

# Chapter 10

## Biosynthesis and Regulation of Flower Scent

B. Piechulla and U. Effmert

### 10.1 Introduction

Scent emission and perception allow inter- and intra-organismic communication over a long distance. The biologically active chemical compounds of such interactions are of small molecular weight (usually less than 300 Dalton), and have a high vapor pressure. The aliphatic and often lipophilic characters of the molecules support the emission from tissues. The volatiles can act through airflow in the atmosphere, as well as through diffusion in aqueous habitats. These properties allow living organisms to rely on these volatile molecules for communication. In seed plants, a complex strategy to ensure reproduction and preservation of species has evolved, which includes flower–animal interactions determined by defined floral traits like color, size, shape, texture, and volatile emission. Floral volatiles represent a crucial element of pollination syndromes, facilitating the attraction of specific pollinators over a wide distance. Floral volatiles underlie natural variations in the number and relative abundance between populations, within populations, within a plant, within a flower, and within different organs and tissues of the flower, which may reflect additional important functions, like defense against enemies and pathogens. An interesting phenomenon is based on mimicked odors to defend and attract plant-interacting organisms (pseudocopulation). In addition, many examples demonstrate that insects use floral scents to communicate with members of their community (e.g., beehives and ant colonies).

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B. Piechulla and U. Effmert  
University of Rostock, Institute of Biological Sciences, Albert-Einstein-Str. 3, 18059 Rostock,  
Germany  
e-mail: birgit.piechulla@uni-rostock.de

## 10.2 Functions of Floral Scents

### 10.2.1 *Floral Scents for Pollination*

Flowers can present animals/pollinators with a virtually unlimited range of species-specific odors. Some compounds are nearly ubiquitous, while others are found only in certain species. This universality, variation, and diversity is contrasted by species-specific and compound-specific flower–animal pollination systems (pollination syndromes). Depending on the plant, species-specific pollinators respond to floral odors. A multivariate analysis of various floral volatile traits was used to characterize distinct groups of pollination syndromes. The survey showed trends of chemical profiles of floral scents that can be attributed to particular animal groups visiting the flowers, but there are no clear-cut boundaries (Dobson 2006).

### 10.2.2 *Floral Scents with Diverse Functions*

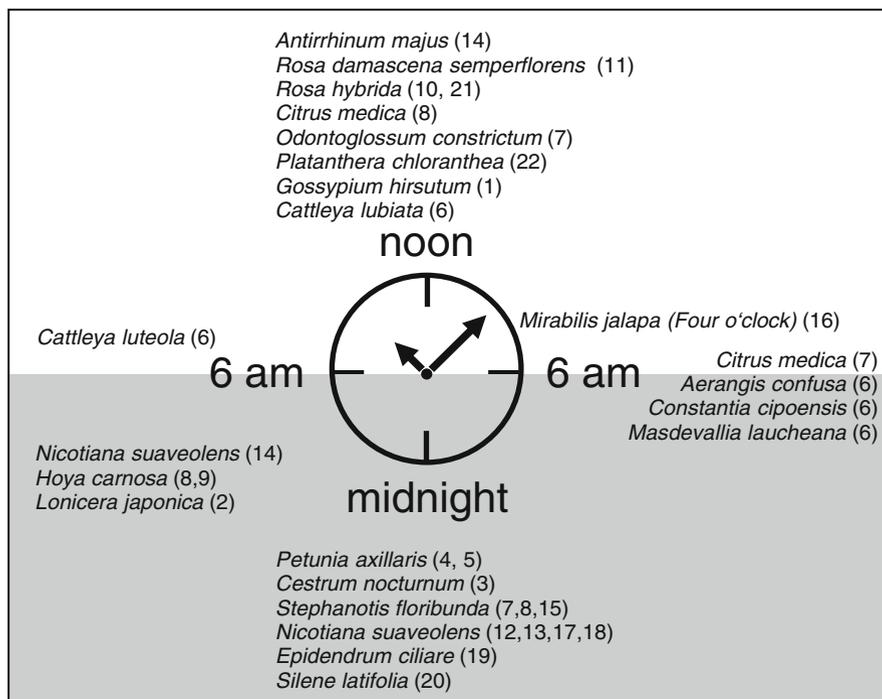
A large proportion of the volatiles within a scent mixture do not correlate with pollinator attraction. There are two conflicting evolutionary pressures facing the plant, namely, volatiles that are needed to advertise an attractive reward to pollinators and those to protect the flower from overexploitation by non-pollinating insects or destructive pollen-feeding animals, and visits by ovipositioning animals, pathogens, herbivores, and other enemies. Although not many studies have addressed the latter possibilities, it is conceivable that floral scent compounds are involved in defense reactions by functioning as, for example, insect repellents and/or antimicrobial compounds. Detailed analysis of volatiles and their spatial allocation in different organs and tissues of the flower is important to understand the complex species-specific host-seeking and host-avoidance strategies. *Mirabilis jalapa* emits dominantly trans- $\beta$ -ocimene, and in minor concentrations myrcene from the petaloid lobes for pollination, while the defense compound (*E*)- $\beta$ -farnesene is localized in the abaxial trichomes of the petals (Effmert et al. 2005a, 2006). Sesquiterpene lactones secreted by anther glands in *Helianthus maximiliani* and terpenoid aldehydes in *Gossypium hirsutum* act detrimentally to the larvae of flower-feeding insects (Dobson and Bergström 2000). In the sunflower moth, *Homoseosoma electellum*, pollen volatiles physiologically affect virgin females by triggering them to initiate calling behavior earlier, resulting in a higher rate of egg maturation. In addition, pollen odor contains a volatile oviposition stimulant that enhances the female's localization of newly opened sunflower heads. Some volatiles might also be involved in the initiation of calling behavior and oviposition by the European sunflower moth *Homoseosoma nebulellum*. Deterrent compounds of pollen odor may also influence pollen selection. Defensive chemicals, such as the lactone protoanemonin in *Ranunculus acris*, or 2-undecanone, 2-tridecanone and  $\alpha$ -methyl ketones in *Rosa rugosa*, are preferentially found in pollen odor (Bergström et al.

1995). Other defense compounds are sesquiterpene lactones, which possess activity against fungi and bacteria (Picman 1986). Compounds often have dual functions, and it is a matter of concentration or dose that initiates a biological reaction. For example, eugenol attracts a variety of insects to *R. acris*, but it also possesses antimicrobial activity.

### 10.3 Patterns of Floral Emission

Most flowers do not employ their entire surface, but use only certain flower parts, floral organs, or even confined areas of a floral organ with distinct morphological characteristics, for volatile production and emission (Bergstöm et al. 1995; Flamini et al. 2003; Dötterl and Jürgens 2005). Although petals often represent the main volatile source, sepals, stamens (anthers, pollen), or pistils (styli, stigmata) can also contribute to, or even dominate, the floral bouquet (Custódio et al. 2006; Effmert et al. 2006). The most apparent morphological feature of emitting tissues is a rugose epidermis often with cells exhibiting a conical or bullate appearance (Effmert et al. 2006; Bergougnoux et al. 2007). The most sophisticated emitting floral tissue is represented by osmophores. These glandular-like floral tissues have been found to be part of the perianth, bracts, appendices of peduncles, or anthers (Effmert et al. 2006). Floral trichomes can also emit volatiles, as shown for *Antirrhinum majus*. However, in many flowers, volatiles released from trichomes do not significantly add to the floral bouquet (Sexton et al. 2005; Effmert et al. 2006).

Besides these spatial differences, floral volatile emission follows temporal variations (Fig. 10.1). Although constant emitters like flowers of *Lathyrus odoratus* (Sexton et al. 2005), *Clarkia breweri* (Pichersky et al. 1994), or *Nicotiana otophora* (Loughrin et al. 1990) are well known, many flowering plants exhibit diurnal (Helsper et al. 1998; Kolosova et al. 2001; Hendel-Rahmanim et al. 2007), crepuscular (Effmert et al. 2005a; Kaiser 2006), or nocturnal (Matile and Altenburger 1988; Loughrin et al. 1990, 1991; Kolosova et al. 2001; Effmert et al. 2008) emission patterns that reflect the time of the plants' main pollinator activities (Levin et al. 2001; Theis and Raguso 2005). Approximately 8% of all flowering plants exhibit a scent emanation that reaches a maximum at night. Many plant species have adapted efficiently so as to be exclusively night-scented. Therefore, it is possible that plants that have been described as scentless may, in fact, be found to be scented at another time of the day. *Aerangis confusa* has been considered scentless during the day, but emits a typical 'white-floral' scent after sunset (Kaiser 2006). Other plant species, such as *Masdevallia laucheana* and *Constantia cipoensis*, have been shown to emanate fragrance only for 1 h during twilight, while *Cattleya luteola* is fragrant only between 4 and 6 a.m., which correlates with the short period of pollination (5.30 to 5.45 a.m.; Kaiser 2006). The precise timing of floral volatile emission is a special phenomenon often controlled by an endogenous clock, as demonstrated for *Cestrum nocturnum* (Overland 1960), *Stephanotis floribunda* (Altenburger and Matile 1990; Pott et al. 2002), *Hoya carnosa* (Altenburger



**Fig. 10.1** Scent clock: plants are arranged according to the time of major scent emission. 1 Loughrin et al. (1994), 2 Miyake et al. (1998), 3 Overland (1960)\*, 4 Hoballah et al. (2005), 5 Oyama-Okuba et al. (2005), 6 Kaiser (2006), 7 Altenburger and Matile (1990)\*, 8 Matile and Altenburger (1988), 9 Altenburger and Matile (1988)\*, 10 Helsper et al. (1998)\*, 11 Picone et al. (2004), 12 Loughrin et al. (1993), 13 Loughrin et al. (1991), 14 Dudareva et al. (2000), 15 Pott et al. (2002), 16 Effmert et al. (2005a), 17 Effmert et al. (2008), 18 Roeder et al. (2007)\*, 19 Kaiser (1993), 20 Jürgens et al. (2002), 21 Hendel-Rahmanim et al. (2007), 22 Nilsson (1978; \*, regulated by the circadian clock)

and Matile 1988), *Nicotiana sylvestris* and *N. suaveolens* (Loughrin et al. 1991), and *Rosa hybrida* (Helsper et al. 1998). This so-called circadian clock is characterized by (1) a recurring rhythm depending on an external signaling cycle (Zeitgeber) within 24 h, (2) a resynchronized rhythm if the Zeitgeber is shifted, (3) a persistent rhythm with a ‘free-running period’ of ca. 24 h (circadian) under constant conditions like continuous light, when the Zeitgeber is missing, and (4) a temperature compensation of the ‘free-running period’. Rhythmicity does not necessarily capture all components of floral mixtures to the same extent. While the majority of volatiles may conform to a nocturnal or diurnal rhythm, some volatiles keep an inverse pattern, or might even be emitted constantly (Loughrin et al. 1991; Nielsen et al. 1995). These temporal variations within a volatile mixture are sometimes linked to spatial variations (Dötterl and Jürgens 2005).

The onset of volatile emission usually corresponds with flower anthesis (Pichersky et al. 1994; Effmert et al. 2008). In flowers with several days of lifespan, the rhythm of volatile release recurs until the amount of volatiles is reduced during senescence, or until successful pollination. A distinct emission can already emerge in young flowers, as documented for *N. suaveolens* (Effmert et al. 2008), but it may also peak only in mature flowers as shown for *A. majus*, where the release of methyl benzoate, as well as terpenoids, reaches a maximum at day 5 to 7 after anthesis (flower lifespan ca. 12 days; Dudareva et al. 2003; Nagegowda et al. 2008). Not all components of a floral volatile mixture appear at the same time during floral development. In *A. majus* flowers, the myrcene and linalool emission is more persistent compared to the emission of methyl benzoate, (*E*)- $\beta$ -ocimene, or nerolidol (Dudareva et al. 2003; Nagegowda et al. 2008). Flowers of *N. alata* released considerable amounts of the sesquiterpene nerolidol at day 5 post-anthesis, while other major constituents like the monoterpenes  $\beta$ -linalool and 1,8-cineol were released at day 2 post-anthesis (Ganz and Piechulla, unpublished data).

Flowering plants are exposed to a constantly changing environment. Hence, abiotic and biotic factors affect the floral metabolism. Increasing temperatures resulted in significantly greater volatile emission (Hanstedt et al. 1994). Elevated temperatures enhanced terpenoid but not benzenoid emission, indicating that temperature has an impact on the biosynthetic pathway, and not only on volatile emanation (Nielsen et al. 1995). Under field conditions, this effect is often superimposed by elevated light intensities, which have a positive effect on volatile emission (Pecetti and Tava 2000). The length of the photoperiod has little influence, as shown for *Mahonia japonica*, where the emission of most floral volatiles remained unchanged (Picone et al. 2002). One of the most influential biotic factors governing floral volatile emission is pollination. In flowers of *Clarkia breweri*, *Cirsium arvense*, *Cirsium repandum*, and *Antirrhinum majus*, volatile emission declined rapidly shortly after pollination (Theis and Raguso 2005). In flowers of *Ophrys sphegodes*, a sexually deceptive orchid, the amount of volatiles decreased only slightly, but a significant increase in all-trans-farnesyl hexanoate has been detected after pollination by a solitary male bee (Schiestl and Ayasse 2001). Floral herbivory on immature *N. attenuata* flowers resulted in a significant decline of benzyl acetone emission, although this has been attributed to a significant reduction in the corolla mass (Euler and Baldwin 1996). However, leaf herbivory did not have a significant impact on floral scent emission in *N. suaveolens* (Effmert et al. 2008).

## 10.4 Biosynthetic Pathways and Key Enzymes

More than 2,000 volatile compounds have been known to be emitted from flowers of 991 plant species (compilation of compounds in ‘SCENTbase’ and ‘Super Scent’; Knudsen et al. 2006; Dunkel et al. 2009). Although the overall diversity of floral volatiles is greater than that detected in vegetative tissue, the biosynthetic pathways involved in both tissues are found to be terpenoid biosynthesis,

phenylpropanoid biosynthesis, and fatty acid ester synthesis. This suggests that derivatization and modification reactions are well established in plants.

### 10.4.1 Terpenoids

Terpenes are formed from C<sub>5</sub> building blocks. C<sub>5</sub> compound biosynthesis occurs in the cytosol via the acetate-mevalonate pathway, and in plastids from pyruvate via methyl erythritol phosphate (MEP). The C<sub>5</sub> compounds, isopentenyl pyrophosphate (IPP) and the isomer dimethyl allyl pyrophosphate (DMAP), are combined to geranylpyrophosphate (GPP) by a GPP synthase. Floral GPP synthases, which are short-chain prenyltransferases, have been isolated from *Antirrhinum majus* and *C. breweri*, and are heterodimeric enzymes (Tholl et al. 2004). Another group of floral enzymes, farnesyl pyrophosphate (FPP) synthases that add an additional C<sub>5</sub> unit to GPP, are relevant for volatile sesquiterpene synthesis.

The most common single compounds in floral scent are monoterpenes, such as limonene, (*E*)- $\beta$ -ocimene, myrcene, linalool, and  $\alpha$ - and  $\beta$ -pinene. GPP is the substrate for monoterpene synthases. The linalool synthase (LIS) was initially isolated from *C. breweri* flowers (Pichersky et al. 1995). This enzyme catalyzes the reaction from GPP to the acyclic monoterpene linalool, without major side products. Therefore, LIS has been considered as a monoproduct enzyme. A multi-product monoterpene synthase is the cineol synthase (CIN). CIN from *N. suaveolens* synthesizes cineol as a major product, together with seven cyclic and acyclic side products ( $\alpha$ - and  $\beta$ -pinene, sabinene, myrcene, (*E*)- $\beta$ -ocimene,  $\alpha$ -terpineole; Roeder et al. 2007). The development of multiproduct enzymes during evolution provides the advantage of simultaneous product synthesis and emission of several volatiles. Beside LIS and CIN, other floral monoterpene synthases have been isolated. These include ocimene, myrcene and nerolidol synthases (Dudareva et al. 2003; Nagegowda et al. 2008). The monoterpenes initially synthesized can be modified further (e.g., acetylation) to form other floral volatiles (Shalit 2003). Irregular terpenoids, such as ionones, are cleavage products of carotenoids.

### 10.4.2 Benzenoids and Phenylpropanoids

The synthesis of benzenoids and phenylpropanoids starts with the deamination of the amino acid phenylalanine. Benzenoids (C<sub>6</sub>–C<sub>1</sub>) are widespread in floral scents. Their synthesis requires the elimination of a C<sub>2</sub> unit. It is not clear as to whether this loss occurs from a phenylpropanoid precursor (C<sub>6</sub>–C<sub>3</sub>), or prior to phenylalanine in the shikimate pathway. The benzenoid and phenylpropanoid pathway is presently being elucidated, and a few genes/enzymes have been identified. These genes include BPBT (benzoyl-CoA:benzyl alcohol/2-phenylethanol benzoyltransferase), IGS (isoeugenol synthase), PAAS (phenylacetaldehyde synthase), BA2H (benzoic

acid 2-hydroxylase), BZL (benzoate:Co A ligase), C4H (cinnamic acid-4-hydroxylase), and SA GTase (UDP-glucose:salicylic acid glucosyltransferase; Boatright et al. 2004).

### 10.4.3 Aliphatic Compounds

This group of compounds (C1 to C25) includes the abundant C6 and C9 aldehydes and alcohols. They are synthesized predominantly from derivatives of fatty acids. Fatty acids are synthesized in plastids. The starting unit is acetyl-CoA, which is the acceptor of a C2 unit from malonyl-CoA. A multienzyme complex eliminates in two reduction reactions, and by elimination reactions, oxygen and double bonds from the new product molecule. Several rounds of C2 unit additions and reduction reactions result in the formation of middle- and long-chain fatty acids.

Many primary products can be modified to increase volatility, as well as to increase the number of diverse compounds with varied olfactory properties. Such modifications or derivatizations are catalyzed by specific enzymes or group of enzymes. In the past decade, an increasing number of floral scent-synthesizing enzymes or genes have been isolated. These include terpene synthases, carboxyl methyltransferases, acyltransferases, and acetyltransferases (Table 10.1).

Typical are methylation reactions. Methylation of hydroxyl groups and hydroxyl groups of carboxyl groups can be distinguished. Many plant compounds contain hydroxyl groups that can be methylated by O-methyltransferases (type I MTs) to reveal the methoxy groups. In general, O'MTs utilize S-adenosylmethionine (SAM) as methyl donor. Members of this MT family catalyze, for example, the formation of methyl eugenol in *C. breweri* flowers, or methyl orcinol in *Rosa chinensis* (Dudareva et al. 2004). The methylation of the hydroxyl group within the carboxyl group results in the formation of esters. Dominant scent compounds of this class are methyl benzoate and methyl salicylate. The enzymes that catalyze this reaction are type III methyltransferases (SABATH methyltransferases). Some accept solely benzoic acid (BAMT), or prefer salicylic acid (2-hydroxy benzoic acid) compared to benzoic acid (SAMTs and BSMTs; Effmert et al. 2005b). Interestingly, active pocket amino acids are highly conserved for the SAMT-type enzymes, while several mutations that result in amino acid changes in the substrate-binding site of the BSMT enzymes allow the binding and catalysis with a wider spectrum of benzoic acid derivatives.

Oxidation reactions result in the introduction of hydroxyl groups. The reactions are catalyzed by cytochrome P450 enzymes, and many of these enzymes have been characterized in plants. The skeletons of monoterpenes and sesquiterpenes are often modified by hydroxylation (e.g., menthol and carvone synthesis). However, a cyt P450 enzyme has not been isolated that catalyzes the derivatization of floral monoterpenes or sesquiterpenes. Similarly, such enzymes have not been reported that are involved in floral phenylpropanoid and fatty acid modifications.

Acylation reactions (including acetylation, butanoylation, and benzoyl acylation) are also common to make compounds more volatile. The basic reaction is the transfer of the acyl group from an acyl-CoA intermediate to the hydroxyl group of

**Table 10.1** Floral scent-synthesizing genes/enzymes

Enzyme	Plant species	References
Carboxyl methyltransferases (SAMT, BSMT, BMT)	<i>Clarkia breweri</i> , <i>Antirrhinum majus</i> , <i>Stephanotis floribunda</i> , <i>Petunia hybrida</i> , <i>Nicotiana suaveolens</i> , <i>Hoya carnosae</i> , <i>Arabidopsis thaliana</i> , <i>Arabidopsis lyrata</i>	Summarized in Effmert et al. (2005b)
Linalool synthase (LIS)	<i>C. breweri</i>	Pichersky et al. (1995)
Cineol synthase (CIN)	<i>N. suaveolens</i> <i>Citrus unshiu</i>	Roeder et al. (2007) Shimada et al. (2005)
Ocimene/myrcene synthase (OCS/MYR)	<i>C. unshiu</i> <i>A. majus</i>	Shimada et al. (2005) Dudareva et al. (2003)
Nerolidol synthase (NER)	<i>A. majus</i>	Nagegowda et al. (2008)
GPP synthase	<i>A. majus</i> , <i>C. breweri</i>	Tholl et al. (2004)
IPP isomerase	<i>A. thaliana</i>	Phillips et al. (2008)
Acetyl-CoA:benzylalcohol acetyltransferase (BEAT)	<i>C. breweri</i>	Dudareva et al. (1998)
Benzyl-CoA: benzylalcoholbenzoyl transferase (BEBT)	<i>C. breweri</i>	D'Auria et al. (2002)
Benzoyl-CoA:benzyl alcohol/2-phenylethanol benzoyltransferase (BPBT)	<i>P. hybrida</i>	Boatright et al. (2004)
Benzoate:CoA ligase (BZL)	<i>P. hybrida</i>	Boatright et al. (2004)
Cinnamic acid-4-hydroxylase (C4H)	<i>P. hybrida</i>	Boatright et al. (2004)
UDP-glucose:salicylic acid glucosyltransferase (SA Gtase)	<i>P. hybrida</i>	Boatright et al. (2004)
Isoeugenol synthase (IGS)	<i>P. hybrida</i>	Boatright et al. (2004)
Phenylacetaldehyde synthase (PAAS)	<i>P. hybrida</i>	Boatright et al. (2004)
Benzoic acid 2-hydroxylase (BA2H)	<i>P. hybrida</i>	Boatright et al. (2004)

an alcohol. A recently discovered plant enzyme family that catalyzes such reactions is BAHD acyltransferase. These BAHD enzymes have been isolated from *C. breweri*, and have been shown to produce benzyl acetate or benzyl benzoate, or acetylate citronellol and geraniol in *R. hybrida* (Dudareva et al. 1998; Shalit 2003).

## 10.5 Regulation of Floral Volatile Biosynthesis

### 10.5.1 Regulation at the Molecular Level

Floral volatile emission seems to rely on de novo-synthesized products, and the site of biosynthesis also represents the site of emission. In the past decade, evidence from several lines of study have revealed that volatile emission directly corresponded

with spatial, temporal, and developmental expression patterns of related floral genes. Furthermore, biosynthesis of floral volatiles was regulated at the transcriptional and/or post-translational level, as well as by the availability of the substrates. It has been reported that genes responsible for floral volatile synthesis are expressed exclusively in floral tissue, more specifically, in the emitting flower part (Wang et al. 1997; Pott et al. 2002, 2004). Cellular immunolocalization demonstrated that corresponding volatile-producing enzymes are detected mostly in epidermal cells, or are membrane-bound (Rohrbeck et al. 2006; Scalliet et al. 2006). At the subcellular level, GFP-fusion to two terpene synthases located the *AmNES/LIS-1* in the cytosol producing nerolidol, and the *AmNES/LIS-2* in plastids producing linalool (Nagegowda et al. 2008).

In nocturnally emitting flowers of *S. floribunda* and *N. suaveolens*, and in diurnally emitting flowers of *A. majus*, the circadian-controlled rhythm of the methyl benzoate release correlates with a circadian-controlled oscillation of the steady-state mRNA levels of the floral methyltransferases *SfSAMT*, *NsBSMT*, and *AmBAMT*, respectively (Kolossova et al. 2001; Pott et al. 2002; Effmert et al. 2005b). The relative transcript level of *NsBSMT* reached its maximum at the day of anthesis (Effmert et al. 2005b), whereas *AmBAMT* showed the highest transcript level at day 4 post-anthesis (Dudareva et al. 2000). Protein levels of these methyltransferases did not show similar pronounced daily oscillation (Effmert et al. 2008), but methylation activities in turn oscillated (Kolossova et al. 2001; Pott et al. 2004).

These results indicate the importance of post-translational modifications and/or the availability of substrates. The determination of substrate concentrations revealed that rhythms in enzyme activities depended on the substrate availability. For example, SAMT in *S. floribunda* flowers in planta methylates benzoic acid, although the in vitro catalytic efficiency for salicylic acid is much greater, because of the substrate salicylic acid that is by far underrepresented in the floral tissue (Pott et al. 2004; Effmert et al. 2005b). Similarly, the mRNA level of an alcohol acetyl transferase (*RhAAT*) expressed in petals of *R. hybrida* followed a diurnal rhythm, which appeared to be controlled by the circadian clock. However, as a result of substrate shortage, the emission of geranyl acetate, as well as germacrene D, ceased under continuous light, indicating that geraniol and germacrene D synthesis is regulated differentially (Hendel-Rahmanim et al. 2007). In contrast, 1,8-cineole synthase *NsCIN* isolated from *N. suaveolens* flowers displayed a pronounced oscillation at the transcript level, and this rhythmicity is controlled by the circadian clock (Roeder et al. 2007).

### 10.5.2 Mechanisms of Regulation

Little is known about the mechanisms of regulation and signaling at the molecular level of floral volatile synthesis. Microarray analysis allowed the identification of a cDNA encoding a floral transcription factor (*odol1*), which has been shown to be up-regulated in a fragrant cultivar of wild-type petunia compared to a non-fragrant cultivar (Verdonk et al. 2005). *Odol1* belongs to the R2R3-type of MYB transcription

factors, which are involved in anthocyanin and phenylpropanoid biosynthesis. Because of the amino acid variation in the R2R3 domain, *Odo1* clusters together with two MYBs of *Arabidopsis thaliana* and one MYB of *Pimpinella brachicarpa* in a new subgroup of MYB proteins. Verdonk et al. (2005) showed that *odo1* suppression modulated the expression of genes belonging to the shikimate pathway, but did not influence the expression of genes responsible for anthocyanin biosynthesis. *Odo1*-suppressed RNAi petunia lines showed partly a dramatic decrease in the emission of floral volatiles such as benzyl benzoate, benzyl acetate, vanillin, and isoeugenol, all of which originated from intermediates of the shikimate pathway, but the purple color of the flower tube remained unchanged. In contrast, Ben Zvi et al. (2008) showed a close link between the scent and anthocyanin biosynthesis. Constitutive overexpression of the anthocyanin pigment1 (*pap1*) MYB transcription factor resulted in an enhanced purple pigmentation in transgenic petunia flowers, and a dramatic increase in the production of nocturnally emitted volatiles. Additional supply of the shikimate pathway intermediate phenylalanine, which is crucial for benzenoid synthesis, abolished nocturnal rhythms of those volatiles in *pap1*-transgenic flowers. These results suggest that phenylalanine is the limiting factor for benzenoid production at daytime when phenylalanine concentrations are down-regulated (Ben Zvi et al. 2008). The constitutive overexpression of *pap1-myb* was superimposed on all subsequent regulatory components (e.g., *odo1*). A linkage between color and scent was also supported by Zuker et al. (2002), who demonstrated that suppression of a flavanone-3-hydrolase, a key enzyme in anthocyanin biosynthesis in *Dianthus caryophyllus*, resulted in a complete loss of petal color, and a marked increase in methyl benzoate emission.

Another factor involved in the regulation of scent emission is the phytohormone ethylene, an important regulator during plant tissue senescence and fruit ripening. Treatment of petunia flowers with exogenous ethylene reduced the emission of seven major volatiles, including methyl benzoate. It also caused a rapid decline in mRNA levels of *PhBSMT1* and *PhBSMT2* in different flower parts like stigmata and styles, ovaries, petal tubes, and petal limbs after 10 h of treatment (Underwood et al. 2005). The decline of mRNA levels in wild-type flowers in response to exogenous ethylene could also be observed for *PhCFAT*, an acyltransferase involved in the biosynthesis of the floral volatile isoeugenol (Dexter et al. 2007). Considering that pollination induces ethylene production in different flower parts starting with stamen and style tissue, followed by ovary and the corolla tissue (Jones and Woodson 1999), it can be concluded that post-pollination processes regulated by ethylene signaling include the down-regulation of volatile biosynthesis and emission (Underwood et al. 2005).

## 10.6 Biotechnological Aspects

Unraveling biosynthetic pathways of floral volatiles and their respective genes and enzymes provides the opportunity to genetically engineer floral scent production. Application of this technique might have practical potential when, for example,

suboptimal pollination rates or even lack of natural pollination could be counterbalanced by reintroducing or improving scent synthesis and emission (Pichersky and Dudareva 2007). Furthermore, ornamental industries, especially the cut-flower industries, have an increasing interest in a genetic approach toward floral scent modulation. During decades of conventional breeding, floral scent has been sacrificed for showy colors or shapes of flowers, long vase life, disease resistance, and endurance of shipment around the world. To date, many commercial flowers lack floral fragrance, although humans still associate flowers with sensual pleasures, and a pleasant fragrance with wellbeing (Vainstein et al. 2001; Pichersky and Dudareva 2007). Driven by this rediscovered desire of consumers, and also driven by the commercial interest of the producer, ornamental industries have to face the challenge to reintroduce floral scents. This implies not only the recovery of native floral volatile traits, but also the modulation of the composition of floral bouquets and timing of volatile production and emission.

In principle, all metabolic pathways of floral volatile biosynthesis are amenable for bioengineering. Advanced functional genomic strategies like high-throughput DNA sequencing (Guterman et al. 2002), targeted transcriptome analyses (Verdonk et al. 2005), and proteomic technologies (Dafny-Yelin et al. 2005) allow the isolation and characterization of a rapidly increasing number of floral genes involved in volatile biosynthesis and related regulatory processes (see Sect. 10.4). Consequently, the increasing number of target genes and a better understanding of pathways have promoted floral scent engineering. However, it is still only just out of infancy, compared to the field of genetic engineering in food crops.

Metabolic engineering of floral scent has been performed using different approaches (Table 10.2). The introduction of a single or multiple transgenes encoding enzymes that are not expressed, or even absent, in the target species has yielded the emission of desired novel volatiles (Lücker et al. 2001, 2004a, b; El Tamer et al. 2003). The wild-type tobacco (*N. tabacum*) was transformed with transgenes encoding three monoterpene synthases native to *Citrus lemon* driven by the cauliflower mosaic virus (CaMV) 35S constitutive promoter. Transgenic plants were shown to emit the main products  $\beta$ -pinene, (+)-limonene and  $\gamma$ -terpinene in a non-tissue-specific manner. However, these transgenic plants emitted (+)-(*E*)-isopiperitenol after they were transformed with an additional transgene encoding limonene-3-hydroxylase that catalyzes hydroxylation of (+)-limonene (Lücker et al. 2004b). In transgenic *Dianthus caryophyllus* (carnation), a transgene comprising the  $\beta$ -linalool-synthase cDNA driven by the CaMV 35S promoter was shown to be expressed in flowers (Lavy et al. 2002). Aranovich et al. (2007) reported transgenic *Eustoma grandiflorum* expressing *C. breweri* benzyl alcohol acetyltransferase (BEAT), but benzyl acetate emission was not observed. Acetate products could be detected in floral and green tissues after feeding the substrates, such as benzyl alcohol, hexanol, or cinnamyl alcohol. Another approach of metabolic engineering involves the down- and up-regulation of native genes associated with floral volatile production. Down-regulation of genes has been achieved by RNA interference (RNAi) techniques (Verdonk et al. 2005; Orlova et al. 2006; Dexter et al. 2007), antisense inhibition of the target gene (Zuker et al. 2002), or virus-based

**Table 10.2** Overview on genetic engineering of floral scent (based on Pichersky and Dudareva 2007; Dudareva and Pichersky 2008)

Gene <sup>a</sup>	Target species	Technique used	Effect	References
<i>CbLIS</i>	<i>Petunia x hybrida</i>	Single-gene introduction	No emission	Lücker et al. (2001)
<i>CbLIS</i>	<i>Dianthus caryophyllus</i>	Single-gene introduction	Emission	Lavy et al. (2002)
<i>DcF3H</i>	<i>D. caryophyllus</i>	Antisense suppression	Up-regulation	Zuker et al. (2002)
<i>FaSAAT</i>	<i>P. hybrida</i>	Single-gene introduction	No emission	Beekwilder et al. (2004)
<i>CILIM, CIPIN, CITER</i>	<i>Nicotiana tabacum</i>	Triple-gene introduction (single-gene introduction and crossings)	Emission	Lücker et al. (2004a)
<i>MspLIM3H</i>	Transgenic <i>CILIM/PIN/TER:N. tabacum</i>	Final quadruple-gene introduction	Emission	Lücker et al. (2004b)
<i>PhODO1</i>	<i>P. hybrida</i>	RNAi	Down-regulation	Verdonk et al. (2005)
<i>PhBSMT</i>	<i>P. hybrida</i>	RNAi	Down-regulation	Underwood et al. (2005)
<i>RhAAT</i>	<i>P. hybrida</i>	Single-gene introduction	Emission	Guterman et al. (2006)
<i>PhBPBT</i>	<i>P. hybrida</i>	RNAi	Down-regulation	Orlova et al. (2006)
<i>PhCFAT</i>	<i>P. hybrida</i>	RNAi	Down-regulation	Dexter et al. (2007)
<i>CbBEAT</i>	<i>Eustoma grandiflora</i>	Single-gene introduction	No emission	Aranovich et al. (2007)
<i>PhCHS</i>	<i>P. hybrida</i>	Virus-induced gene silencing	Up-regulation	Spitzer et al. (2007)
<i>AthPAP1</i>	<i>P. hybrida</i>	Overexpression	Up-regulation	Ben Zvi et al. (2008)

<sup>a</sup>*CbLIS*, linalool synthase (*Clarkia breweri*); *DcF3H*, flavanone 3-hydroxylase (*D. caryophyllus*); *FaSAAT*, strawberry alcohol acyltransferase (*Fragaria x ananassa*); *CILIM*, limonene synthase (*Citrus lemon*); *CIPIN*, pinene synthase (*C. lemon*); *CITER*,  $\gamma$ -terpinene synthase (*C. lemon*); *MspLIM3H*, limonene-3-hydroxylase (*Mentha spicata* ‘Crispa’); *PhODO1*, transcription factor ODORANT1 (*P. hybrida*); *PhBSMT*, benzoic acid/salicylic acid carboxyl methyltransferase (*P. hybrida*); *RhAAT*, alcohol acetyltransferase (*Rosa x hybrida*); *PhBPBT*, benzylalcohol/phenylethanol benzoyltransferase (*P. hybrida*); *PhCFAT*, coniferyl alcohol acyltransferase (*P. hybrida*); *CbBEAT*, benzyl alcohol acetyltransferase (*C. breweri*); *PhCHS*, chalcone synthase (*P. hybrida*); *AthPAP1*, Anthocyanin Pigment1 MYB transcription factor (*Arabidopsis thaliana*)

gene silencing methods (Spitzer et al. 2007). In a recent study, Ben Zvi et al. (2008) reported a new approach of metabolic engineering by the introduction of foreign regulators, which were superimposed on the native downstream regulators of

volatile biosynthesis. Results showed that modulation of regulatory components changed the rhythms of volatile production and emission.

## 10.7 Conclusions

Successful floral scent engineering allows (1) the introduction of novel scent components, (2) the enhancement of underrepresented components in a floral bouquet, (3) a decrease in the amount of unpleasant or removal of unwanted components, and (4) the modulation of floral scent traits in seed plants, including changes in temporal emission patterns of volatiles. Although progress in floral scent engineering has been considerable, floral scent production remains relatively unpredictable. Floral volatile biosynthesis is a complex network of overlapping and competing pathways, in which the regulatory mechanisms are poorly understood (Dudareva and Pichersky 2008). Up-regulating and overexpression of genes, as well as the introduction of genes of an underrepresented pathway, might result in substrate shortage (Beekwilder et al. 2004; Aranovich et al. 2007; Ben Zvi et al. 2008), or disposal of toxic gene products by glucosylation (Lücker et al. 2001). Down-regulation of native genes may lead to unexpected results due to the re-channeling of metabolites (Zuker et al. 2002). Nevertheless, transgenic flowers releasing appropriate amounts of engineered metabolites may enhance human pleasure. As the flower is the target organ for floral scent production, modulation of transgene expression may be achieved more efficiently by the use of floral promoters instead of the CaMV 35S promoter. Floral scent engineering not only has a good potential in floral biotechnology, but it can also serve as an important tool for the elucidation of floral volatile metabolism.

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