acetate         | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adrsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | –                         |
| Isoeugenol              | Flavored | Flavoring agent                                                            | Fragrance ingredients, manufacturing vanillin                            | –                         |
| Methyl chavicol         | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | Cleaning and washing products, personal care products |
| *(E)*-Methyl cinnamate  | Flavored | Flavoring agent                                                            | Fragrance ingredients, absorbent, adsorbent, fungicides, insecticide, air treatment, colorant, fluid property modulator, manufacturing drug, chemical, furniture, paint, polish, softener, water treatment | Cleaning and washing products, air care products |
| *(Z)*-Methyl cinnamate  | Flavored | Flavoring agent                                                            | Fragrance ingredients, pesticide                                          | –                         |
| Methyl eugenol          | Flavored | Flavoring agent                                                            | Fragrance ingredients, pesticide                                          | –                         |
| Vanillin                | Flavored | Flavoring agent                                                            | Fragrance ingredients, Absorbent, adsorbent, air treatment, fluid property modulator, lubricant, manufacturing chemical, beverages, pesticide, paint, polish | Cleaning and washing products, personal care products, cosmetics, automotive care products |

\(^a\) Phenylpropanoid compounds which are present in *Ocimum* species but their use is not yet reported; \(p\)-Methoxy cinnamyl alcohol; \(p\)-Methoxy cinnamyl aldehyde

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- Not reported for use
them for such purposes. As plants are sessile, they communicate with the surrounding environment using a chemical language by emitting diverse VOCs. These are typically lipophilic compounds that evaporate into the atmosphere because of high vapor pressure (Dudareva et al. 2013). Plant-derived VOCs function in arrays of ecological contexts, such as plant reproduction, protection, communication, and adaptation to abiotic stresses (Dudareva et al. 2013; Vivaldo et al. 2017; Vranova et al. 2013) (Fig. 6). Plants that cannot self-pollinate are dependent on pollinators for their reproductive success (Dudareva and Pichersky 2000). Plant VOCs serve as an attractant for various pollinators like beetles, moths, bees, etc. For example, compounds like linalool, methyl eugenol attract pollinators, whereas esters released by the ripened fruit attract seed-dispersing agents (Gang 2005; Raguso and Pichersky 1995). As *O. basilicum* is entomophilous, during flowering, it benefits the other neighboring plant by increasing the frequency of pollinator visits (Jiang et al. 2016). In response to the damage or herbivory, plants release VOCs to deter or repel the insect or animal herbivores (Kessler and Baldwin 2002). Furthermore, for their self-defense, plant VOCs aid in recruiting various parasitic or predatory insects that are enemies of attacking herbivores (Dicke and van Loon 2000; Shiojiri et al. 2002). Some of the *Ocimum* species can be used as a companion plant with crops for biological control of pests, such as aphids, beetles, moths and whiteflies, as

| Monoterpenoids       | Sesquiterpenoids          | Phenylpropanoids         |
|----------------------|---------------------------|--------------------------|
| Camphor             | α-Muuroline               | Eugenol                  |
| γ-Terpinene         | β-Bisabolene              | Methyl chavicol          |
| (E)-β-Ocimene       | β-Selinene                | (E)-Methyl cinnamate     |
| Geraniol            | T-Muurolol                | (Z)-Methyl cinnamate     |
| Geranial            | (E)-β-Farnesene           | Anethole                 |
| Thymol              | Germacrene-D              | Isoeugenol               |
| Neral               | β-Cadinol                 | Eugenol acetate          |
| Limonene            | α-Cadinol                 | Chavicol                 |
| (E)-β-Farnesene     | β-Caryophyllene           | Methyl eugenol           |
| Germacrene-D        | β-Selinene                | (E)-Methyl cinnamate     |
| Chavicol            | β-Bisabolene              | (Z)-Methyl cinnamate     |
| Methyl chavicol     | Thymol                    | Anethole                 |
| (E)-Methyl cinnamate| β-Bisabolol               | Isoeugenol               |
| (Z)-Methyl cinnamate| Germacrene-D              | Eugenol acetate          |
| Methyl chavicol     | Germacrene-D              | Chavicol                 |
| (Z)-Methyl cinnamate| Germacrene-D              | Methyl eugenol           |
| Eugenol             | Germacrene-D              | (E)-Methyl cinnamate     |
| Methyl eugenol      | Germacrene-D              | (Z)-Methyl cinnamate     |
| Isoeugenol          | Germacrene-D              | Anethole                 |
| Eugenol acetate     | Germacrene-D              | Isoeugenol               |
| Chavicol            | Germacrene-D              | Eugenol acetate          |

**Fig. 1** Major specialized metabolites of monoterpenoid, sesquiterpenoid and phenylpropanoid classes reported from the essential oil of various *Ocimum* species.
well as for attracting predatory insects. For instance, *Amaranthus hybridus* plant had lower aphid infestation when surrounded with either *O. basilicum* or *O. gratissimum* than alone (Yarou et al. 2020). Similarly, *Aphis citricola* aphid abundance was decreased by 38%, and the number of its natural enemies also increased when apple trees intercropped with *O. basilicum* (Song et al. 2013). Also, the presence of *O. basilicum* and *O. gratissimum* in the vicinity of the tomato reduced oviposition of leafminer *Tuta absoluta* on tomato plants (Yarou et al. 2018). In another study, *O. basilicum* attracted the generalist predatory insect green lacewing *Ceraeochrysa cubana* and benefited the larval and adult survival (Batista et al. 2017). Moreover, many volatile metabolites possess antibacterial and antifungal activities that may protect the plant from pathogens (Dicke and Baldwin 2010; Quintana-Rodriguez et al. 2018). For example, methyl chavicol-rich essential oil of *O. ciliatum* has been shown to possess in vitro antifungal activity, against phytopathogenic fungi *Colletotrichum gloeosporioides* (MIC ≥ 1000 ppm) and *Moniliophthora perniciosa* (MIC = 250–500 ppm) (Costa et al. 2015), while methyl chavicol (at 1000 ppm) and linalool (at 300 ppm) chemotypes of *O. basilicum* reduced the mycelial growth of *Botrytis fabae* by 78 and 49%, respectively (Oxenham et al. 2005). Upon the herbivore attack, VOCs released by the infested plant may further induce the volatile emission from healthy leaves of the same plant or adjacent unchallenged plants (Baldwin et al. 2006). In *Ocimum* species, there is no such report of the metabolic priming on the neighboring plant; however, this metabolic priming results in a more rapid and intense defense response that can be mounted by healthy adjacent plants upon any subsequent herbivory attack (Engelberth et al. 2004; Kim and Felton 2013). Plants also compete with other nearby plants because of the allelopathic effect on their germination and growth through VOCs (Romagni et al. 2000). Aqueous leaf extract of *O. basilicum* has been shown to have an inhibitory effect on the growth of weeds, *Anagallis*
arvensis and Phalaris minor (El-Rokiek et al. 2018). Moreover, plants throughout their life cycle are exposed to a variety of abiotic stress conditions. Different VOCs can serve as photoprotective, thermotolerant, and antioxidant agents (Fig. 6) by safeguarding plants against light, heat, cold, and oxidative stress conditions (Cofer et al. 2018; Loreto and Schnitzler 2010; Loreto and Velikova 2001). For instance, volatile terpenoids improve plant tolerance to the abiotic stresses by quenching reactive oxygen species (ROS) or stabilizing the cell membrane (Brilli et al. 2019). In O. basilicum, drought stress resulted in the enhanced production of α-bergamotene, β-myrcene, methyl eugenol, and methyl chavicol (Mandoulakani et al. 2017). Similarly, microwave exposure led to higher emission of α-bergamotene, 1,8-cineole, caryophyllene oxide, and methyl chavicol (Lung et al. 2016). However, decrease in the eugenol and methyl eugenol levels was observed in O. tenuiflorum under cold, drought, flood and salt stress conditions (Rastogi et al. 2019).

Potential adverse health effects of specialized metabolites present in Ocimum species

Though terpenoids and phenylpropanoids have been widely used in various applications, they enter the human body through oral, dermal, and nasal routes. Despite their many health benefits, some specialized metabolites like camphor, methyl eugenol, and methyl chavicol present in the essential oil of Ocimum species could have toxic effects after particular concentrations based on the data from in vivo and in vitro studies in model organisms or cell lines (Bristol 2011; Johnson et al. 2000; Zuccarini 2009) (Table 4). For example, methyl eugenol and methyl chavicol are reported of having genotoxic or carcinogenic potential at specific levels (Table 4). Interestingly, Ocimum species aqueous or organic extracts of a specific tissue or whole plant were found to be less toxic (Table 4). Most of the terpenoids have shown some cytotoxic effects primarily on vital organs, such as the liver, lungs, kidney, and neuronal tissues at higher concentrations when used in pure form. These cytotoxic studies have shown that these terpenoids exert adverse effects by disrupting plasma membrane, producing ROS, impairing
mitochondrial function, and/or causing lipid peroxidation (Agus 2021). These specialized metabolites mainly exhibit hepatotoxicity as the reactive metabolites and ROS are formed during their metabolism in the liver (Zárybnický et al. 2018). Consequently, such specialized metabolites must be carefully utilized in various applications due to their acute or chronic adverse effects beyond a specific level. However, up to certain levels, these metabolites either in purified form or plant extracts as a bouquet of compounds, might be safe for human usage. Further, these plant-based natural molecules are recommended to be used in formulations and not in pure form.

**Strategies to improve the chemotypes in Ocimum species**

For many aromatic crops, genetic enhancement using various approaches aims to improve chemical composition, essential oil and herb yield. The classical breeding methods, along with biotechnological interventions, can facilitate such improvement in the yield and quality of essential oil in important and popular Ocimum species. This includes various approaches like metabolic engineering, transgenic, and in vitro culture techniques (Fig. 7), as further described.

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**Fig. 4** Species-wise predominant occurrence of specialized metabolites of monoterpenoid, sesquiterpenoid and phenylpropanoid classes in the essential oil from different Ocimum species. More number of predominant metabolites is listed from the most studied Ocimum species with the higher number of chemotypes, whereas less number of principal metabolites is listed from the least studied Ocimum species.
Breeding methods and polyploidy induction approach

Over the years, varieties of several Ocimum species with high essential oil yield and desired metabolite profiles have been developed through traditional breeding methods. For instance, high essential oil yielding, eugenol-rich O. tenuiflorum (CIM Ayu), methyl chavicol and linalool-rich O. basilicum (CIM Saumya), and O. basilicum with a flavor similar to Piper betle due to the presence of chavibetol have been developed (Lal et al. 2018). Also, morphologically similar but chemically distinct breeding lines of O. basilicum have been established, with eugenol (line SW) and methyl chavicol (line EMX-1), as the only phenylpropanoid components in their essential oil (Gang et al. 2001). Similarly, O. basilicum lines distinguished with camphor, methyl chavicol, and eugenol chemotypes have been developed with high essential oil content and herbage yield (Gupta 1994). The allelic basis behind the inheritance of such specialized metabolites has been discussed in a few studies (Dudai and Belanger 2016; Gupta 1994). Additionally, some breeding studies have focused on the development of O. basilicum varieties with improved agronomic traits, such as cold tolerance (Ribeiro and Simon 2007; Römer 2010) and disease resistance against basil Fusarium wilt (caused by...
Fusarium oxysporum) (Dudai et al. 2002) and downy mildew (Peronospora species) (Römer 2010). For ornamental purposes, O. basilicum lines with compact inflorescence have been generated (Dudai et al. 2002; Morales and Simon 1996). Interspecific hybridization can be employed to generate stable new varieties within the three to four generations (Dudai and Belanger 2016).

Earlier studies have indicated the effect of ploidy levels on essential oil production such that polyploid plants have significantly higher essential oil accumulation than diploid ones (Dhawan and Lavania 1996; Lavania 2005). The polyploidy induction approach by treating the seeds or other propagating material with colchicine has been used for decades in crop improvement programs. Omidbaigi et al. (2010) induced tetraploidy in O. basilicum by colchicine treatment to seeds and apical meristem of seedlings. This resulted in a 69% increment in the essential oil content in the tetraploid plant compared to the diploid. Additionally, polyploidy was generated in an interspecific hybrid between eugenol-rich O. gratissimum and thymol-rich O. viride that could produce eugenol (50–55%) and thymol (7–10%). Further, their selfing and selection led to the development of two lines, one with 80–85% eugenol, while another with 82–85% thymol chemotypes (Khosla et al. 1990). Thus, interspecific hybridization and polyploidy generation offer additional ways for the targeted breeding to modulate chemotypes and essential oil yield.

Metabolic engineering through transgenic approaches

Metabolic engineering requires an in-depth understanding of the specialized biosynthetic pathways. To improve the yield and composition of some of the most valuable terpenoids and phenylpropanoids in the essential oil of specific Ocimum species, pathway engineering can be explored as a targeted approach.
(Fig. 8). The identification and functional characterization of genes entailed in the biosynthesis of specialized metabolites as chemotypes is crucial to manipulate any steps in their biosynthetic pathways through metabolic pathway engineering. Recently, key genes involved in the specialized metabolite biosynthesis have been characterized from different Ocimum species. These include 4-COUMARATE-CoA LIGASE (Ok4CL7 and Ok4CL15) (Lavhale et al. 2021) of the phenylpropanoid pathway, 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE (OkHMGR) (Bansal et al. 2018) of the MVA pathway, and β-CARYOPHYLLENE SYNTHASE (OkBCS), a sesquiterpene synthase (Jayaramaiah et al. 2016) characterized from O. kilimandscharicum. Also, several genes from O. basilicum have been characterized, such as PHENYLALANINE AMMONIA-LYASE (ObPAL) (Khakdan et al. 2018) of the phenylpropanoid pathway, 4-HYDROXYPHENYL-PYRUVATE REDUCTASE (ObHPPR) and TYROSINE AMINOTRANSFERASE (ObTAT) involved in the rosmarinic acid biosynthesis (Li et al. 2019), OXIDOSQUALENE CYCLASES (OCS) and cytochrome P450s (CyP450s) in the ursolic acid and oleanolic acid biosynthesis (Ghosh 2018). Anand et al. (2016) have characterized EUGENOL SYNTHASE (EGS) involved in the phenylpropanoid biosynthesis from several Ocimum species. The metabolic engineering techniques can facilitate particular manipulation in metabolite flux to achieve higher levels of the desired metabolites (Dudareva et al. 2013; Lange and Ahlami 2013; Marchev et al. 2020). HMGR enzyme from the MVA pathway and 1-deoxy-d-xylulose-5-phosphate synthase (DXS) and 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR) enzymes from the MEP pathway determine the metabolite flux for isoprenoid biosynthesis (Rodríguez-Concepción 2006). In the study conducted by Xie et al. (2008), higher activity of enzymes from the MEP pathway has been correlated well with the high level of citral in O. basilicum (line SD). On the contrary, the high activity of PAL has been observed in O. basilicum (line EMX-1), which is rich in methyl chavicol. The transcriptomic, proteomic, and biochemical approaches have revealed the reduced carbon flux into the phenylpropanoid pathway resulting in the terpenoid-rich chemical profile of O. basilicum (line SD) (Xie et al. 2008). In a similar context, the higher level of terminal enzymes from the terpenoid biosynthetic pathways along with low levels of PAL could be attributed to the increased flux in the terpenoid biosynthesis (Iijima et al. 2004). Thus, directing carbon flux through the overexpression or silencing of critical enzymes from the entry, key intermediate or terminal points (Fig. 8) of either phenylpropanoid or terpenoid pathways will help to modify or improve the key chemotypes in Ocimum species.

Many transgenic plant species have been developed that produce increased levels of monoterprenoids through overexpression of TPSs using the constitutively expressing promoters in heterologous system (Aharoni et al. 2003, 2006). Recently, overexpression of HMGR from terpenoid-rich O. kilimandscharicum (OkHMGR) in different phenylpropanoid-rich Ocimum species (O. basilicum, O. gratissimum, and O. tenuiflorum) has led to terpenoid accumulation with increased essential oil content (Bansal et al. 2018). Further, many enzymes of the metabolic pathway occur as isoforms. As PAL isoforms are localized in different subcellular sites, such as microsomal and cytosolic, it results in the differential subcellular distribution of cinnamic acid and, in turn, can partition phenylpropanoid biosynthesis into different end-product-specific pathways, such as flavonoids, lignin, etc. (Achnine et al. 2004). Also, different 4CL isoforms can regulate the flux of various hydroxycinnamates into other branches of phenylpropanoid biosynthesis (flavonoids, anthocyanins, phenylpropanes, lignins, coumarins, etc.) and thus, makes it a promising target for metabolic engineering in Ocimum species (Lavhale et al. 2018). For instance, the silencing of a specific 4CL isoform (OS4CL) through RNAi in O. tenuiflorum has led to a reduction in the eugenol level without affecting lignin and sinapic acid contents (Rastogi et al. 2013). Recently, characterization of two 4CL isoforms (Ok4CL7 and Ok4CL15) from O. kilimandscharicum have revealed that Ok4CL7 utilizes p-coumaric acid, ferulic acid and caffeic acid. In contrast, Ok4CL15 uses p-coumaric acid, ferulic acid and sinapic acid as substrates, indicating their potential role in lignin and phenylpropanoid biosynthesis (Lavhale et al. 2021). Overall, such reports have demonstrated that the desired change in the chemotypic profile can be achieved by targeting the specific isoform of the enzyme.

Furthermore, the transcription factors (TFs) play a pivotal role in regulating a specialized metabolic
**Table 4** Reported toxic effects of representative specialized metabolites from *Ocimum* and crude extracts of two *Ocimum* species

| Compounda | Type of toxicity | LC$_{50}$/LD$_{50}$/IC$_{50}$ value$^b$ or dose (D) given | Organism/cell line used | References |
|-----------|------------------|--------------------------------------------------------|------------------------|------------|
| 1,8-Cineole | Cytotoxicity | IC$_{50} = 4.02$ mM | Balb/C 3T3-A31 fibroblast | Mendanha et al. (2013) |
|           | Hemolysis | IC$_{50} = 18.4$ mM | Human RBC suspension |           |
| D-Camphor | Convulsion, piloerection, decreased motility & weight gain | D = 1000 mg/kg body weight/day | Rats | Zuccarini (2009) |
|           | Decreased body weight and food consumption | D = 681 mg/kg body weight/day | Rabbits |           |
| Citral | Cytotoxicity in liver, cholestasis, toxic effect on glandular stomach | IC$_{50} = 0.008–0.014\%$ (w/v) at 4 h; 0.003–0.012\% (w/v) at 24 h | HepG2 human liver cells; F1-17 skin cells; human skin fibroblast | Zárybnický et al. (2018) |
| D-Limonene | Hepatocellular lesions with Kupffer cell hyperplasia, hydropic degeneration, microvesicular steatosis and necrosis, incipient fibrosis | D = 25–75 mg/kg body weight | Wistar rats | |
| (+)-Limonene | Cytotoxicity | IC$_{50} = 1.58$ mM | Balb/C 3T3-A31 fibroblast | Mendanha et al. (2013) |
|           | Hemolysis | IC$_{50} = 23.8$ mM | Human RBC suspension |           |
|           | Dermal erythema | D = 250–4000 mg/kg/day | Sprague–Dawley rats |           |
|           | Decreased food intake and body weight in male rats, increased liver weight, epithelial hyperplasia, erythema | D = 4000 mg/kg/day | Male and female Sprague–Dawley rats | Bickers et al. (2003) |
|           | Dermal irritation, lethargy, ataxia, piloerection, erythema, edema, epithelial hyperplasia | D = 125–4000 mg/kg/day | Wistar albino rats |           |
| x-Terpineol | Cytotoxicity | IC$_{50} = 0.13$ mM | Balb/C 3T3-A31 fibroblast | Mendanha et al. (2013) |
|           | Hemolysis | IC$_{50} = 6.1$ mM | Human RBC suspension |           |
| Eugenol and isoeugenol | Cytotoxicity in hepatocytes | LC$_{50} = 200–300$ $\mu$M | Male Fischer 344 rat and female B6C3F1 mice | Burkey et al. (2000) |
| Methyl cinnamate | Cytotoxicity | LC$_{50} = 12.07$ mM | RAW264.7 cells | Murakami et al. (2018) |
| Methyl chavicol | Hepatoma, hepatic angiosarcoma | D = 23,000–46,000 ppm | CD-1 female mice | Smith et al. (2002) |
|           | Genotoxicity (Unscheduled DNA synthesis) | D = 500–2000 mg/kg body weight; D = 10$^{-6}$–10$^{-2}$ M | Hepatocytes isolated from rats; Hepatocytes from F344 male rats |           |
|           | Increased platelet count, bile duct and oval cell hyperplasia, chronic perportal inflammation, hepatocellular hypertrophy and degeneration, cholangiofibrosis | D = 37.5–600 mg/kg body weight | F344/N rats and B6C3F1 mice | Bristol (2011) |
| Compound          | Type of toxicity                                                                 | LC$_{50}$/LD$_{50}$/IC$_{50}$ value$^b$ or dose (D) given | Organism/cell line used                                      | References                        |
|-------------------|----------------------------------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------------------|-----------------------------------|
| Methyl eugenol    | Liver neoplasm, benign and malignant neuroendocrine tumor                        | D = 150–300 mg/kg body weight                            | F344/N rat and B6C3F1 mice                                   | Johnson et al. (2000)             |
|                   | Genotoxicity (Unscheduled DNA synthesis)                                         | D = 10–500 μM                                            | Male Fischer 344 rats and female B6C3F1 mice                 | Burkey et al. (2000)              |
|                   | Hepatocyte cytologic alterations with cytomegaly, Kupffer cell pigmentation, bile duct hyperplasia and atrophy, chronic inflammation in mucosa of the glandular stomach | D = 10–1000 mg/kg body weight                            | Male and female F344/N rats                                  | Abdo et al. (2001)                |
|                   | Cytologic alterations with cytomegaly, inflammation of liver and atrophy, necrosis, edema of the fundic region of the glandular stomach | D = 10–1000 mg/kg body weight                            | Male and female B6C3F1 mice                                  |                                   |
|                   | Chronic gastritis of the glandular stomach                                        | D = 300 mg/kg body weight                                | Male and female F344/N rats                                  | Smith et al. (2002)               |
|                   | Cytologic alterations with necrosis, bile duct hyperplasia, inflammation in the liver | D = 300–1000 mg/kg body weight                            | Male and female B6C3F1 mice                                  |                                   |
|                   | Genotoxicity (Unscheduled DNA synthesis)                                         | D = 10$^{-4}$–10$^{-2}$ M                               | Hepatocytes isolated from F344/N male rats                   |                                   |
|                   | Hepatocellular adenoma and carcinoma, hepatocholangioma, hepatocholangiocarcinoma, non-neoplastic lesion of the glandular stomach | D = 37–300 mg/kg body weight                            | Male and female F344/N rats                                  |                                   |
| O. gratissimum    | Increased aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) in kidney and serum, decreased total bilirubin and conjugated bilirubin | LD$_{50}$ = 4.24 μg/kg body weight; D = 100–400 mg/kg body weight | Albino rats                                                   | Ojo et al. (2013)                 |
| aqueous leaf extract |                                                                                |                                                          |                                                              |                                   |
| O. tenuiflorum    | No toxic effect reported                                                         | D = 5 g/kg                                               | Wistar rats                                                   | Chandrasekaran et al. (2013)      |
| aqueous crude extract |                                                                              |                                                          |                                                              |                                   |

$^a$Table prepared with metabolites (also occurring in Ocimum species) using their purified, organic or crude extract form based on in vivo and/or in vitro toxicity studies

$^b$LC$_{50}$—Median lethal concentration; LD$_{50}$—Median lethal dose; IC$_{50}$—Median inhibitory concentration
content by activating or repressing a set of the gene(s) in the chosen pathway (Grotewold 2008; Iwase et al. 2009). Over 40 TF families such as MYB, WRKY, bZIP, bHLH, HB, NAC, etc., have been discovered through transcriptome sequencing from O. basilicum and O. tenuiforum that are known regulators of the specialized metabolism in plants (Rastogi et al. 2014). Recently, Rastogi et al. (2020) have studied the expression patterns of TFs from the specialized metabolism to understand the regulation of terpenoid and phenylpropanoid biosynthesis. The similarity observed among the expression patterns of bHLH1_25905, EREB, MADS box_50254, MYB3, MYB5, MYC, and TTG1 TFs; and PAL, 4CL, and trans-cinnamate-4-hydroxylase (C4H) enzymes of phenylpropanoid pathway from three Ocimum species (O. gratissimum, O. kilimandscharicum, and O. tenuiforum) have supported the hypothesis that regulation of gene(s) expression occurs through TFs binding to their respective promoters owing to broad structural and functional similarity (Rastogi et al. 2020). Moreover, O. basilicum plants ectopically expressing peltate glandular trichome-specific TFs MsYABBY5
and MsMYB from *M. spicata* resulted in the reduced production of specialized metabolites, indicating their role as repressors (Reddy et al. 2017; Wang et al. 2016). Thus, metabolite flux analysis and interventions for suppressing the expression of TFs, which negatively impact the pathway, could also enhance the yields.

The recently discovered and Nobel-winning RNA-guided genome editing technique, clustered regularly interspaced short palindromic repeats/CRISPR-associated 9 endonuclease (CRISPR/Cas9), is a potential tool for crop improvement owing to its high efficiency, simplicity, and specificity (Arora and Narula 2017). Metabolic engineering by targeting multiple genes can be achieved through the multiplex CRISPR/Cas9 system to turn plants into bio-factories for specialized metabolite biosynthesis (Bhambhani et al. 2021; Karkute et al. 2017). Several genes that encode for the enzymes involved in the biosynthesis of many specialized metabolites are present in a cluster on the chromosomes, and the CRISPR/Cas9 tool has proven as an efficient method for knock-in or knock-out of gene clusters (Bhambhani et al. 2021). Plants with polyploidy show multiple homologs of the gene of interest can be targeted through sgRNA-based CRISPR/Cas9-mediated genome editing (Wilson Fig. 8 Metabolic engineering approaches for manipulating the desired chemotype by either increasing or decreasing the levels of specific metabolites include overexpression or silencing of the enzyme(s) (represented by W, X, Y, and Z, which are involved in the biosynthesis of specialized metabolites represented by B, C, D and E) either at the entry point, important middle step(s), branch or terminal points in a specialized metabolic pathway. Metabolic engineering approaches for the overexpression of enzymes include target gene expression under constitutive promoter and CRISPRa for gene upregulation. In contrast, downregulation of enzymes in the pathway can be achieved through RNAi at the translational level, CRISPRi at the transcriptional level or CRISPR knock-out. Expression of TFs, which act as either positive or negative regulators of the pathway, can also be manipulated to achieve the desired level of chemotype in the chosen *Ocimum* species. CRISPRa: CRISPR activation, CRISPRi: CRISPR interference, dCas9: Nuclease-deactivated Cas9, gRNA: guide RNA, miRNA: micro RNA, RISC: RNA-induced silencing complex. This figure is created using BioRender.Com
et al. 2019). Thus, a similar approach can be used in the Ocimum species where polyploidy is reported for enhancing desired metabolites. The CRISPR/Cas9 has been applied recently in metabolic engineering to produce specific metabolites (Fig. 8) in medicinal plants; for example, knocking out 4′-O-METHYL-TRANSFERASE 2 (4′-OMT2) gene from the benzylisoquinoline alkaloid pathway has resulted in the reduced production of morphine, thebaine, etc. in Papaver somniferum (Upadhyay et al. 2015), which diverted geranylgeranyl pyrophosphate (GGPP) to taxol biosynthesis (Li et al. 2017). Recently, Navet and Tian (2020) have utilized CRISPR/Cas9 to generate downy mildew resistant lines in O. basilicum. The genome sequence of O. tenuiflorum (Upadhyay et al. 2015), O. gratissimum chloroplast genome sequence (Balaji et al. 2021), and different transcriptome sequences from O. basilicum, O. tenuiflorum (Rastogi et al. 2014), O. americanum (Zhan et al. 2016), O. gratissimum, and O. kilimandscharicum (Anand et al. 2019; Singh et al. 2020) are available. Consequently, such resource availability can boost the mass production of crucial chemotypes from the selected Ocimum species by targeting specific biosynthetic pathway genes through CRISPR/Cas9 technology or other genome editing approaches where sequence information is a prerequisite. Thus, CRISPR/Cas9 studies will have an enormous potential for chemotype improvement in these Ocimum species. Additionally, the integrative analysis using transcriptomic, proteomic, and metabonomic approaches will give a system-level framework for identifying crucial genes or pathways involved in the biosynthesis of specialized metabolites and their regulation, and subsequently, this may speed up the process of advancement in Ocimum species to improve the quality and yield of essential oil. Recently, the integration of transcriptomics with metabolomics has helped to discover the tissue-specific biosynthesis and compartmentalization of major metabolites, like camphor and eugenol in O. kilimandscharicum (Singh et al. 2020), which needs to be further explored.

The anatomical structures, where the essential oil (represents only the content of volatiles in the anatomical structures recovered by steam distillation) is biosynthesized and stored in Ocimum species, can be targeted to improve chemotype contents. The types of glandular trichome (peltate and capitate), their size and density can affect the net efficiency of essential oil accumulation (Maurya et al. 2019) as the level of secretion is relative to trichome size (Huchelmann et al. 2017) and density (Deschamps et al. 2006). The methyl chavicol accumulation pattern in O. basilicum leaf tissue correlated well with the peltate gland density and CVOMT expression in the peltate glands at different developmental stages (Deschamps et al. 2006). Recently, Maurya et al. (2019) have demonstrated that the essential oil content is dependent on the density and size of peltate and capitate glandular trichomes using microscopic analysis in different Ocimum species (O. basilicum, O. gratissimum, O. kilimandscharicum, and O. tenuiflorum). Subsequently, the high essential oil content in O. basilicum was associated with a larger size of peltate trichomes despite their low density compared to other Ocimum species (Maurya et al. 2019). Additionally, several TFs governing glandular trichome development have been identified (Huchelmann et al. 2017). For instance, Matías-Hernández et al. (2017) exhibited an increase in the trichome density along with increased artemisinin content when a MYB TF (AaMYB1) was overexpressed in Artemisia annua plant. Also, exogenous treatment of phytohormones (gibberellic acid and calliterpenone) in M. arvensis induced the formation of a greater number of trichomes with the increased diameter, which resulted in an increased essential oil accumulation with high menthol and menthone contents (Bose et al. 2013). Recently, transcriptomic analysis of O. basilicum and O. tenuiflorum was carried out to identify genes involved in the glandular trichome development concerning the essential oil biosynthesis. Most of the transcripts belonged to the TF families, such as bHLH, C2H2, R2R3MYB, and R3MYB, which regulate trichome development. Their higher expression in O. basilicum than O. tenuiflorum may be associated with the high essential oil content of O. basilicum (Chandra et al. 2020). Thus, all such reports reveal that higher accumulation of essential oil can be facilitated by the large size and high density of trichomes. Moreover, the development of the glandular trichome is driven by a TF interactome network, which can either act as an activator or inhibitor (Lange and Turner 2013). Consequently, the characterization of
such interactome to modulate anatomy and density of glandular trichomes in Ocimum species for the biosynthesis and storage of higher quantities of essential oil with important chemotypes would be a great biotechnological challenge in the future.

In vitro tissue-culture techniques for Ocimum species

Many Ocimum species have been successfully regenerated using in vitro propagation (Dode et al. 2003; Manan et al. 2016; Rady and Nazif 2005; Saha et al. 2010; Singh and Sehgal 1999). In addition to this, the use of elicitors in callus, cell, and organ cultures for the overproduction of the specialized metabolites is an effective strategy (Fig. 7) for chemotype improvement (Namdeo 2007). For example, the callus culture has been more influential in the production of betulinic acid than in vitro derived leaves from O. basilicum, O. kilimandscharicum, and O. tenuiflorum (Pandey et al. 2015). The light quality also has strongly influenced the phenylpropanoid biosynthesis (Nadeem et al. 2019; Nazir et al. 2020b), while exogenous melatonin is effective in phenolics production from the callus cultures of O. basilicum (Duran et al. 2019; Nazir et al. 2020a). Furthermore, differentiated plantlets or organ culture is beneficial for metabolite production with higher and stable essential oil yield (Karuppusamy 2009). Particularly, shoot culture has proven the best option for the higher accumulation of specialized metabolites than cultivated plants (Murthy et al. 2014). Methyl chavicol level was higher in the essential oil from the in vitro propagated O. basilicum than ex vitro and in vivo plants (Manan et al. 2016). Also, in vitro grown leaves and somatic embryos had higher quantities of eugenol than field-grown O. basilicum and O. tenuiflorum leaves (Bhuvaneshwari et al. 2016). However, cell culture can be superior for the production of metabolites with a higher yield by scaling up the cell culture (Nitzsche et al. 2004). Mathew and Sankar (2014) have reported higher total terpenoid content in cell culture in the presence of an elicitor than field-grown O. basilicum, O. gratissimum, and O. tenuiflorum plants. Similarly, leaf-derived suspension cultures accumulated 11-fold higher rosmarinic acid than callus cultures or leaves from the field-grown O. basilicum plants (Kintzios et al. 2003). With the treatment of elicitors and precursor feeder, the accumulation of total phenylpropanoids has been elevated in suspension cell cultures with correlated PAL expression in O. tenuiflorum (Vyas and Mukhopadhyay 2018). Likewise, recently, higher triterpenoids (such as betulinic acid, ursolic acid, oleanolic acid, and rosmarinic acid) production has been achieved in the O. basilicum suspension culture (Pandey et al. 2019). In O. basilicum, high levels of nepetoidins have accumulated in callus and suspension cultures (Berim and Gang 2020). Subsequently, 2.7-fold high linalool and a 50% rise in methyl chavicol have been observed with silver nitrate as an elicitor in cell suspension cultures from O. basilicum (Açıkgoz 2020).

The hairy roots induced by Agrobacterium rhizogenes mediated transformation are efficient for specialized metabolite production (Murthy et al. 2008). These are genetically stable and can grow in media devoid of growth regulators. They have a high growth rate and can produce particular metabolites from the plant’s aerial part (Srivastava and Srivastava 2007). For instance, the enhanced levels of ursolic acid and eugenol in hairy root cultures of O. tenuiflorum have well corresponded with concentrations and duration of exposure of elicitors and the age of the cultures (Sharan et al. 2019). Biswas (2020) has shown the enhanced rosmarinic acid content using methyl jasmonate as an elicitor in the non-transformed O. basilicum root culture. Further, under both light and dark conditions, rosmarinic acid accumulation is higher in hairy root cultures from the green basil cultivar of O. basilicum than those of the purple basil cultivar (Kwon et al. 2021). Previously, elite hairy root lines have been developed with significantly higher rosmarinic acid levels than non-transformed roots of O. basilicum (Srivastava et al. 2016). In addition to this, somatic hybridization is used to produce hybrids from related species or distant genera (Grosser et al. 2000). Somaclonal variations can be helpful to enhance the essential oil profile of Ocimum species. These variations, if genetically stable for many generations, can be incorporated through plant breeding techniques (Krishna et al. 2016). Biotransformation is another approach that can be used to accumulate metabolites of particular stereospecificity and regioselectivity, utilizing cell or organ culture (Giri et al. 2001).
Conclusion and future perspectives

Several chemotypes from different Ocimum species have been reported with a multitude of medicinal, culinary, and industrial applications. The approaches of classical breeding, interspecific hybridization, and tissue culture have been fruitful in increasing the total essential oil content as well as developing specific chemotypes in Ocimum species till now. Still, globally there is a high demand for naturally occurring specialized metabolites. Although several of such commercially important metabolites can be chemically produced, synthetic products are often left with racemic mixtures, while the natural compounds are free of such manufacturing defects and leftovers. It is important to understand adverse effects (if any) of these metabolites to fine tune the concentrations in the final products or define dose. Hence, to mitigate such market needs, the recent biotechnological interventions and synthetic biology tools have an outstanding potential for the chemotypic improvement of Ocimum species for their economic expansion. An enhanced chemotypic profile in Ocimum species could also improve other traits, such as tolerance to abiotic stresses, disease resistance to phytopathogens, pest control, an allelopathic effect for weed control and phytoremediation potential. The current genome editing tools will also help us to understand the biosynthetic pathways of specialized metabolites and provide an ideal option to improve essential oil yield and quality. However, the lack of whole genomic and transcriptomic sequences from important Ocimum species will be a challenge to exploit hidden chemopotentail and chemotype advancements using genome editing tools. Further, identification and characterization of the TF networks regulating specialized biosynthetic pathways and correlating them with the metabolome will be necessary for effective TF manipulation in chemotype improvement. Nevertheless, comprehensive metabolomic profiling of various organs and organelles will bring more exciting information on fine-tuning of biosynthetic pathways for important specialized metabolites. Thus, extensive research aimed at the functional analysis of genes involved in the biosynthesis, regulation, and transport of specialized metabolites would be indispensable to enhance the market value of several Ocimum species and their chemotypes.

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