

Review

Recent Advances in Flower Color and Fragrance of *Osmanthus fragrans*

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Abstract: *Osmanthus fragrans* is an evergreen shrub or tree of the Oleaceae family with a long history of cultivation in Asian countries and is one of the ten traditionally famous flowers in China, with important cultural and economic value. The unique floral color and fragrance of *O. fragrans* are formed by a variety of endogenous metabolites that distinguish it from other flowers and exhibit extraordinary ornamental value. However, many studies on the flower color and fragrance of this plant have been mainly based on bioactive extracts and physiological characteristics, leading to a notable lack of molecular machinery and systematic research. In this review, recent advances in bioactive ingredients associated with the underlying regulatory mechanisms, as well as the prospect for industrial utilization, are comprehensively presented and critically evaluated. In particular, the isolated components and essential genes required for flower color and fragrance are also well summarized, which will provide a scientific basis for molecular breeding for ornamental applications and facilitate the discovery of novel natural products for the future industrial development of *O. fragrans*. In prospect, we plan to use genetic research and high-throughput omics to analyze the genes related to the flower color and fragrance of *O. fragrans*, and at the same time, we will hybridize and breed excellent *O. fragrans* varieties that are resistant to low temperature.

Keywords: *Osmanthus fragrans*; flower color; floral fragrance; gene regulation; valorization



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1. Introduction

1.1. *Osmanthus fragrans* Flower Cultivar Diversity

Osmanthus fragrans Lour. is a famous tree species with a long cultivation history, as well as flowers of high medicinal and edible value [1]. Bibliometric analysis of research on *O. fragrans* flower color and fragrance based on the Web of Science Core Collection database reveals that the number of *O. fragrans* studies has increased in the last decade. In Figure 1, years and keywords are juxtaposed in a heatmap, where flower color and fragrance are the main research highlights. Figure 1 is helpful for understanding the hot trends in *O. fragrans* research, updating research techniques, and accelerating research on the flower color and fragrance of *O. fragrans*. According to the “Flora of China”, *O. fragrans* is native to southwest China, and its distribution is concentrated in the vast central and subtropical areas north of the South Ridge to the south of the Qinling Mountains [2]. In other parts of the world, *O. fragrans* was first introduced from Guangzhou to England, and was planted at Ningjia Kew in England in 2007, followed by other European countries one after another; finally,

some countries along the Mediterranean were successful in introducing it, and grow it in open fields [3]. After long-term breeding and hard cultivation by many gardeners, the *O. fragrans* flower has formed a rich variety of cultivars. Generally, it can be divided into four major cultivar groups, namely, the Albus group, Asiaticus group, Luteus group, and Aurantiacus group. *O. fragrans* plants are often low and bushy shrub-like; their growing environment is warm and humid, with high temperatures and sunshine. Shorter sunlight periods and lower humidity are conducive to the opening of *O. fragrans*. The four groups are divided by their flower color and flowering time (Figure 2). Except for the Asiaticus group, which can bloom throughout the year and appears light yellow, the flowering period among the other three groups is mainly in autumn, and the flower color becomes the main difference instead of the flowering time. The Albus group flowers are a light silver white color. The Luteus group flowers are golden yellow [4]. The Aurantiacus group flowers are darker in color, an orange-red. In recent years, new colored groups have emerged with high ornamental value that are differentiated by leaf color phenotypes. The main technique for forming colored groups is cutting and grafting. Leaf color, leaf width, and leaf cross-sectional area are generally the main criteria for colored group classification. A new cultivar, ‘Qiannan Guifei’, changes leaf color from red to yellow-green and ultimately to green [5], enriching the landscape function of *O. fragrans*.

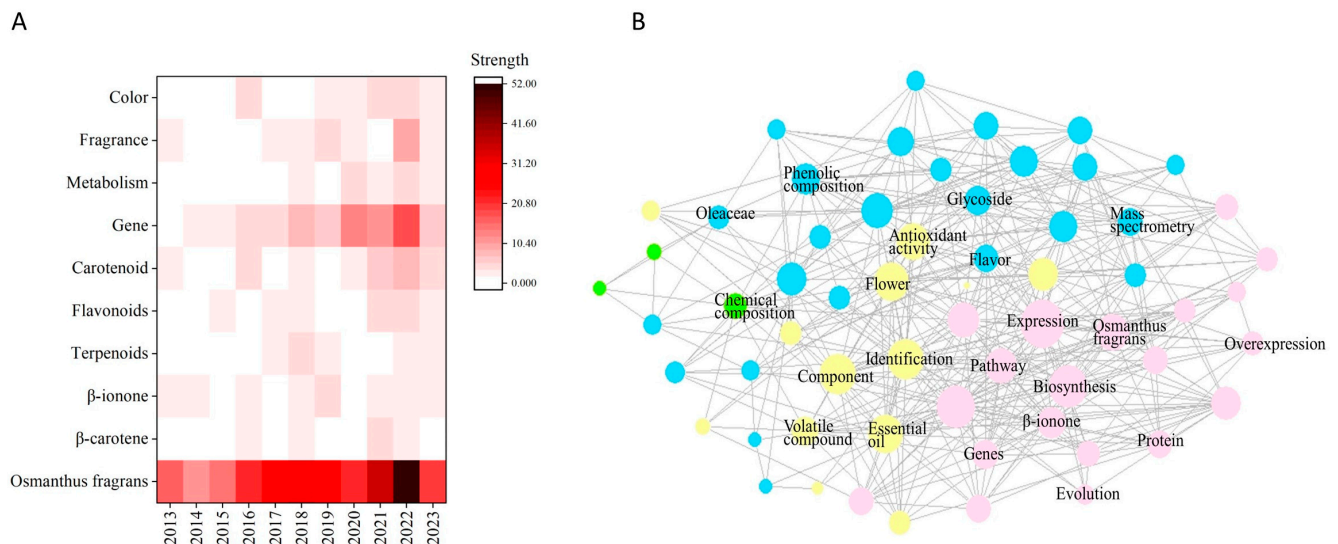


Figure 1. Bibliometric analysis of research on *O. fragrans* flower color and fragrance based on the Web of Science Core Collection database. (A) Heatmap of bibliometric analysis, the number of documents with “*Osmanthus fragrans*”, “ β -carotene”, “ β -ionone”, “Terpenoids”, “Flavonoids”, “Carotenoid”, “Gene”, “Metabolism”, “Fragrance”, and “Color” as the keywords in the Web of Science Core Collection database. (B) Network diagram for bibliometric analysis of *O. fragrans* research based on the Web of Science Core Collection database.



Figure 2. Picture of petals from four representative *O. fragrans*. Based on color and flowering time, *O. fragrans* is divided into four species groups, (A) the Albus group; (B) Asiaticus group; (C) Luteus group; (D) Aurantiacus group. Scale bar = 5 mm.

1.2. *Osmanthus* Resource Distribution

Osmanthus are mainly distributed in eastern and southeastern Asia, with a few species extending southeast to the islands of New Caledonia in Oceania, and subtypes distributed intermittently in tropical Asia, Oceania, and central South America. There are approximately 35 species of *Osmanthus*, two of which are hybrids (*O. × fortunei* and *O. × burkwoodii*), and 33 natural species. According to the geographical distribution characteristics, four main distribution areas are distinguished within their worldwide distribution in East Asia and Southeast North America [6] and the Asian distribution type is the main distribution center of *Osmanthus*. The total number of plants in this region is 28, accounting for 84.9% of the total plants.

China is the country with the most abundant *O. fragrans* resource in the world. The species of *O. fragrans* is native to the southwestern region of China, and a small amount is distributed in Southeast Asian countries such as Vietnam, Cambodia, and India. *O. fragrans* is rarely planted in the northern regions due to its cold intolerant growth characteristics, and the southern regions are not suitable for the vernalization of *O. fragrans* due to the high temperature in winter, where potted *O. fragrans* is often used. After continuous cultivation, breeding, climate change, and other factors, the area suitable for *O. fragrans* has begun to gradually shift to the southwest [7]. Subsequently, through continuous on-site inspections, researchers discovered wild *O. fragrans* varieties mainly in Zhejiang, Anhui, Jiangxi, Hunan, Guangxi, Fujian, and other places. The discovery of new wild varieties can expand the understanding of *O. fragrans* and has a positive effect on breeding new excellent cultivated varieties to expand the group of *O. fragrans* varieties for further functional genome research [8].

1.3. The Value and Utilization of *Osmanthus fragrans*

O. fragrans is one of the ten famous traditional Chinese flowers. The cultivation history in China began in the Han Dynasty and lasted for 2500 years. *O. fragrans* flowers often appear in Chinese traditional myths and ancient Chinese poems, deriving many images that are deeply rooted in the heart of people, such as “Wu Gang cuts Gui”, “Chan Gong Zhe Gui”, and other myths that have been circulating in the folks. The image of *O. fragrans* harbors many noble spirits in modern times, including friendship, honor, and auspiciousness [9,10]. *O. fragrans* has great economic value with wide applications. Ancient Chinese medicine uses the organs of *O. fragrans*, including roots, branches, flowers, and fruits, as a prescription to relieve dampness and cold [11]. Food made from *O. fragrans* flower is very popular due to its unique flavor, gradually forming a rich and diverse food industry in modern times. The various secondary metabolites contained in *O. fragrans* have great potential for the cosmetics industry, and the acetone extract of *O. fragrans* used as a raw material has antioxidant and antiseptic functions, which can postpone the aging of skin. *O. fragrans* can also be used in garden landscaping. Different varieties often have different floral characteristics, including beautiful shapes, sweet aromas and auspicious symbols [12].

2. *Osmanthus fragrans* Flower Color

The color of *O. fragrans* flowers is one of the most important ornamental features during the flowering period. The floral components contained in different varieties of *O. fragrans* are highly associated with their colors. The main pigment substances that form the flower color of *O. fragrans* are carotenoids and flavonoids, the orange flowers are synthesized mainly by carotenoids, while the yellow and white flowers are mainly caused by flavonoid compounds, and carotenoids have a greater impact on the formation and variation of the floral color [13,14]. The main biosynthetic pathways involved in *O. fragrans* color formation are phenylpropanoid biosynthesis, flavonoid biosynthesis, terpenoid backbone biosynthesis, carotenoid biosynthesis, astaxanthin biosynthesis, and zeatin biosynthesis (Figure 3), which are described in detail below. The classic bioactive

substance and the physicochemical effects related to *O. fragrans* flower color is shown in Table 1 [15,16].

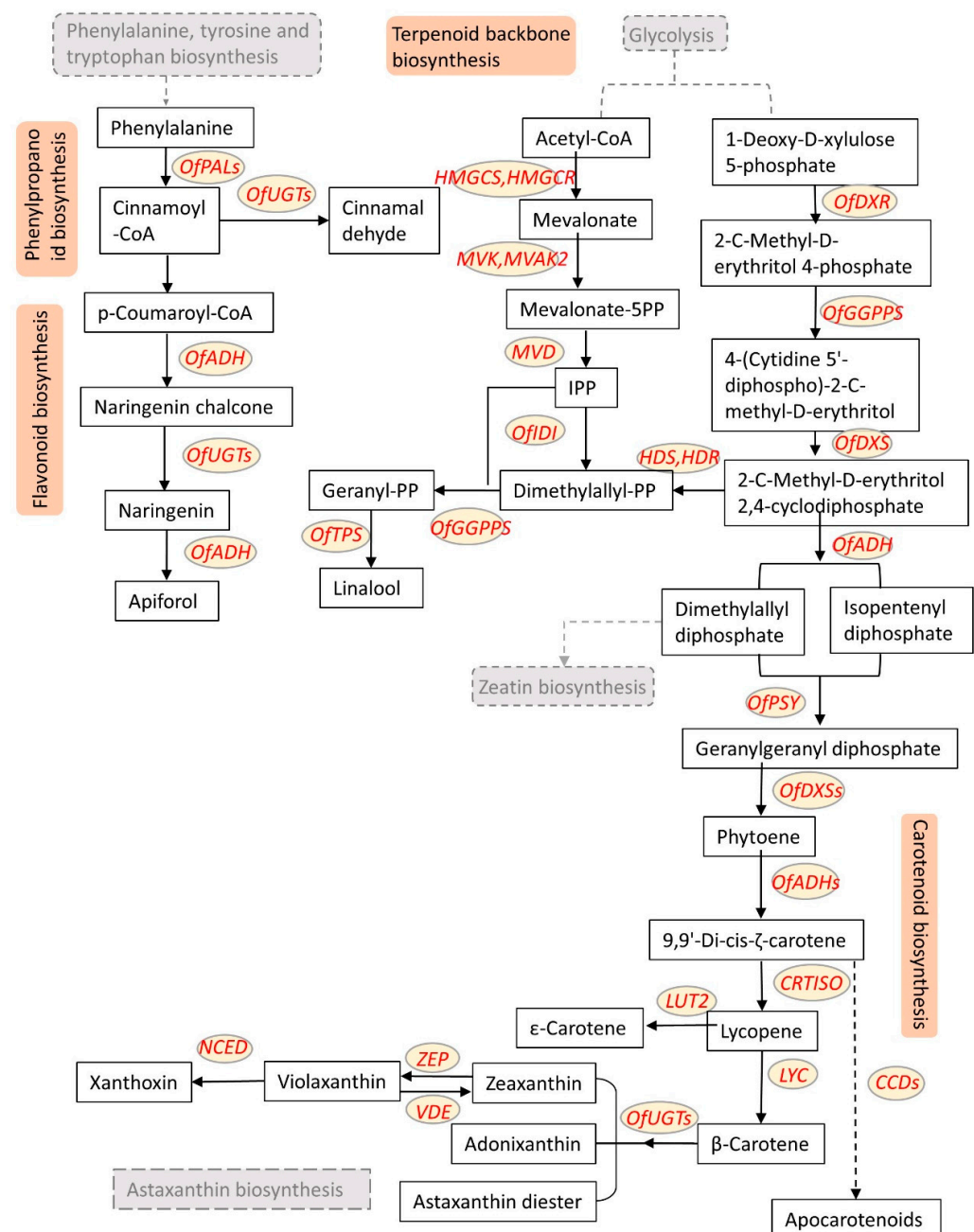


Figure 3. The basic pathway map for the regulation of *O. fragrans* flower color and floral fragrance. The main biosynthetic pathways involved are phenylpropanoid biosynthesis, flavonoid biosynthesis, terpenoid backbone biosynthesis, carotenoid biosynthesis, astaxanthin biosynthesis, and zeatin biosynthesis. The genes involved are *OfPSY* (Phytoene synthase), *OfZ-ISO* (ζ-carotene isomerase), *OfCRTISO* (Carotenoid isomerase), *OfPAL* (Phenylalanine ammonia-lyase), *OfCHI* (Chalcone isomerase), *OfTPS* (Terpene synthase), *OfUGTs* (UDP-glucosyl transferase), *OfADH* (Alcohol dehydrogenase), *OfMECS* (2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase), *OfHDR* (4-hydroxy-3-methylbut-2-enyl diphosphate reductase), *OfDXS* (1-Deoxy-D-xylulose-5-phosphate synthase), *OfGGPPS* (Geranylgeranyl pyrophosphate synthase), *OfNCED* (9-cis Epoxycarotenoid dioxygenases), *OfCCDs* (Carotenoid cleavage dioxygenase), etc.

Table 1. Bioactive substance and the physicochemical effects related to flower color and fragrance.

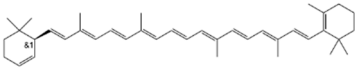
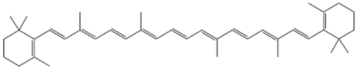
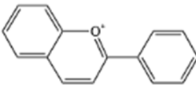
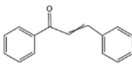
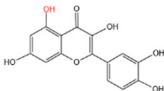
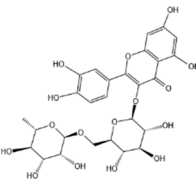
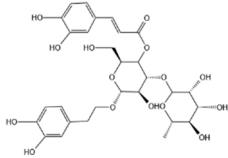
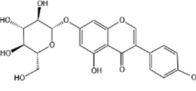
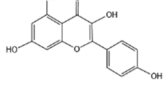
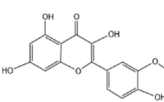
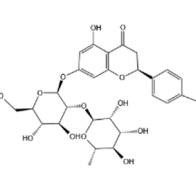
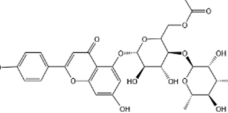
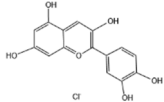
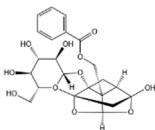
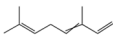
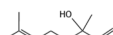
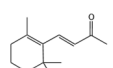
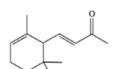
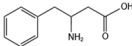
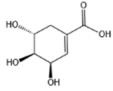
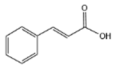
Substance	Structure	Function
α -carotene		Antioxidants destroy free radicals and prevent chronic diseases such as heart disease and cancer.
β -carotene		Reduces lipid peroxidation, scavenges free radicals, and prevents aging and disease in the body.
anthocyanins		Flavonoids, anti-staphylococcus aureus and hemolytic streptococcus, anti-aging and hypoxic, anti-adverse irritant, anti-fatigue, inhibition of blood sugar elevation.
chalcone		Flavonoids, one of the largest groups of bioactive natural products, anticancer, anti-inflammatory, antibacterial, antioxidant and anti-parasitic.
quercetin		Flavonoids, with a variety of biological activities, are antioxidants and play an important role in the clinical treatment of cancer and cardiovascular diseases.
rutin		Anti-inflammatory, maintains vascular resistance, promotes cell proliferation, prevents blood cell coagulation, diuretic, cough suppressant, hypolipidemic, hypotensive, protects ulcer surface.
verbascoside		Pharmacological effects include immune modulation, antioxidant, energy enhancement and anti-fatigue.
genistin		Improve insulin resistance, anti-diabetic effect, inhibit cancer cell activity, inhibit prostate cancer and antioxidant activity.
kaempferol		Anticancer, fertility inhibitor, anti-epileptic, anti-inflammatory, antioxidant, antispasmodic, anti-ulcer, choleric diuretic, cough suppressant.
isorhamnetin		Various biological effects include antitumor, anti-myocardial hypoxia and ischemia, relieving angina pectoris, anti-arrhythmia, treating coronary heart disease and hypertension, scavenging oxygen free radicals, lowering serum cholesterol, etc. It is widely used in clinical practice.
naringin		Naringin can significantly improve local microcirculation and nutrient supply, inhibit the proliferation and migration of melanocytes, and effectively fight against dark circles caused by hyperpigmentation.
camellianin A		Flavonoids, anticancer activity and anti-angiotensin-converting enzyme (ACE) activity.
cyanidin chloride		Strong antioxidant, anti-inflammatory, anti-allergic, antimicrobial, anticancer, vision protection and cardiovascular disease prevention.

Table 1. Cont.

Substance	Structure	Function
paeoniflorin		Hepatoprotective effect; immune modulating effect and anti-hyperglycemic effect, inhibits nerve cell damage and improves motor retardation, can be used to treat Alzheimer's disease, Parkinson's disease, epilepsy and other brain disorders.
ocimene		Ocimene is used in the production of spices and in daily chemical flavors.
linalool		Important spice, a raw material for the blending of various artificial essential oils, used in the formulation of cosmetics, soap, detergent, food and other flavors
β -ionone		Used in daily cosmetics, beauty products, food flavors, and in large quantities for the production of vitamin A, E and carotene
α -ionone		Permission to use edible spice temporarily by GB 2760-96, mainly used to formulate longan, raspberry, blackberry, cherry, citrus and other types of spices.
phenylalanine		Amino acid derivative, used as a spice for food.
shikimic acid		Inhibit platelet aggregation, inhibit arterial and venous thrombosis and cerebral thrombosis, have anti-inflammatory and analgesic effects, can also be used as antiviral and anticancer drug intermediates.
trans-cinnamic acid		With fragrance, sterilization, antiseptic, preservation, can be used in the pharmaceutical industry, beauty, pesticide industry, organic synthesis chemical.

2.1. Major Compounds of Coloration in *Osmanthus fragrans*

2.1.1. Carotenoids

Carotenoids are compounds that include carotenoids and lutein, which are the main color-presenting substances for yellow and orange types of *O. fragrans* [14,17]. Carotenoids have medicinal value in antioxidant, immunomodulatory, antiaging, and anticancer activities [18]. Carotenoids are also an important class of photosynthetic pigments that play an indispensable regulatory role in photosynthesis and protection of plants from bright light [19]. Carotenoids in *O. fragrans* are mainly synthesized in the plastids of cells, and the carotenoid content in plants can be regulated through the hydroxylase pathway, the dioxygenase cleavage pathway and the oxidase pathway, resulting in color changes in different plant tissues [20]. Currently, the carotenoid biosynthesis pathway (CBP) has been well characterized in plants. Carotenoid compositions in *O. fragrans* consist of α -carotene, β -carotene, α -cryptoxanthin, β -cryptoxanthin, lutein, and zeaxanthin, but carotenoid accumulation patterns during the flowering process are different. The petals of the yellow-white cultivars exhibited high contents of β -carotene, lutein and α -carotene, whereas the petals of the orange-red cultivars mainly contained β -carotene and α -carotene [14]. *O. fragrans* cultivars vary in pigment composition and concentrations due to differential expression regulation of the downstream genes involved in the carotenoid synthesis and degradation pathway [21]. Therefore, carotenoids play an indispensable role in floral color formation, and a detailed metabolic pathway can be found in Figure 3.

2.1.2. Flavonoids

Flavonoids are a major class of secondary metabolites in plants, which have the basic structure of phenylchromenes and are mainly found in the vesicles of plant cells and to a lesser extent in various organs of plants, such as roots, stems, leaves, and flowers [22]. Some studies have proven that flavonoids have important medicinal effects in inhibiting free radical oxidation reactions and preventing tumors and cardiovascular diseases [23]. Flavonoids are also the most important pigment group in flower color, and flavonoids

are involved in the widest range of colors [24]. All flower colors in the world, from pale yellow to blue-violet, are regulated by flavonoids, which are important pigment substances in the petals of *O. fragrans* flowers [25]. Among the flavonoids [26–28], there are mainly two categories of red and yellow compounds, among which red substances are mainly anthocyanins and other flavonoids basically belong to the yellow composition, such as dark yellow chalcone and aurora ketone, light yellow as well as nearly colorless flavonoids and flavonols. It has been shown that the content of flavonoids in *O. fragrans* varies with the flowering period [29], the total flavonoid content in unopened *O. fragrans* is significantly higher than that in opened *O. fragrans* and dried *O. fragrans*, and the amount of total flavonoids in dried *O. fragrans* decreases significantly, probably due to the decrease in the content of secondary metabolites produced in *O. fragrans* when it enters the aging stage. Six flavonoids and one phenylethanoid glycoside were isolated from the ethanol extract of *O. fragrans* flower residues, identified as quercetin (1), rutin (2), verbascoside (3), genistin (4), kaempferol (5), isorhamnetin (6) and naringin (7) [30]. Eleven flavonoids were identified or tentatively identified. ‘Xiaoye Sugui’, ‘Boye Jingui’, ‘Wuyi Dangu’, ‘Yingye Dangu’, ‘Danzhuang’, ‘Foding Zhu’, and ‘Tianxiang Taige’, which are enriched in rutin and total flavonoids, and ‘Sijigui’ contained the highest amounts of kaempferol glycosides and apigenin 7-O-glucoside and could be selected as potential pharmaceutical resources [13]. Liquid chromatography-mass spectrometry (LC-MS) was used to detect the main flavonoids contained in the floral extract of *O. fragrans* [31], including miconioside B, egonol gentiobioside, and camellianin A.

Anthocyanins are the main chromogenic substances among flavonoids [32], involving the red, blue and purple coloration of flowers. There are more than 100 species of anthocyanins known worldwide, and there are 6 species of anthocyanins commonly found in *O. fragrans* flowers, including geranophyllin, cornflowerin, delphiniumin, paeoniflorin, petuniain, and mallowin [33]. Among them, geranophyllin, cornflowerin, and delphinidin are the core pigments of anthocyanins, while paeoniflorin, petunidin, and mallowin are the three derived pigments. Among them, geranophyllin dominating the orange-red presentation.

2.2. Regulation of Genes Involved in *Osmanthus fragrans* Flower Color Formation

A number of genes associated with the formation of flower color in *O. fragrans* have been isolated and identified. For example, β -ring hydroxylase (HYB) and zeaxanthin epoxidase (ZEP) are more strongly expressed in ‘Jingui’ petals than in ‘Chenghong Dangu’ petals, the flower color of ‘Jingui’ is golden yellow and ‘Chenghong Dangu’ is orange red, resulting in greater β -carotene contents. ϵ -ring cyclase (LCYE) is most strongly expressed, leading to lutein accumulation. The overexpression of carotenoid cleavage dioxygenase especially *OfCCD1* and *OfCCD4* in ‘Jingui’ is responsible for the near nonexistence of α -carotene and β -carotene in petals. The coloration of *O. fragrans* is positively correlated with carotenoid content. It shows The 15-cis- ζ -carotene isomerase gene (15-cis- ζ -carotene isomerase, Z-ISO) *OfZ-ISO1* and *OfZ-ISO2* are involved in carotenoid accumulation, and both genes have important practical significance to fully reveal the mechanism of coloration in *O. fragrans* [34] (in Chinese with English abstract).

CCD includes four CCD subfamilies (*CCD1*, *CCD4*, *CCD7*, and *CCD8*) and five NCED subfamilies (*NCED2*, *NCED3*, *NCED5*, *NCED6*, *NCED9*) with a total of 9 members [35], especially *CCD1* and *CCD4*, are key genes catalyzing many carotenoids to produce β -violagenone, α -violagenone, and uncinolide [36], determining pigmentation and the formation of aroma substances in *O. fragrans*. *CCD1* is mainly an enzyme-activated protein that cleaves carotenoids to produce aroma substances [37]. *CCD4* is involved in both the degradation pathway of carotenoids and the synthesis pathway of aroma components, including saffronin and β -violagenone [38]. The expression of *CCD4* is strongly correlated with the difference between colored varieties [39]. The expression of *OfCCD4a* in *O. fragrans* is negatively correlated with carotenoid accumulation [40] (in Chinese with English abstract). The *ERF2* transcription factor positively regulates *CCD1* and *CCD4*, apparently

changing flower color to form fragrance [41]. The overexpression of *MYB43* promote the genes relating to the accumulation of β -carotenoids. The *OfWRKY3* is a positive regulator of the *OfCCD4* gene, and might partly account for the biosynthesis of beta-ionone in *O. fragrans*. the *OfMYB1* may acted as a transcriptional activator to regulate expression of *OfPAL* gene [42–44] (Figure 4).

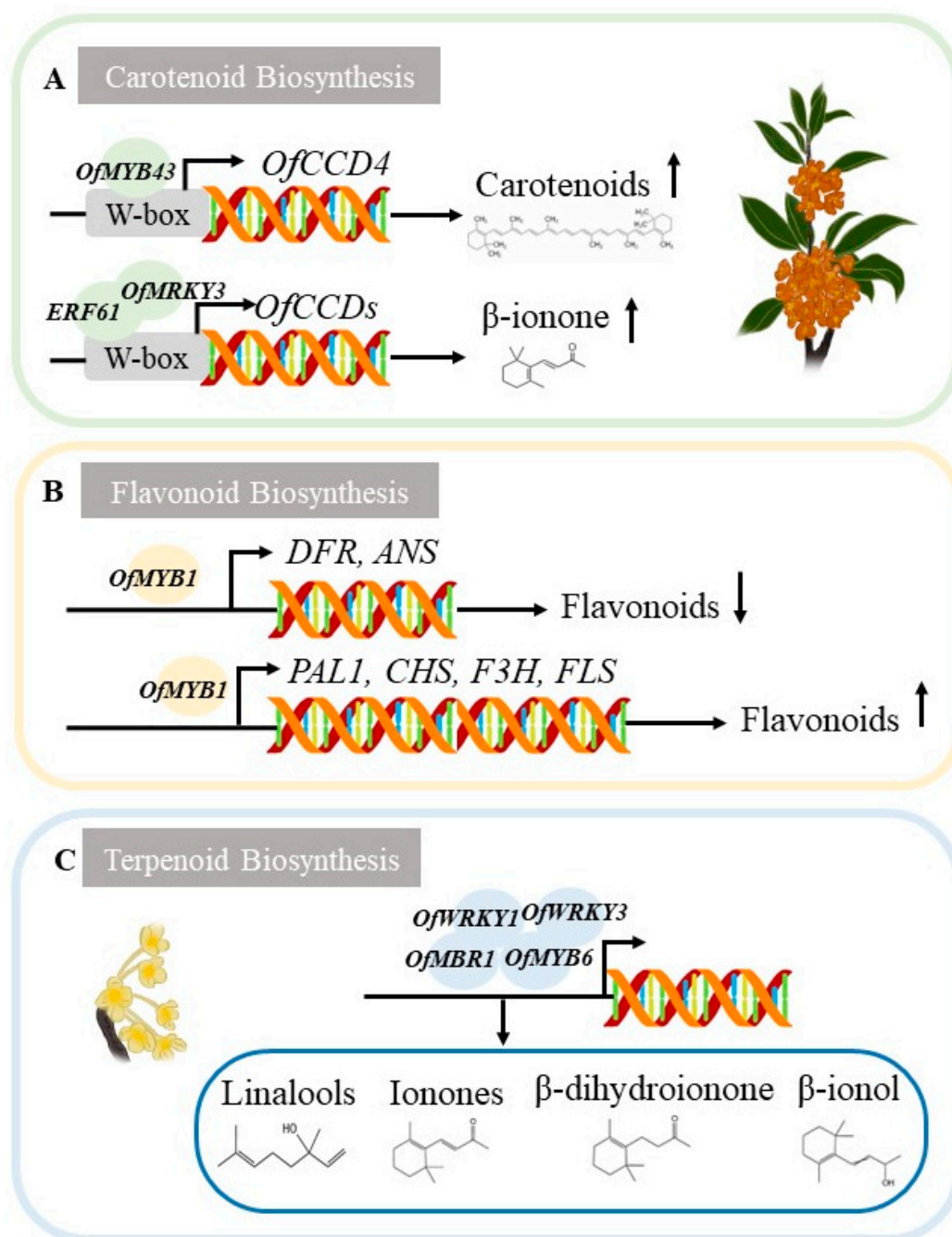


Figure 4. Examples of gene regulation essential for *O. fragrans* flower color and floral fragrance. (A) Regulation of transcription factor *OfMYB43* (MYB-related transcription factors), *OfERF61* (Ethylene response factor), *OfWRKY3* (WRKY transcription factors) associated with *OfCCDs* for carotenoid metabolism. (B) Regulation of transcription factor *OfMYB1* involved in flavonoid biosynthesis. (C) Regulation of transcription factors and their roles in terpenoid biosynthesis.

Genes related to anthocyanin synthesis mainly include *CHS*, *CHI*, *F3H*, *F3'H*, *F3'5'H*, *DFR*, *ANS*, and *UGT* [45–47]. The expression abundance of related genes directly affects anthocyanin accumulation in plants, further influencing the color phenotype [48]. Chalcone

isomerase (CHI) is the rate-limiting enzyme in anthocyanin biosynthesis. Taking ‘Orange-red Dangui’, ‘Jingui’, and ‘Early Yingui’ as samples to analyze the expression of *CHI* [49] (in Chinese with English abstracts), of which ‘Orange-red Dangui’ belongs to Aurantiacus group, ‘Jingui’ belongs to Luteus group, ‘Early Yingui’ belongs to Albus group. *CHI* expression positively correlates with the anthocyanin content among *O. fragrans* varieties and tissue specificity [49] (in Chinese with English abstracts), and the anthocyanin content aggravates flower color formation, causing the darkest color in ‘Orange-red Dangui’. The related genes involved in the flower coloration of *O. fragrans* are shown in Table 2.

Table 2. List of genes involved in the regulation of *O. fragrans* flower color and floral fragrance.

Genes	Description	Gene_ID	Homeosis Gene	Function	Refs
<i>PSY</i>	phytoene synthase	ofr.gene9262	AT5G17230	Color	[44]
<i>Z-ISO</i>	ζ-carotene isomerase	ofr.gene50192	AT1G10830	Color	[50]
<i>CRTISO</i>	carotenoid isomerase	ofr.gene51545	AT1G06820	Color	[50]
<i>PAL</i>	phenylalanine ammonia-lyase	ofr.gene722	AT3G10340	Color	[51]
<i>CHI</i>	chalcone isomerase	ofr.gene57827	AT3G55120	Color	[51]
<i>TPS</i>	Terpene Synthase	ofr.gene51840	AT4G20230	Fragrance	[52]
<i>CCDs</i>	carotenoid cleavage dioxygenase	ofr.gene58593	AT4G19170	Fragrance & color	[21,43,44,53–56]
<i>UGT</i>	UDP-glucosyl transferase	ofr.gene54750	AT2G36780	Fragrance	[57]
<i>ADH</i>	Alcohol dehydrogenase	ofr.gene58337	AT3G15880	Fragrance	[58]
<i>MECS</i>	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	ofr.gene9262	AT1G63970	Fragrance	[39]
<i>HDR</i>	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	ofr.gene5420	AT4G34350	Fragrance	[39]
<i>DXS</i>	1-Deoxy-D-xylulose-5- phosphate synthase	ofr.gene30635	AT4G15560	Fragrance	[59]
<i>GGPPS</i>	Geranylgeranyl pyrophosphate synthase	ofr.gene53350	AT4G36810	Fragrance	[44]
<i>NCED1</i>	9-Cis-epoxycarotenoid dioxygenases	ofr.gene29722	AT3G63520	Fragrance & color	[60]

3. *Osmanthus fragrans* Floral Fragrance

The fragrance of *O. fragrans* is popular among the public, giving it high ornamental and economic value. There is a difference between varieties because the aroma substances contained are different [61]. The main aroma substances in *O. fragrans* include ocimene, linalool and its oxides, and ionone compounds. According to the structure, the aroma components can be divided into alcohols, ketones, esters, and terpenes. The main substances include linalools, ionones, small amounts of alcohols, fatty acids, and esters [62]. The relative contents of β-ionone, α-ionone, and cis-linalool oxide accounted for 44.55%, 13.88%, and 3.96%, respectively [63] (in Chinese with English abstract). The classic bioactive substance and the physicochemical effects related to *O. fragrans* floral fragrance are shown in Table 1.

3.1. Fragrance Component Analysis

The main components of most *O. fragrans* flowers include linalool, ionone, a small amount of alcohols, fatty acids and esters and other compounds, resulting in *O. fragrans* having a unique floral fragrance compared with other flowers, and the difference in the floral fragrance between *O. fragrans* varieties is mainly attributed to a small amount of terpenoids and fatty acids.

3.1.1. Terpenoids

Terpenoids are important aroma active substances in *O. fragrans*. Its synthesis pathway consists of two metabolic pathways. One is the mevalonic acid pathway (MVA), which occurs in the cytoplasm, endoplasmic reticulum, and peroxidation. Among the targets, sesquiterpene compounds are mainly synthesized. The other is the methylerythritol phos-

phate pathway (MEP), which occurs in plastids and is mainly involved in the synthesis of monoterpenoids [64]. The MVA and MEP pathways in upstream section were separated. Recent studies have shown that isopentenyl pyrophosphate (IPP), the common precursor of terpenes, can be used as an energy source for monoterpenes or sesquiterpenes. Therefore, the MVA and MEP pathways can be crossed together [65].

3.1.2. Fatty Acid Compounds

The biosynthesis of fatty acid compounds is mainly synthesized through α -oxidation, β -oxidation, and lipoxygenase metabolism pathways [64]. Lipoxygenase (Lipoxygenase, LOX) can catalyze the production of fatty acid derivatives from phenolic glycerides in plants [66]. The fatty acid derivatives undergo α -oxidation and β -oxidation to further form alcohols, aldehydes, and ester compounds. Among them, α -ionone and β -trans-ionone are the main aroma active substances in *O. fragrans* [67].

3.2. Regulation of Genes Related to Fragrance Formation

Studies on the MEP pathway related to monoterpene biosynthesis have shown that most of the gene expression levels in the MEP pathway, such as the *MCT2* gene and *CMK2* gene, are consistent with the level of monoterpene release. The expression of the key gene *TPS* in the last step of monoterpene synthesis is significant. The expression level of the *TPS* gene in different flowering stages and different tissues is significantly different, in the two cultivars 'Gecheng' and 'Liuye' there were 33 volatile compounds at the beginning of flowering (S2), increasing from tight bud stage (S1) to full flowering stage (S3) and decreasing at late full flowering stage (S4) [52], and the expression of some *TPS*s also has circadian rhythm differences [68]. Studies have shown that *CCDs* are one of the most important floral regulation genes. The transcription level of *CCD4* is closely related to the accumulation of carotenoids. In *O. fragrans*, *CCD4* is the key gene that causes carotenoid degradation, which in turn produces β -ionone, which is the key substance of floral fragrance [55]. The synthesis of floral scents involves the participation of multiple transcription factors. Studies have shown that the overexpression of the *OfWRKY3* transcription factor promotes the expression of the *OfCCD4* gene [43]. The expression trend of *OfRAP2-12* is consistent with the release law of the floral substance ocimene and its derivatives [69] (in Chinese with English abstract). The *ERF2* transcription factor combines with the related elements of the *CCD1* and *CCD4* gene promoters to promote the expression of the *CCDs* [70] (in Chinese with English abstract). *OfMYB19* and *OfMYB20* have a positive effect on the accumulation of the aroma substance cis-linalool oxides, while *OfMYB51*, *OfMYB65*, and *OfMYB88* show a negative effect on one or more linalool oxides [71,72]. The related genes regulating the fragrance of *O. fragrans* are shown in Table 2. Several major pathways for fragrance component synthesis were summarized as shown in Figure 5.

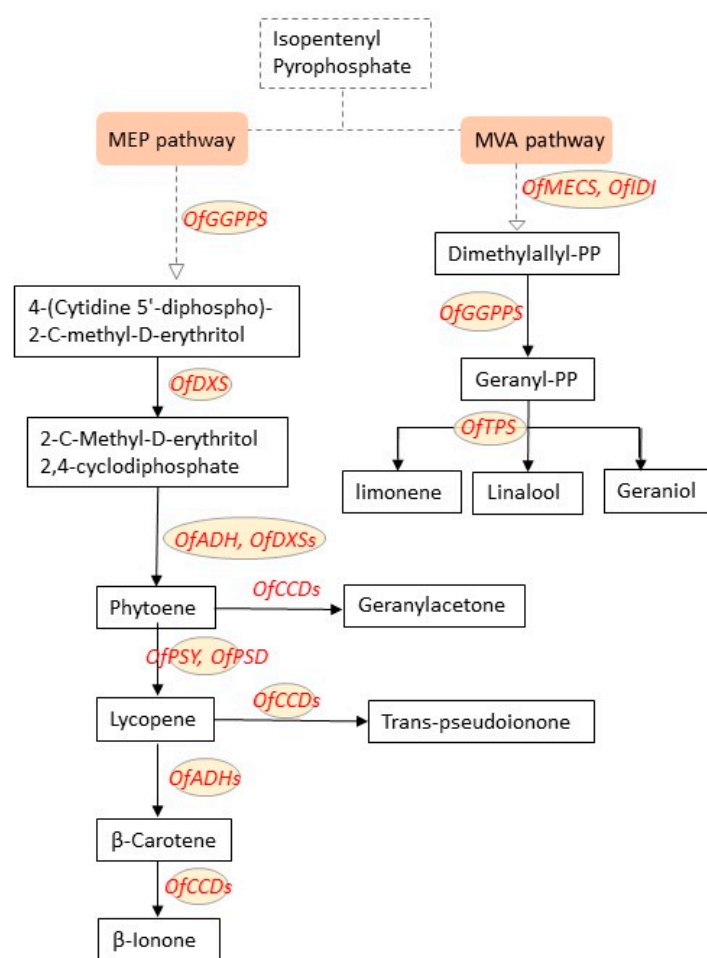


Figure 5. Two main metabolic pathways involved in the formation of floral fragrance, where the compounds produced are mainly classified as terpenoids. The genes involved in the pathways are *OfTPS*, *OfADH*, *OfMECS*, *OfDXS*, *OfGGPPS*, *OfCCDs*, *OfIDI* (Isopentenyl diphosphate isomerase), *OfPSY*, *OfPSD*, etc.

4. The Relationship between Color and Floral Fragrance

Flower color and floral fragrance are important phenotypic traits of *O. fragrans* essential for its ornamental quality and economic value. Current studies have shown that there is an emerging connection between the metabolic pathways that regulate flower color and floral fragrance. The first is that the synthesis of anthocyanin and the benzene ring type/phenylpropanoid compound share the same predecessor on the shikimic acid pathway [73]. The second is that the MEP biosynthesis pathway in plants can simultaneously produce carotenoids and volatile terpenoids. Among them, the carotenoid cleavage dioxygenase gene *CCDs* are crucial regulatory genes that play a fine-tuning role in mediating flower color and floral fragrance [39,55]. *CCD4* is a key enzyme in the degradation pathway of the petal pigment substance β-carotenoid, which catalyzes the formation of the floral substance β-ionone simultaneously. β-Ionone is the main aroma substance in *O. fragrans*, and the transcriptome sequencing and qRT-PCR results indicated that the high β-ionone content in the *O. fragrans* petals was accompanied by the upregulation of *OfCCD1* and *OfCCD4*. Transcription factor *ERF61* [39] can be combined with the *OfCCD4* gene promoter to regulate the expression of *OfCCD4* to coordinate the transformation from flower color to fragrance. The enzymatic activity assay confirmed that *OfCCD4* functioned in catalyzing β-carotenoid into β-ionone [56].

5. High Value-Added Products of *Osmanthus fragrans*

With the trend of consumption upgrades becoming increasingly obvious, the demand for high-end consumer goods has shown explosive growth. As an *O. fragrans* product industry, the traditional advantages include ecological, catering and sustainable tourism development, and new development spaces should be expanded, such as essential oils and health care products, taking advantage of their medicinal and edible value [74]. High value-added products of *O. fragrans* are derived from at least four directions, including seedlings, primary processed products, tourism, and high value-added processed products (Figure 6). In some applications in the seedling industry, gene editing has been used to advance or delay the flowering of *O. fragrans*, while at the same time giving different flower colors on a single plant during different growth periods. Osmanthus extract is a brownish-yellow paste-like spice with a strong aroma that is extracted with petroleum ether, becoming a raw material for making perfumes. After mass production, the domestic price of osmanthus extract is more than 10,000 RMB per kilogram, and the price abroad is 1600 dollars per kilogram. Using different preservation methods will affect the extraction rate and the ratio of aroma components of *O. fragrans* essential oil. Compared with the traditional single enzyme extraction of *O. fragrans* essential oil, compound enzyme extraction is better. It increases the content of the main aroma substances, such as β -ionone, linalool, β -ionol, β -dihydroionone, geraniol, γ -decalactone, nerol, and perillyl alcohol, while reducing the content of harmful substances as phthalates, thereby improving the quality of *O. fragrans* essential oil to provide a basis for improving the extraction process of *O. fragrans* essential oil [16,75,76]. *O. fragrans* essential oil is an important raw material for perfumes, and high-grade cosmetics still have many unexplored effects. Potential neuroprotective and antitumor effects of the essential oil were also found. essential oil, which would benefit the development and utilization of *O. fragrans* [77]. How to adapt to the current market environment and seize market opportunities is a strategic issue that must be of great importance to the development of *O. fragrans* essential oil production enterprises in the future [74].

Research has implied that flowers are important natural sources of bioactive components with higher antioxidant capacities for use in the food and pharmaceutical industries [78]. *O. fragrans*, as a food flavor additive, is used extensively in the food industry, frequently appearing on the market, which includes wine, pastry, and beverages, and is widely praised by the public because of its seasonal characteristics and special floral flavor. The flower is often used as a food ingredient to add some color and fragrance and has nutritional value to food because of the bioactive substance it contains [79]. *O. fragrans* tea looks similar to other teas, but the unique scent of *O. fragrans* distinguishes it. Pharmacological studies have demonstrated that *O. fragrans* has a wide range of biological activities, such as antioxidant, antitumor, anti-inflammatory, anti-hyperglycemic, anti-thrombotic, anti-melanogenesis, neuroprotective, and hepatoprotective activities [1]. The medicinal value promotes the development of high value-added health care medicines of *O. fragrans*, which have the therapeutic effects of relieving cough, phlegm, dispelling cold, and pain. As an ornamental garden plant, *O. fragrans* has great potential in gardening and viewing. The stems of *O. fragrans* seedlings are twisted and wound, growing into a peculiar craftsmanship tree, which is used to provide creativity for garden design. With a great abundance of flower resources and massive potential in the tourism industry, China has seen the rise of flower-themed tourism markets. References can be made to form *O. fragrans* theme tourism, for instance, the China International Jasmine Cultural Festival in Hengxian and the Dounan Flower Market in Kunming all achieve great success around the world, leading to a substantial improvement of the local socioeconomic benefits [80]. The *O. fragrans* cultural heritage is used to create a themed industrial chain famous for its ornamental, medicinal, and edible characteristics.

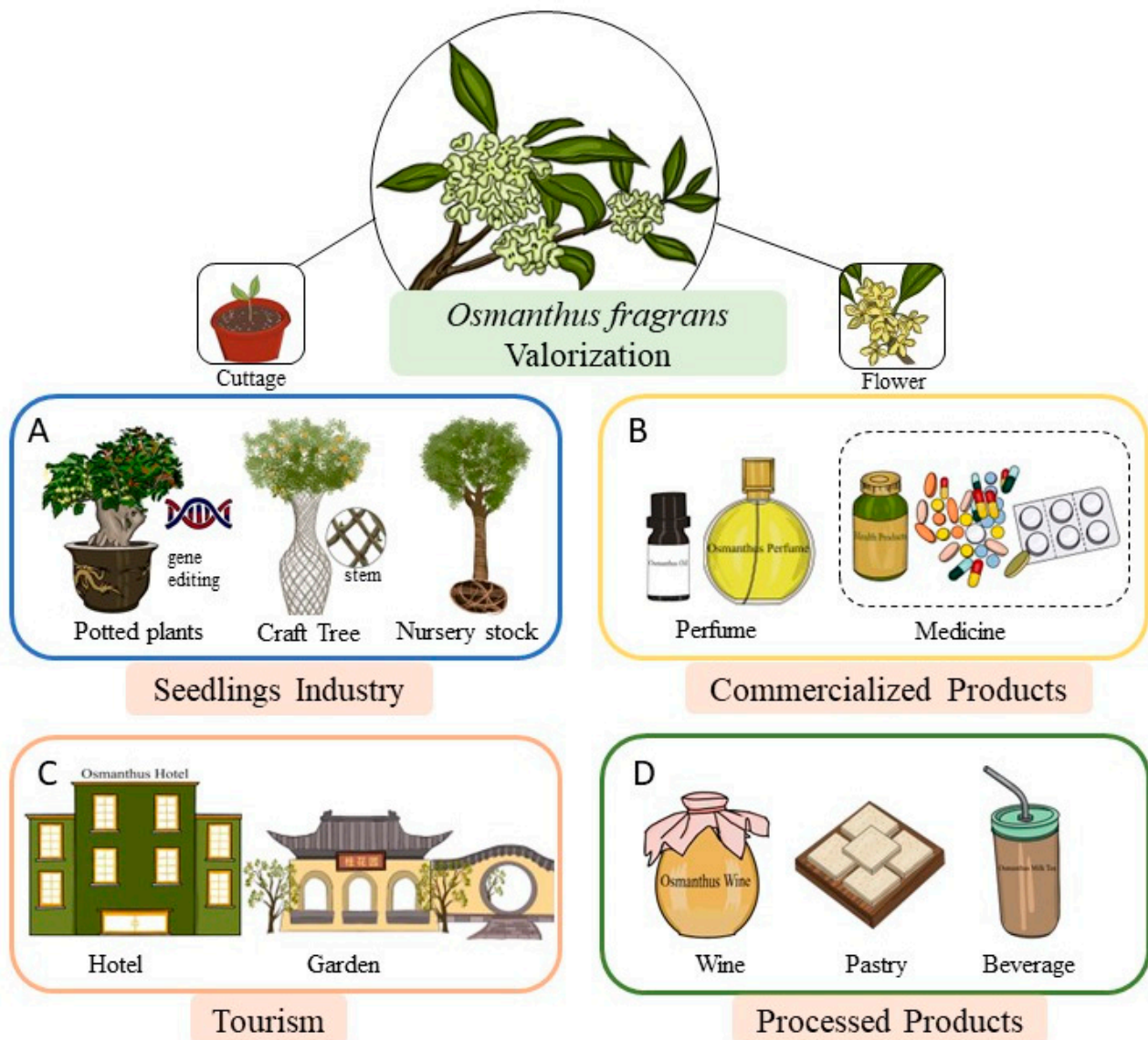


Figure 6. High value-added products of *O. fragrans*. Products are developed from four directions, including (A) osmanthus seedlings, (B) primary commercialized products, (C) osmanthus tourism, and (D) high value-added processed products.

6. Conclusions and Prospect

6.1. Conclusions

This article reviews *O. fragrans* flower color and floral fragrance from 3 aspects: material component analysis, gene regulation, and characteristic valorization. Flower color substances in *O. fragrans* are mainly flavonoids and carotenoids, and the genes that regulate color formation mainly include *CCDs*, *OfCRTISO*, *CHI*, etc. The aroma substances leading to the unique fragrance of *O. fragrans* are mainly terpenes, benzene ring/phenylpropanoids, and fatty acids, and the genes that regulate the formation of floral aromas mainly include *TPS*, *CCD*, *OfWRKY3*, *OfRAP2-12*, *ERF2*, etc. Some transcription factors regulate genes to affect flower color and fragrance. Members of essential bioactive substances in *O. fragrans* have good pharmacological activities potential for clinical use to human health. Therefore, exploring the molecular regulatory mechanism responsible for these valuable metabolites would facilitate the effectiveness utilization from this important edible and medicinal plants and help interested researchers discover food and medicinal natural products from *O. fragrans* for further industrial development.

6.2. Prospect

With the development of next-generation sequencing and nanopore sequencing, sequencing data are accumulated rapidly and more and more analytical techniques are used for data mining. The flower color and fragrance of *O. fragrans* can be mined and analyzed by sequence characterization and chain analysis using bioinformatics [81,82]. The integration of genomics, in combination with various histological tools such as transcriptomics, proteomics and metabolomics, may be the most powerful approach to reveal the molecular and biochemical basis for the biosynthesis of flower color and fragrance metabolites in *O. fragrans*. This line of research requires the identification of genes encoding enzymes involved in the synthesis of flower color and fragrance substances and the determination of how the expression of these genes is regulated, which may require the use of transgenic model plants [83]. Meanwhile, existing research shows that calli and others have carried out tissue culture of *O. fragrans* [84,85], but due to the low efficiency of callus tissue proliferation and the difficulty of differentiation of indefinite buds, the tissue culture and regeneration system of *O. fragrans* has not been successfully established [86]. The lack of complete sequencing of the *O. fragrans* genome may limit the cloning of *O. fragrans* genes and the development of gene transformation requires a stable *O. fragrans* tissue culture technology. Shen [87] used SWATH-MS to discover the post-transcriptional regulation of poplar response to lead. The use of genetic studies and high-throughput proteomics approaches to analyze the genes involved in flower color and fragrance in *O. fragrans* will further our understanding of the regulation of which in the plant. To date, the biosynthetic pathways associated with the flower color and fragrance of *O. fragrans* have been intensively analyzed. However, the extremely short flowering period and poor cold resistance of *O. fragrans* make it difficult to grow in areas north of the Yangtze River, which limits its ornamental and fragrant functions. It can be domesticated by planting some excellent *O. fragrans* species in the south and north area and selecting excellent low temperature tolerant species through hybridization to extend the flowering period to realize the southern species planted in the north.

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