The Characteristics of Flower Scents in Carnations

11

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Abstract

Benzenoid aromatic compounds are the most important scent components of *Dianthus*, although some wild *Dianthus* species also produce terpenoids and fatty acid derivatives as principal scent components, and thereby having diverse scents. The scent of *Dianthus* carnations is described as spicy and is generally ascribed to eugenol. Our research shows lower diversity of carnation scents than wild *Dianthus* scents and differing main scent types among cultivars for cut and potted flowers. We also found that scents of most of the current cultivars for cut flowers were not spicy and had fruity notes derived from methyl benzoate. In addition, scent emissions decreased sharply after harvesting, and were almost absent after a few days. In this chapter, we describe the chemistry of scents from the current carnation cultivars. In plants, methyl benzoate is usually synthesized from benzoic acid by benzoic acid carboxyl methyltransferases (BAMT). Hence, the corresponding gene *BAMT* is considered important for scent biosynthesis in carnations. From nucleotide sequence analyses of the carnation genome, more than 10 candidate *BAMT* genes were found. We describe the characteristics of this gene and its homologs in the second half of this chapter.

11.1 Introduction

In reviews of fragrance-related publications, it is clear that the scent of carnations (*Dianthus caryophyllus* L.) has long been used as a fragrance (Anonis 1985; Ghozland and Fernandez 2010). Moreover, perfumes that imitate carnation scents are widely marketed on the internet. But the real scent of fresh carnation flowers may be familiar to few people. In our questionnaire survey of about 1,000 ordinary people in Japan, less than 8% could recall the actual scent of carnations (Kishimoto et al. 2012), whereas 20, 70, and 66% of people claimed familiarity with the scents of chrysanthemums, roses, and lilies, respectively. Hence, although carnations are widely perceived as aromatic flowers in books and other literature, few Japanese people have experienced the scent of carnations.

The carnation scent is described as spicy and similar to that of cloves (Ghozland and Fernandez 2010), and the benzenoid aromatic compound eugenol is identified as a major source of this scent (Clery et al. 1999). Therefore, to experience the scent of carnations described in the literature, one merely needs to open the lid of a clove bottle and smell the spicy fragrance of

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eugenol. At the florist, carnations are available as red, white, yellow, green, and brown flowers that have been produced by traditional breeding, and blue flowers that were developed through genetic engineering (Fukui et al. 2003). With knowledge of the scent of eugenol, one could ask whether the scents of these flowers are as diverse as their colors, and whether the spicy scent of cloves is present?

In this chapter, we summarize the scent characteristics of currently marketed carnations.

11.2 Scent Diversity in Carnations

Carnations are flowers of the *Dianthus* family, which originate from an evolutionary hot spot on the Mediterranean coast (Valente et al. 2010). In this region, wild *Dianthus* species have adapted to dryness (Valente et al. 2010) and the rich variety of scents from wild *Dianthus* reflects species diversity in the genus.

11.2.1 Wild Dianthus Scents

The scents of many wild *Dianthus* species are thought to originate from benzenoid aromatic compounds such as isoeugenol, methyl salicylate, methyl benzoate, and benzyl benzoate (Kishimoto et al. 2011, 2013). These benzenoids are synthesized from sugar metabolites of glycolysis and the pentose phosphate cycle (Fig. 11.1). These substrates are converted to phenylpropanoid/benzenoids via the shikimate pathway (Fig. 11.1; Muhlemann et al. 2014). Methyl salicylate is generally used as a fragrance in foods and beverages (Burdock 2010). Moreover, because this compound has anti-inflammatory activities, it is frequently used as a medicine. The scent of methyl salicylate is often perceived as sweet or medicinal (Burdock 2010; Clery et al. 1999). For many Japanese, this scent is that of poultice, yet in the USA, this scent is commonly associated with root beer. The scent of methyl benzoate is described as fruity or floral (Burdock 2010). It is also the main component of the sweet scent of petunia (*Petunia hybrida*) and snapdragon (*Antirrhinum majus*; Negre et al. 2003). Benzyl benzoate also has a pleasant scent and is used in perfumes (Burdock 2010). Various other scent compounds have been detected in wild *Dianthus*, and most are fragrant benzenoids (Kishimoto 2012; Kishimoto et al. 2011).

The fatty acid derivatives (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate are principal scents in several minor wild *Dianthus* species (Kishimoto et al. 2011). These compounds are synthesized via the hydroperoxide lyase pathway of oxylipin metabolism and are often called green leaf volatiles (Matsui 2006). (*Z*)-3-hexenol smells of chopped leaves and the scent of (*Z*)-3-hexenyl acetate is described as green and fruity with a floral note that is reminiscent of banana (Burdock 2010).

Dianthus superbus flowers are widely distributed from Europe to the Far East and their scents, which are based on terpenoids (Galbally and Galbally 1997; Kishimoto et al. 2011), reportedly increase at night (Erhardt 1991). This feature is considered suitable for nocturnal pollinators (Erhardt 1991). The major scent component β-caryophyllene is a bicyclic sesquiterpene that sits among essential oils of various herbs (Alma et al. 2007; Calvo-Irabien et al. 2009), and its relaxing odor is often described as woody, spicy, dry, or camphoraceous, with a citrus background (Burdock 2010). Another major component, (*E*)-β-ocimene, is found in various flowers and fruits, and is experienced as a warm herbaceous odor or a woody odor with a floral scent (Burdock 2010). Like benzenoids, these terpenoids are products of sugar metabolism. Recent studies show that monoterpenes and sesquiterpenes are mainly synthesized by plastidial methylerythritol phosphate and cytosolic mevalonate (MVA) pathways, respectively (Muhlemann et al. 2014).

Although exceptions have been described, groups of plants generally use the same biosynthetic pathways to produce scents. For example,

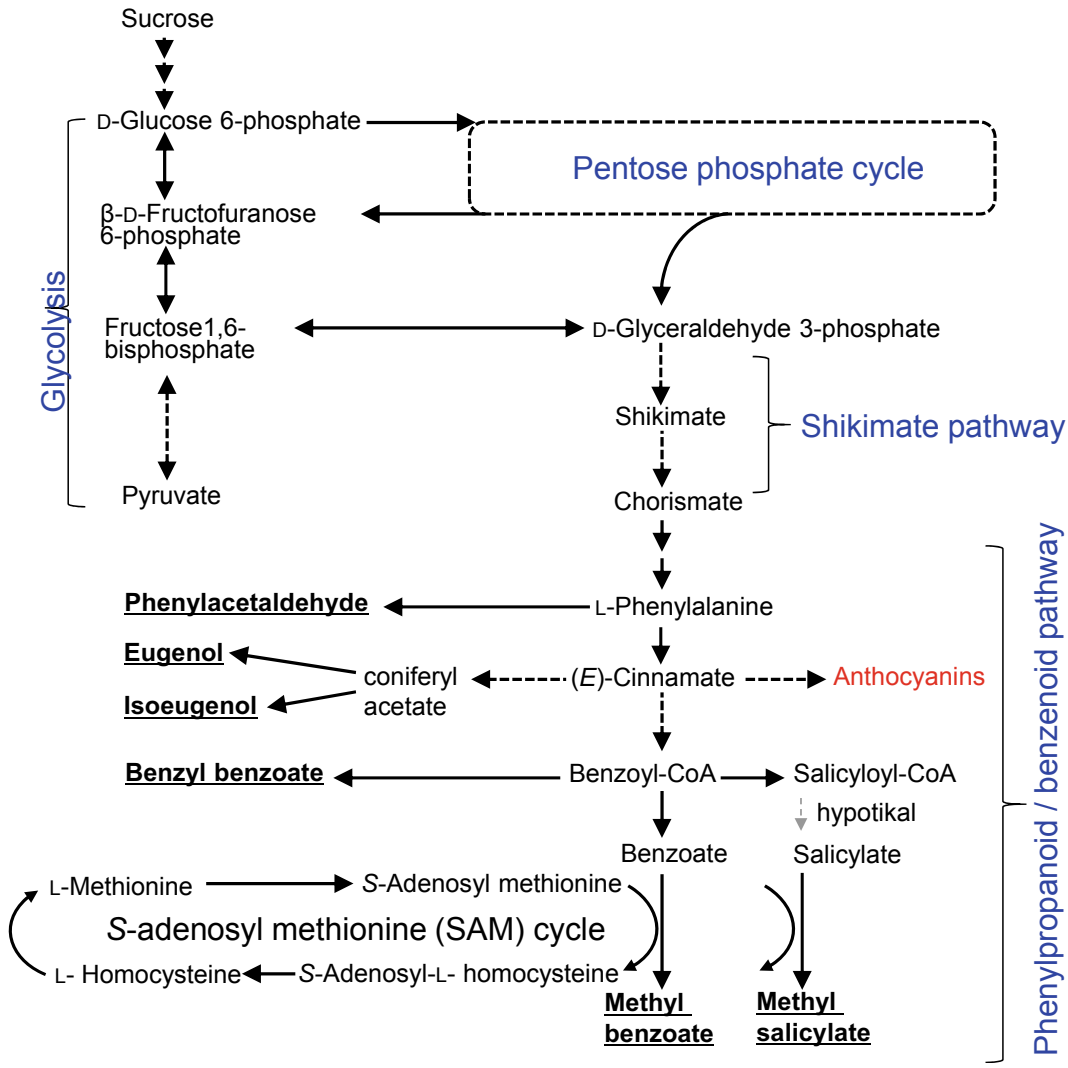

Fig. 11.1 Biosynthetic pathway for benzenoid aromatic compounds; benzenoid aromatic compounds are principal scent components of most *Dianthus* species and are synthesized in the phenylpropanoid/benzenoid biosynthetic pathway via the shikimate pathway. Solid and dotted arrows indicate single and multiple catalytic processes, respectively. Underlined and red letters indicate scent and color components, respectively. Blue letters indicate the names of biosynthesis pathways

Petunia and *Chrysanthemum* are excellent sources of benzenoids and terpenoids, respectively (Kondo et al. 2006; Sun et al. 2015). Among scent compounds of *Dianthus*, aromatic benzenoids do not always play greater roles than fatty acid derivatives and terpenoids. Hence, the primary metabolic pathways of dominant scents differ between species, leading to diverse scents of *Dianthus* flowers.

11.2.2 Carnation Scents

Marketed carnations can be red, white, yellow, pink, orange, green, purple, brown, or blue. This color diversity is likely comparable to or greater than that of wild *Dianthus* species. To clarify the diversity of carnation scents, we investigated emitted volatiles of 25 carnation cultivars for cut flowers in Japan (Kishimoto et al. 2019).

Cultivars were randomly selected and their shapes included standard and spray types. In all cultivars, the main scent components were aromatic benzenoids, as indicated by the typical scent compositions shown in Fig. 11.2 (Upper panel). The dominant scent component of 21 cultivars (84%) was methyl benzoate, which produces a fruity odor. The common cultivar Francesco was also classified into this type, but only one cultivar carried the spicy scent of eugenol. The principal scents in the other three cultivars were weak-scent benzenoids, such as benzyl benzoate and benzyl alcohol. We also collected cultivars that were deliberately considered to have characteristic scents. Their principal scents were eugenol or benzyl benzoate and no carnation cultivars had dominant fatty acid derivative or terpenoid scents (Kishimoto et al. 2019). Hence, most carnations in Japan have fruity scents derived from methyl benzoate and sometimes have the spicy scent of eugenol or the weak floral scent of benzyl benzoate.

Clery et al. (1999) compared the emitted scents from traditional carnation cultivars (registered before 1970) and modern cultivars (registered after 1994) and suggested that modern cultivars have lost the spicy fragrance of eugenol. Although their sample numbers were small, the results of this European study indicated that methyl benzoate is the major scent component of modern carnation cultivars, as described for carnations in Japan.

The diversity of carnation scents is much less than that of wild *Dianthus*. In a review of floral fragrance, Vainstein et al. (2001) suggested that modern cultivars have been unintentionally selected against fragrance, reflecting the negative correlation between longevity and fragrance. However, no positive or negative correlations between types of scent and floral longevity have been reported for carnations.

A relationship between the scents of volatile benzenoids and anthocyanin-derived color was previously reported in carnations. In their study, Zuker et al. (2002) suppressed the anthocyanin biosynthesis gene for flavanone 3-hydroxylase using antisense technology, and observed petal color changes from red to white and increased production of volatile benzenoids. Hence, color- and scent-biosynthetic pathways may compete in carnation flowers. Accordingly, biosynthetic processes for these phenotypes generally overlap in plants (Fig. 11.1), suggesting that the selection of darker colored flowers that are rich in anthocyanins will favor the loss of fragrant strains. In contrast, our transcriptome analysis showed that expression periods of anthocyanin- and scent-biosynthetic genes do not overlap at the carnation flowering stage (unpublished data). Similar pattern of gene expression was also observed in *Petunia hybrida* flowers (Verdonk et al. 2005). This strongly suggests that the processes of developing color and scent do not overlap during flower development. Thus, the phenomenon described by Zuker et al. (2002) may be unique to transgenic carnations.

In carnations, wilt (*Fusarium oxysporum* f. sp. *dianthi*)-resistant cultivars of the Mediterranean-type were rapidly distributed during the 1980s (Onozaki 2018, Yagi et al. 2014b). Perhaps methyl benzoate was the dominant scent component of this variety. In any case, why current carnation scents differ from those described in books about perfume is an interesting theme of carnation research.

Most scents of cut carnation flowers that are sold at florists are fruity and are derived from methyl benzoate. However, we found that carnation scents are lost rapidly after harvest (Kishimoto et al. 2019), with scent emissions from cut flowers decreasing by 15%–50% over the 2 days after harvest (Fig. 11.2 lower panel). In addition, we compared sensory tests for the carnation scent in 80 subjects and quantitatively investigated scent emissions over time (Kishimoto et al. 2019). For the average subject scent emissions from most cut flowers decreased to almost undetectable levels within a few days (Fig. 11.2 lower panel; Kishimoto et al. 2019). Hence, carnations at florists are almost unscented. Our transcriptome analyses of Francesco flowers showed rapid decreases in expression levels of several scent-related genes after cutting of the flowers (unpublished data), suggesting that rapid declines in scents of cut flowers are in part related to gene expression levels. Commercial

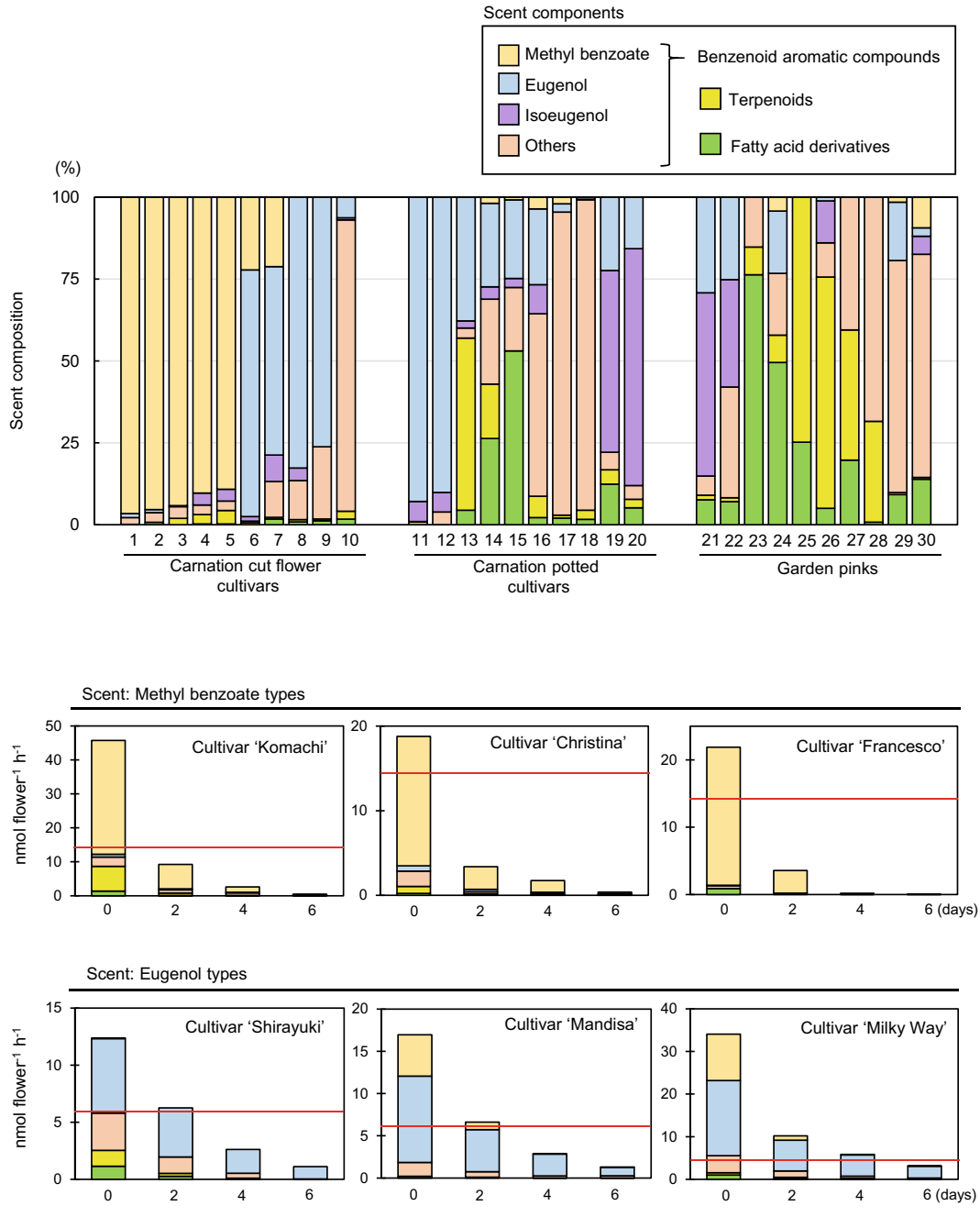
Scent components
Methyl benzoate
Eugenol
Isoeugenol
Others
Benzenoid aromatic compounds
Terpenoids
Fatty acid derivatives
(%)
Scent composition
100
75
50
25
0
1 2 3 4 5 6 7 8 9 10
Carnation cut flower cultivars
11 12 13 14 15 16 17 18 19 20
Carnation potted cultivars
21 22 23 24 25 26 27 28 29 30
Garden pinks
Scent: Methyl benzoate types
nmol flower⁻¹ h⁻¹
Cultivar 'Komachi'
Cultivar 'Christina'
Cultivar 'Francesco'
0 2 4 6 (days)
Scent: Eugenol types
Cultivar 'Shirayuki'
Cultivar 'Mandisa'
Cultivar 'Milky Way'

Fig. 11.2 Scent components of carnations and changes in relative emissions; upper panel, comparison of scent compositions between carnations and garden pinks; emitted scent compounds from flowers were collected using a dynamic headspace method with a Tenax TA column (Oka et al. 1999). The collected scent compounds were analyzed using gas chromatography–mass spectroscopy (GC–MS) and were identified and quantified using corresponding standards. The graphs show the ratios (%) of each scent component relative to total emissions (nmol flower^{-1} h^{-1}). Cultivar names: Komachi C10 (1), Francesco (2), Komachi (3), Chiquita (4), Christina (5), Mandisa (6), Milky Way (7), Shirayuki (8), Across (9), Siberia (10), Precious (11), Fosset Red (12), Rafale (13), Cheerful (14), Orfica (15), Milky Salmon Pink (16), Shantery (17), Memorial White (18), Bambino (19), Magical White (20), Raspberry Thunder (21), Coconut Sunday (22), Diana Crimson Picoty (23), Matsuzaka Nadeshiko (24), Dynasty Red (25), Supra Purple (26), Telstar Orchid (27), Telstar Burgundy (28), Kaori (29), Saint First (30). Lower panel; changes in emission quantities and compositions of scents in carnation cut flowers from the day of harvesting until 6 days later; intensities of flower scents were evaluated by 80 subjects as very scented, scented, slightly scented, or unscented. Red lines indicate boundaries at which more than 70% of subjects gave the positive evaluations "very scented" or "scented". This figure is a modification of a figure published by Kishimoto et al. (2019)

cut carnations are generally treated with ethylene inhibitors such as silver thiosulfate (STS) to suppress flower senescence. Yet, it was confirmed that this treatment was not the cause of scent reductions (Kishimoto et al. 2019).

After harvesting of carnation flowers, eugenol-type scents were shown to last longer than those of methyl benzoate (Fig. 11.2 lower panel). Accordingly, the aroma threshold of eugenol was shown to be lower than that of methyl benzoate in humans. Moreover, eugenol-type scents decreased more slowly than those of methyl benzoate (Fig. 11.2 lower panel). Nonetheless, for use as aromatic cultivars, improved persistence of scents is required. If you come across a fragrant carnation cut flower, I strongly encourage you to look up the cultivar name, because it is a very valuable experience.

Carnation scents are lost during processing into cut flowers, but remain present in potted flowers. Accordingly, no aromatic carnation cultivars for cut flowers are known, whereas several potted carnations are sold as aromatic cultivars in Japan. We investigated the scent compositions of 25 potted carnation cultivars (Kishimoto et al. 2015), as presented in Fig. 11.2 (upper panel). Sensory tests of the scents of these cultivars were also performed previously (Kishimoto et al. 2015). These studies show that some cultivars produce eugenol and isoeugenol as principal scent components and are sufficiently scented for sensual uses. Isoeugenol scents were also preferred by subjects, and 13%–32% of them identified the scent as a vanilla-like fragrance. The chemical structures of isoeugenol and vanillin, which has the fragrance of vanilla, are very similar, and isoeugenol is often used as a precursor for vanillin synthesis (Priefert et al. 2001). In fragrance-related books, the isoeugenol scent is described as spicy (Burdock 2010), but people who are accustomed to the scent of vanillin may recognize it as vanilla.

In our investigations, benzenoid aromatic compounds such as eugenol, isoeugenol, benzyl benzoate, phenylacetaldehyde, or methyl salicylate and terpenoids such as β-caryophyllene or nerolidol were detected as major scent components in the pot cultivars (Kishimoto et al. 2015). Figure 11.2 shows some of the results (upper panel). Scents based on eugenol or benzyl benzoate were also found in the cut flower cultivars but these findings were rare (Kishimoto et al. 2019). Other scent compounds were also detected in cut flower cultivars but were not the principal components (Kishimoto et al. 2019). On the other hand, methyl benzoate, which is the most principal scent of cut flower cultivars, was a minor component in pot cultivars (Kishimoto et al. 2015; Fig. 11.2 upper panel). Hence, differing scents of cut flower cultivars and pot cultivars of carnations may reflect different genetic backgrounds.

We also investigated scent emissions from horticultural *Dianthus* cultivars other than carnations, such as garden pinks (Kishimoto et al. 2015; Fig. 11.3). These flowers better resemble their wild ancestor species than carnations, and their scent components were clearly more diverse than those of carnations. Among randomly selected cultivars of garden pinks, we found

flowers with benzenoids, terpenoids, and fatty acid derivatives as principal scent components (Fig. 11.2 upper panel). These data suggest that the diversity of scents from wild species is preserved in garden pinks.

Our research shows that carnation scents are less diverse than those of wild *Dianthus*. Therefore, we explored the possibility of introducing new scents into carnations by crossing with wild species. The wild species *Dianthus superbus* var. *longicalycinus* has high terpenoids, β-caryophyllene, and β-ocimene contents (Kishimoto et al. 2011). After interspecific mating between this wild species and a carnation species lacking terpenoids, these terpenoids were acquired as principal scent components in F_1 hybrids (Kishimoto et al. 2013). Additionally, the benzenoids eugenol, benzyl alcohol, methyl *o*-anisate, and methyl salicylate were acquired by interspecific hybrids between carnations and the fragrant wild species, and their scents were perceptible (Kishimoto et al. 2013). Hence, it is possible to breed scents of various benzenoids and terpenoids into carnations.

11.2.3 Scent-Biosynthetic Genes in Current Cultivars

As described above, benzenoid aromatic compounds are the most important sources of scent in *Dianthus* (Kishimoto and Yagi (2015); Kishimoto et al. 2011, 2015, 2019), and methyl benzoate is the most common and principal scent component in current carnation cultivars for cut flowers (Kishimoto et al. 2019). The scent of Francesco is also typical of current cultivars, and about 90% of the emitted scent is due to methyl benzoate (Fig. 11.2 upper panel). In plants, methyl benzoate is commonly synthesized from benzoic acid by BAMT (Fig. 11.1). In this catalytic process, a methyl group is supplied from the *S*-adenosyl-L-methionine (SAM) cycle (Fig. 11.1). Generally, methylation of carboxyl groups reduces the boiling points of target compounds, leading to improved transpiration efficiencies. Therefore, this methylation reaction is one of the most important processes for the production of flower scents. Salicylic acid carboxyl methyltransferases (SAMTs) and jasmonic

Fig. 11.3 Garden pink flowers; scent compositions of these flowers are shown in Fig. 11.2

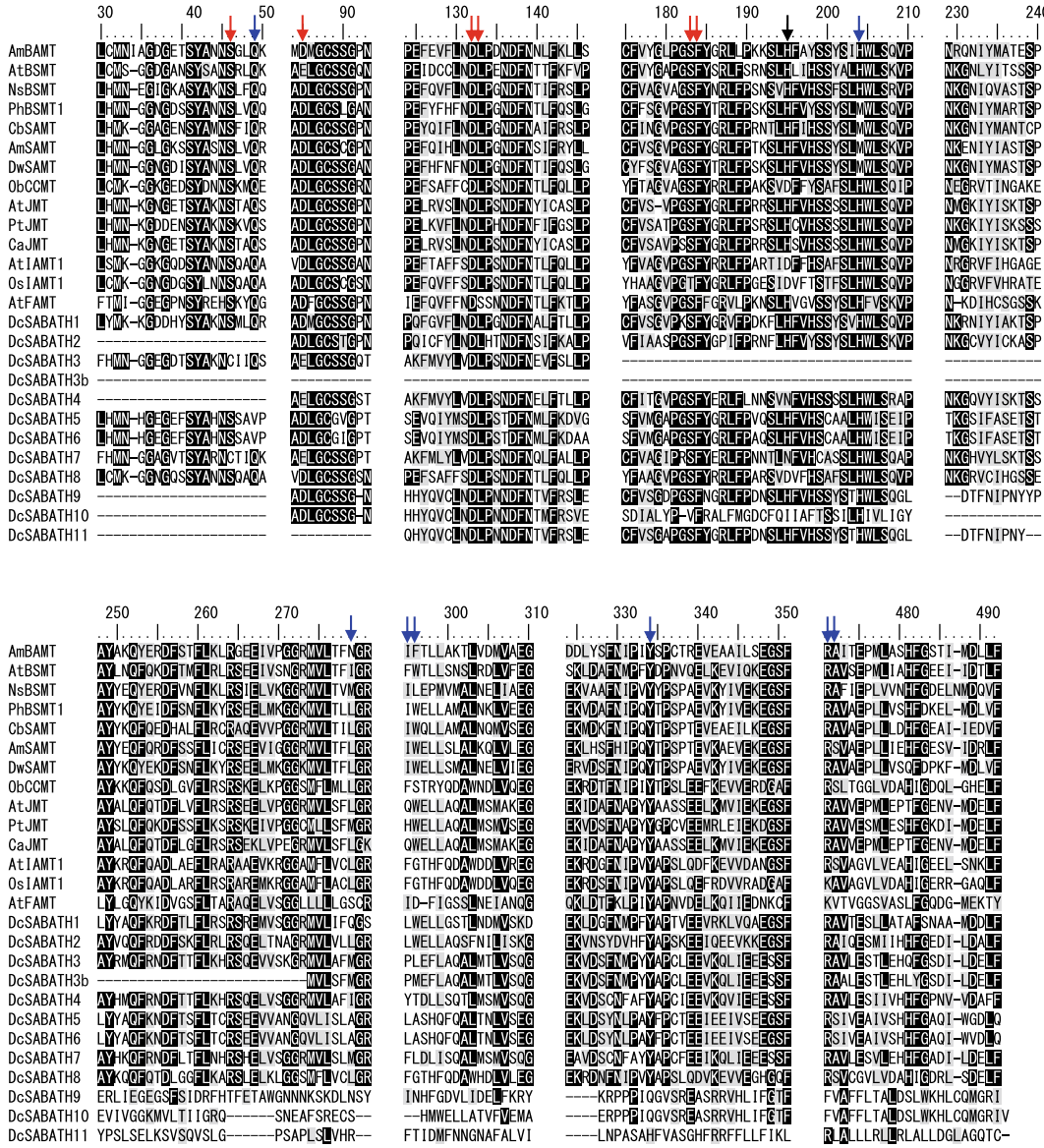

Fig. 11.4 Comparisons of common motifs between *S*-adenosyl-L-methionine-dependent benzoic acid/salicylic acid carboxyl methyltransferases (SABATHs) of *Dianthus caryophyllus* (DcSABATHs) and other plant SABATH family proteins; AmBAMT, *Antirrhinum majus* benzoic acid carboxyl methyltransferase (AAF98284); AtBSMT, *Arabidopsis thaliana* benzoic acid/salicylic acid methyltransferase (AAY25461); NsBSMT, *Nicotiana suaveolens* BSMT (CAF31508); PhBSMT1, *Petunia hybrida* BSMT1 (AAO45012); CbSAMT, *Clarkia breweri* salicylic acid methyltransferase (AAF00108); AmSAMT, *A. majus* SAMT (AAN40745); DwSAMT, *Datura wrightii* SAMT (ABO71015); ObCCMT1, *Ocimum basilicum* cinnamate/*p*-coumarate methyltransferase (ABV91100); AtJMT, *A. thaliana* jasmonic acid methyltransferase (AAG23343); PtJMT, *Populus trichocarpa* JMT (AGR50489); CaJMT, *Capsicum annuum* JMT (ABB02661); AtIAMT1, *A. thaliana* indole acetic acid methyltransferase 1 (BAD43349); OsIAMT1, *Oryza sativa* IAMT1 (ABZ04474); CaCAS1, *Coffea arabica* caffeine synthase 1 (BAC43760);CsTCS1, *Camellia sinensis* theobromine and caffeine synthase 1(BAB12278). The black arrow indicates the position related to substrate specificity for benzoic acid. Red arrows indicate *S*-adenosyl-L-methionine binding residues. Blue arrows indicate residues that interact with carboxyl moieties of salicylic acid or indole-3-acetate

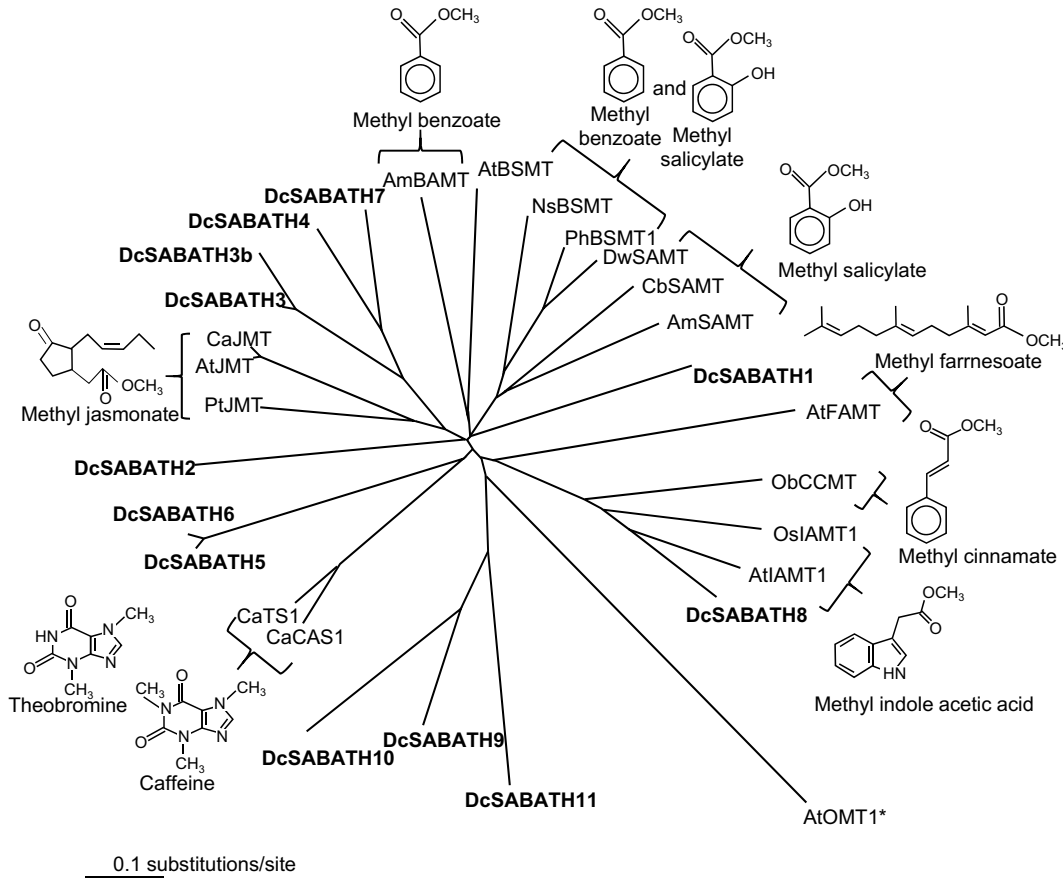

Fig. 11.5 Phylogenetic relationships between known *S*-sdenosyl-L-methionine (SAM)-dependent benzoic acid/salicylic acid carboxyl methyltransferases (SABATHs) and SABATHs from *Dianthus caryophyllus* (DcSABATHs); AmBAMT, *Antirrhinum majus* benzoic acid methyltransferase (AAF98284); AtBSMT, *Arabidopsis thaliana* benzoic acid/salicylic acid methyltransferase (AAY25461); NsBSMT, *Nicotiana suaveolens* BSMT (CAF31508); PhBSMT1, *Petunia hybrida* BSMT1 (AAO45012); CbSAMT, *Clarkia breweri* salicylic acid methyltransferase (AAF00108); AmSAMT, *A. majus* SAMT (AAN40745); DwSAMT, *Datura wrightii* SAMT (ABO71015); AtJMT, *A. thaliana* jasmonic acid methyltransferase (AAG23343); CaJMT, *Capsicum annuum* JMT (ABB02661); PtJMT, *Populus trichocarpa* JMT (AGR50489); AtIAMT1, *A. thaliana* indole acetic acid methyltransferase 1 (BAD43349); OsIAMT1, *Oryza sativa* IAMT1 (ABZ04474); ObCCMT1, *Ocimum basilicum* cinnamate/*p*-coumarate methyltransferase (ABV91100); CaCAS1, Coffea arabica caffeine synthase 1 (BAC43760); CsTCS1, *Camellia sinensis* theobromine and caffeine synthase 1 (BAB12278). **Arabidopsis thaliana O*-methyl transferase1 (AtOMT1) is not a SABATH family member. This figure is a modification of a figure published by Yagi et al. (2014a)

acid carboxyl methyltransferases (JMTs) are known as similar enzymes (Ross et al. 1999; Seo et al. 2001). These SAM-dependent methyltransferases are collectively referred to as the SABATH family (D'Auria et al. 2003) and are found only in the plant kingdom. They also lack significant sequence similarity with other methyltransferases (Wang et al. 2017). We previously identified 11 primary sequence structures (*DcSABATH1-11*) that were similar to SABATH, and these were candidate *BAMT* genes in the Francesco genome (Yagi et al. 2014a). Yet it remains unclear which DcSABATHs contribute to carnation scents. Figure 11.4 shows the amino acid sequences of DcSABATHs and known SABATHs in flowering plants. The 204th amino acid residue is notable in these SABATHs (black arrow in Fig. 11.4), because substrate specificity

for benzoic acid, a precursor of methyl benzoate, is dramatically improved by substituting the amino acid at this position with histidine (black arrow in Fig. 11.4; Barkman et al. 2007). Because many DcSABATHs retain this histidine, they are expected to synthesize methyl benzoate preferentially over other methylated volatiles. Yet about half of the known DcSABATHs lack the amino acid residues that are important for binding to SAM (red arrows in Fig. 11.4). Hence, these DcSABATHs may not function as BAMT.

In phylogenetic analyses, most DcSABATHs are dissimilar to known BAMT (Fig. 11.5), in part because known SABATHs are derived from plants other than Caryophyllales. Many members of the BAMT subfamily can methylate both salicylic acid and benzoic acid, and therefore have weak substrate specificities (Fig. 11.5; Barkman et al. 2007; Negre et al. 2003). Moreover, many DcSABATHs have retained the target site of the salicylic acid carboxyl group (blue arrows in Fig. 11.4). Comparisons of DcSABATHs, which produce almost no methyl salicylate, with SABTHs of wild *Dianthus*, which are rich in methyl salicylate, may reveal substrate specificities of the SABTH family. We believe that sequence analysis of the Francesco genome (Yagi et al. 2014a) has led the way for further research on scent biosynthesis-related genes in carnation.

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