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Volatile Constituents in the Scent of Roses

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ABSTRACT

The cultivation of roses as an ornamental plant has a long history. Apart from floricultural purposes, roses also have an economic importance as a source of natural fragrance in perfume and cosmetic industry and for medical applications. Rose hips, flowers, and their extracts are used for tea infusions and for flavouring of a broad variety of foods. In the scent of roses, more than 400 volatiles have been identified and this review focuses on the key classes of these compounds. There are several possibilities to classify rose volatile compounds, for example by their biosynthetic pathways or their chemical structures. Beginning with cellular compartmentalization, we summarize the recent findings of volatiles derived from the methylerythritol phosphate- and mevalonic acid pathways, the shikimate pathway, and the formation of fatty acid and carotenoid derived volatiles. Additionally, we review recent knowledge about the enzymes involved in these pathways, i.e. methyltransferases, decarboxylases, reductases, carotenoid cleavage enzymes, and β-glycosidases. In the light of recent findings, we also summarize the rhythmic release of volatile compounds. Finally, the evolution of scent metabolic pathways of roses and future research aspects are discussed.

Keywords: biosynthesis, enzymes, precursors, rhythmic emission
Abbreviations: **AA**, amino acid; **AADC**, L-aromatic amino acid decarboxylase; **AAT**, alcohol acetyl transferase; **CCD**, carotenoid cleavage dioxygenase; **DMAPP**, dimethylallyl diphosphate; **DXP**, desoxyxylulose phosphate; **FPP**, farnesyl diphosphate; **GPP**, geranyl pyrophosphate; **GGPP**, geranylgeranyl pyrophosphate; **IPP**, isopentenyl phosphate; **LOX**, lipoxygenase; **MEP**, methylerythritol phosphate; **MVA**, mevalonic acid; **OMT**, *O*-methyltransferase; **PAR**, phenylacetaldehyde reductase

CONTENTS

INTRODUCTION

Today more than 150 *Rosa* species and 25,000 cultivars are known (Cairns *et al.* 2000). The huge number of cultivars has been obtained by the interbreeding of a relatively small number of European and Chinese species. The scent of European cultivars is characterized by 2-phenylethanol and monoterpene alcohols, whereas Chinese roses emit mainly 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene along with lipid derived alcohols and esters (such as hexanol and hexenyl acetate) (Scalliet *et al.* 2002; Charri-Martin *et al.* 2007). Roses play an economically important role as ornamental flowers and raw materials for rose oils and rose water. Essential rose oils are of great importance for the perfume, culinary, and medicinal industries, but the production is complex and time consuming with low yield. For the production of essential oils from *Rosa damascena* Mill., 3,000 kg of flower petals are necessary to produce1 kg of oil. Therefore rose oil is one of the most expensive essential oils on the world market (Baydar *et al.* 2005). Due to the high expense of rose essential oil production, the scent of rose flowers has been intensively studied and considerable advancements in the understanding of the volatile formation have been achieved within recent decades.

Starting with cellular compartmentalization (**Fig. 1**), we review the recent findings related to the formation of key aroma compounds in rose flowers. Metabolic pathways involved in the volatile formation such as the methylerythritol phosphate pathway, the shikimate pathway; and the formation of fatty acid and carotenoid derived volatiles are discussed. In addition, we summarize the knowledge about the enzymes involved in the formation of the different classes of volatile compounds.

TERPENOIDS AND DERIVATIVES OF GPP/GGPP

Terpenoids belong to a class of natural compounds derived from $(C_5)_n$ isoprene units. In addition to monoterpenes (C_{10}),

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Invited Review

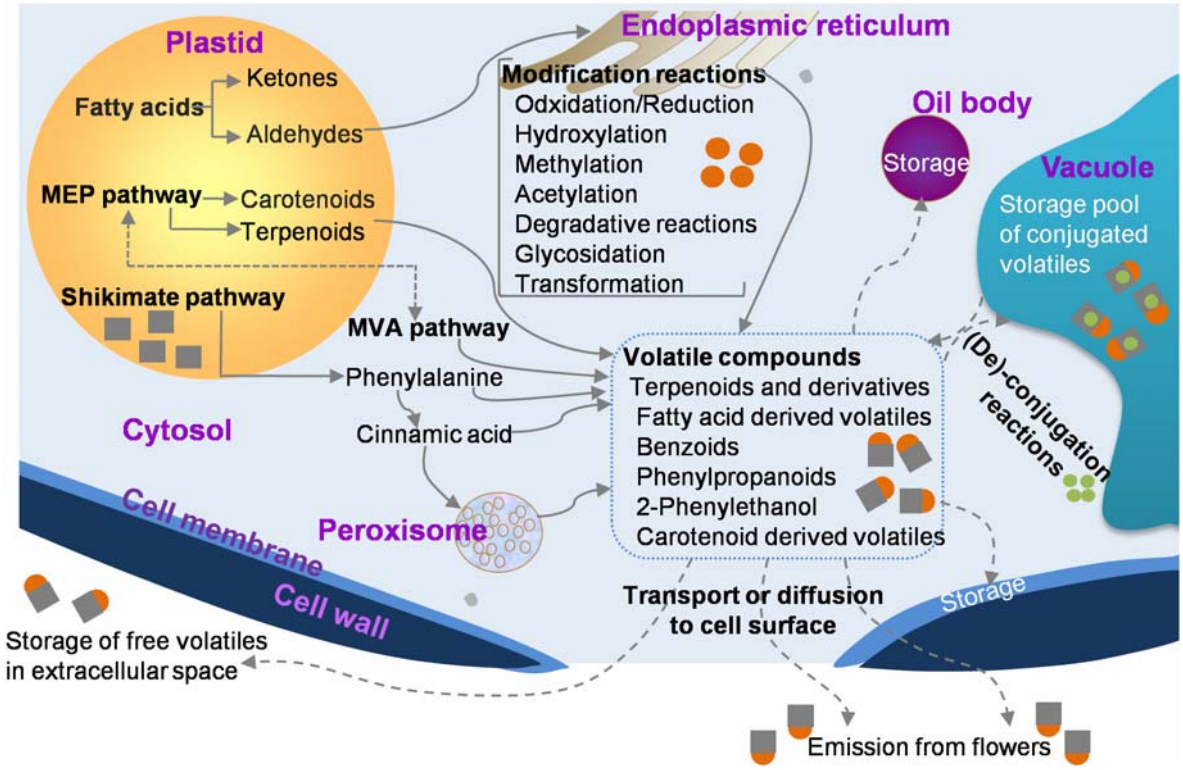

Fig. 1 Simplified scheme of cellular processes involved in the synthesis of rose scent compounds based on the review of Pichersky *et al.* (2006).

hemiterpenes (C_5), sesquiterpenes (C_{15}), and even some diterpenes (C_{20}) have high enough volatilities to contribute to floral scents. The monoterpenes and to a lesser extent the sequiterpenes comprise an important class of volatiles in the essential oils extracted from peppermint, lemon, caraway, chamomile, dill, eucalyptus, thyme, sandalwood, rosemary, lavender, and roses. They exhibit important ecological functions as defence compounds against herbivores or competing plants, and pollinator attractants (Little and Croteau 1999; Dewick 2002).

Two pathways for the formation of the C_5 units, IPP (isopentenyl phosphate) and DMAPP (dimethylallyl diphosphate), are elucidated in plants: the long known mevalonate pathway by the intermediate mevalonic acid (MVA) and the methylerythritol phosphate pathway by the intermediates desoxyxylulose phosphate (DXP) and methylerythritol phosphate (MEP) (Dewick 2002). The MEP pathway is localized in the plastids and provides IPP and DMAPP for hemiterpene, monoterpene, and diterpene biosynthesis. The C_5 units from the MVA pathway, localized in the cytosol, mainly form sequiterpenes (Dudareva *et al.* 2004) (**Fig. 1**). However, an exchange of intermediates between the two pathways, and thus a contribution of isoprene units derived from the MVA- or the MEP pathway, are prevalent (Dewick 2002; Schuhr *et al.* 2003). The second step of biosynthesis, the condensation of IPP and DMAPP, results in geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP), the precursors of monoterpenes, sequiterpenes, and diterpenes, respectively.

The subclass of monoterpenes comprises the largest class of volatile terpenoids in rose flowers. A large number of volatile monoterpenes such as citronellol, nerol, geraniol, and linallol have been identified and contribute to the odour of roses (**Fig. 2A**). The concentrations of citronellol, geraniol, nerol, and linallol constitute 60% of the oil, but their aroma contribution is relatively low at 6%. These low values are due to the high odour thresholds of citronellol, geraniol, nerol, and linallol with 40, 75, 300 and 6 µg/kg in water, respectively (Ohloff 1990). Moreover, polyhydroxylated monoterpenediols are important compounds involved in the formation of rose volatiles (Knapp *et al.* 1998; Winterhalter *et al.* 1999; Knapp *et. al.* 2000). Knapp *et al.* (1998) elucidated the structures of 22 monoterpendiols, whereas 19 of them were firstly reported in roses and confirmed the importance of terpenoiddiols to the rose odour formation. In addition they isolated and clarified the structure of the labile genuine precursor ((*S*)-3,7-dimethyl-5-octene-1,7-diol) of the isomeric rose oxides (**Fig. 2A**).

Further modifications such as hydroxylation, oxidation, and acylation can increase the diversity of volatiles as summarized by Dudareva *et al.* (2004). P450 oxidases located in cellular endoplasmic reticulum are involved in these modifications; cytochrome P450 oxidases for example are responsible for the hydroxylation of terpenoids and are involved in the formation of volatiles derived from fatty acids. NAPD/NAD-dependent oxidoreductases like the non-specific alcohol dehydrogenases convert aldehydes to alcohols, i.e. hexanal to hexanol. Further important modifications are methylation reactions by methyltransferases. Those enzymes have a high substrate specificity, which will be discussed in detail in the next section. Modification reactions mainly occur in the cytosol, but some may take place in other subcellular compartments such as plastids, mitochondria, peroxisomes, and the endoplasmic reticulum (Pichersky *at al.* 2006).

Recently it was elucidated that the volatiles from *R. hybrida* L. 'Papa Meilland' such as terpenoids, volatiles derived from the shikimate pathway, and volatiles from fatty acids are emitted from both epidermal layers (adaxial, abaxial), even though the tissues are morphologically different (Bergougnoux *et al.* 2007). Additionally, some morphological differences of the cell layers were explored, but scented and unscented rose cultivars showed no major difference in petal anatomy. The terpenoids were histochemically localized and showed an accumulation of droplets in both epidermal layers (Bergougnoux *et al.* 2007).

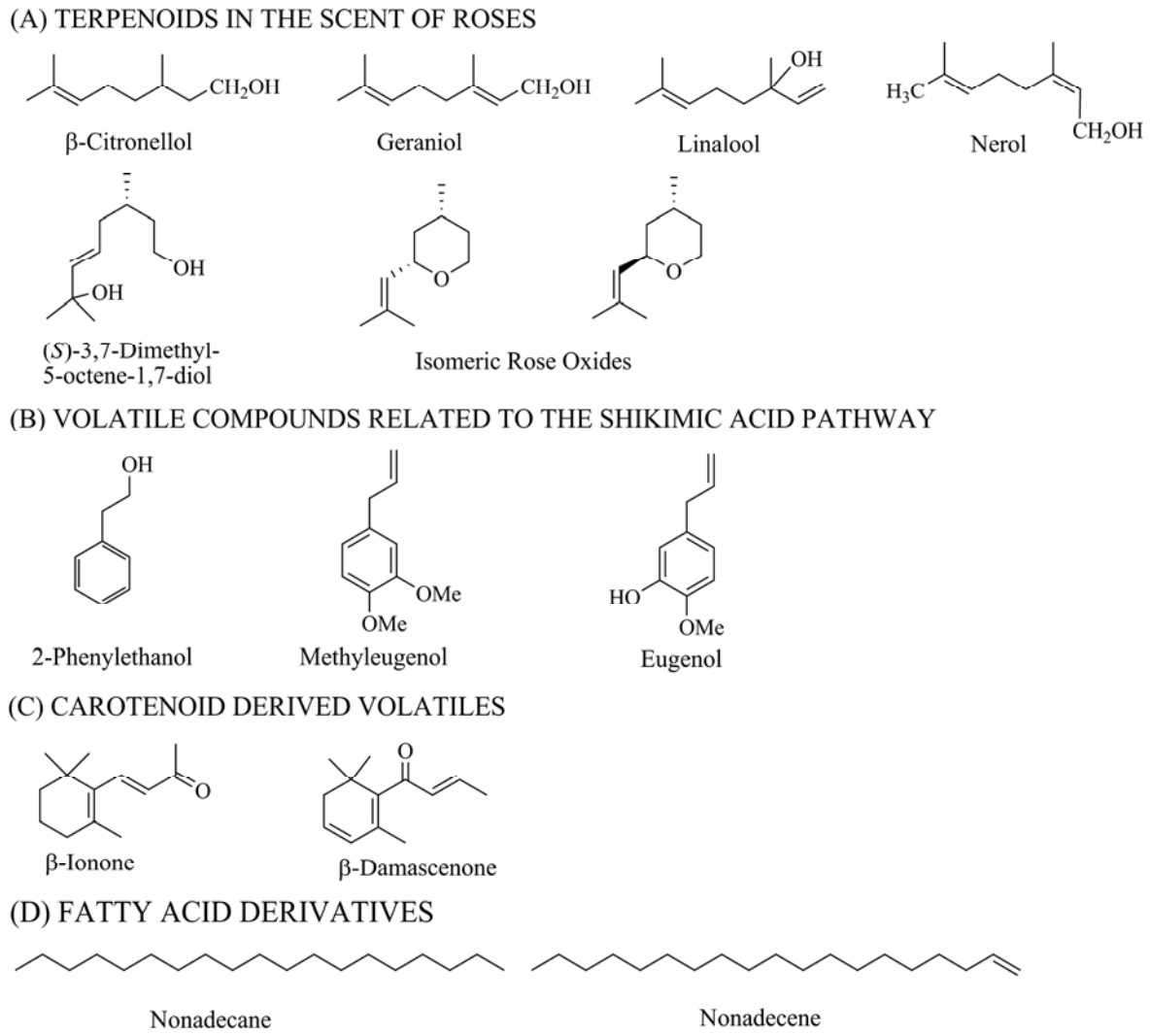

Fig. 2 Important scent compounds in roses.

VOLATILE COMPOUNDS DERIVED FROM THE SHIKIMIC ACID PATHWAY AND THEIR FORMATION

In plants approximately 20% of the carbon flows through the shikimate pathway and most of the carbon is used for the synthesis of secondary metabolites. The shikimate pathway provides precursors for the biosynthesis of primary metabolites such as aromatic amino acids and folic acid. In addition, several side reactions from the primary metabolic pathways provide aromatic precursors for the biosynthesis of secondary metabolites. Most phenylpropanoids and benzenoids contributing to floral scent derive from shikimic acid via chorismic acid, L-phenylalanine, and *trans*-cinnamic acid.

2-Phenylethanol

In European rose flowers such as *R. damascena*, the most dominant volatile compound derived from the shikimic acid pathway is 2-phenylethanol (**Fig. 2B**). 2-Phenylethanol is an aromatic alcohol having a rose-like odour (odour threshold 750 µg/L in water (Ohloff 1990); 12-24 ng/L in air (Blank *et al.* 1989). It is one of the dominant scent compounds emitted from European roses such as *R. damascena* and *R. hybrida* 'Hoh-Jun' (Sakai *et al.* 2007), which are used for the production of essential rose oils. Therefore, the biosynthetic pathway of 2-phenylethanol was intensively studied. In 1978, Bugorskii and Zaprometov proposed the formation of 2-phenylethanol from L-phenylalanine via phenylpyruvate and phenylacetic acid as intermediate products in roses. Subsequently, Watanabe *et al.* (2002) could confirm L-phenylalanine as a precursor of 2-phenylethanol using labelled isotopes ([$^2H_8$] L-phenylalanine). Interestingly, it has been shown that the α-hydrogen atom of L-phenylalanine was retained during the reaction and therefore reaction pathways different from those proposed by Bugorskii and Zaprometov (1978) via phenylacetaldehyde were put forward (Hayashi *et al.* 2004). Recent investigations of the biosynthesis of 2-phenylethanol in 'Hoh-Jun' and *R. damascena* confirmed the intermediate compound 2-phenylacetaldehyde. Two enzymes, namely L-aromatic amino acid decarboxylase (AADC) and phenylacetaldehyde reductase (PAR), were found to be involved in the biosynthesis of 2-phenylethanol (Sakai *et al.* 2007).

L-aromatic amino acid decarboxylases catalyze the decarboxylation of aromatic L-amino acids in plants, mammals, and insects. In plants, these enzymes are involved in the biosynthesis of secondary metabolites, i.e. biosynthesis of alkaloids (Facchini *et al.* 2000). The L-aromatic amino acid decarboxylase isolated from rose flowers ('Hoh-Jun' and *R. damascena*) showed high substrate specificity towards L-phenylalanine and no activity against the other aromatic amino acids (L-tryptophane, L-tyrosine, and L-arginine) (Sakai *et al.* 2007). The amino acid sequence is 99% identical to that of the L-aromatic amino acid decarboxylase isolated from petunia (Kaminaga *et al.* 2006). Under aerobic conditions, ammoniac and hydrogen peroxide are released during the reaction. Consequently, it can be concluded that the first reaction step in the biosynthesis of 2-phenylethanol is the formation of phenylacetaldehyde from L-phenylalanine catalyzed by the substrate specific L-aro-

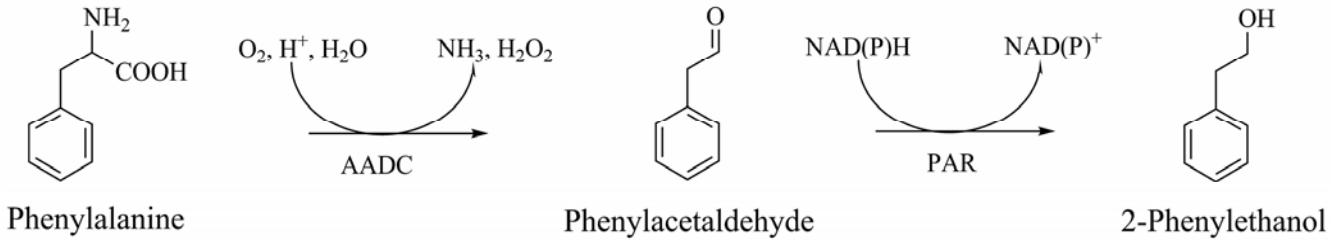

Fig. 3 Biosynthetic pathway of 2-phenylethanol from L-phenylalanine via phenylacetaldehyde.

matic amino acid decarboxylase (Sakai *et al.* 2007) (**Fig. 3**). The final step of biosynthesis from phenylacetaldehyde to 2-phenylethanol is catalyzed by 2-phenylacetaldehyde reductases (**Fig. 3**). The necessary hydrogen atoms are provided by the co-substrate NAD(P)H. Whereas the enzyme isolated from 'Hoh-Jun' showed catalytic activity with NADPH and NADH, the recombinant phenylacetaldehyde reductases from *Lycopersicon esculentum* Mill. (LePAR1 and LePAR2), which were introduced into transgenic *Petunia hybrida* Hort. ex Vilm. flowers, did not use NADH (Sakai *et al*. 2007; Tieman *et al.* 2007). Both compounds (phenylacetaldehyde and 2-phenylethanol), with their fruity and floral flavour, contribute to the scent of roses. In flowers the balance between both compounds is responsible for attracting the appropriate pollinating insects (Tieman *et al.* 2007). Hence the dehydrogenase activities of the 2-phenylacetaldehyde reductases were also investigated. The enzymes isolated from 'Hoh-Jun' showed 10 times higher reductase- than dehydrogenase-activity (Sakai *et al*. 2007). Furthermore, for the enzyme isolated from tomato, a reverse reaction with 2-phenylethanol was not detected (Tieman *et al.* 2007). All reductases showed the highest catalytic activity towards 2-phenylacetaldehyde as substrate, but differed in the activity using other substrates. For example, while the recombinant enzymes based on LePAR1 and LePAR2 could reduce benzaldehyde and cinamylaldehyde, the enzyme from 'Hoh-Jun' could not (Sakai *et al*. 2007; Tieman *et al.* 2007). The broad range of substrates tested for the enzymes from rose flowers suggests that the enzymic affinity is affected by the stereo structure of the substrates, for example in the C-6 position (Sakai *et al*. 2007).

1,3,5-Trimethoxybenzene and 3,5-dimethoxytoluene

1,3,5-Trimethoxybenzene and 3,5-dimethoxytoluene (**Fig. 4**) are the key scent compounds of tea-scented modern roses and ancient Chinese roses. 3,5-Dimethoxytoluene is one of the major volatiles emited by *R. hybrida* 'Grand Mogul', 'Lady Hillingdon', and 'Diorama' with 73, 61 and 26% of the total volatiles detected in the head space, respectively (Joichi *et al.* 2005). 1,3,5-Trimethoxybenzene is with 60% one of the major scent compounds emitted from *R. chinensis* var. *spontanea* Rehder & Wilson (Joichi *et al.* 2005). *O*-Methyltransferases (OMTs) were found to be responsible for the formation of these two scent compounds. Plant OMTs play an important role in secondary metabolism. They are responsible for the methylation of hydroxyl and carboxyl moieties. Although all OMTs transfer methyl groups, they differ in structure and substrate specificity. The first group is solely involved in the methylation of phenylpropanoid-based compounds such as isoflavones. The second group is found in lignin producing plants and methylates, for example, the phenylpropanoidesters of coenzyme A. The third class is responsible for the methylation of carboxyl groups of small molecules like phenolic compounds (Effmert *et al*. 2005).

Lavid *et al.* (2002) and Scalliet *et al*. (2002) isolated OMTs involved in the biosynthesis of 3,5-dimethoxytoluene and 1,3,5-trimethoxytoluene in roses (*R. hybrida* 'TANellis', syn. 'Fragrant Cloud'; *R. hybrida* 'Golden Gate'; 'Lady Hillingdon'; and *R. chinensis* Jacquin 'Old Blush') and characterized their function by recombinant enzymes. The cell free extract of *R. damascena* 'Summer Damask' showed no OMT activities as could be expected from roses of European origin. The recombinant orcinol *O*-methyltransferases (OOMTs) mainly catalyze the reaction from orcinol via 3-methoxy-5-hydroxytoluene to 3,5-dimethoxytoluene (**Fig. 4A**), but also react with the intermediate 3,5-dihydroxyanisole of the reaction pathway from phloroglucinol to 1,3,5-trimethoxybenzene (**Fig. 4B**). The purified OOMTs showed the highest affinity towards orcinol. The activities of the cell free extracts and the recombinant enzymes differed in their relative activities against various substrates. The activities of the cell free extracts towards the substrate phloroglucinol were higher than the ones determined with recombinant enzymes. Another kind of OMTs, namely caffeic acid *O*-methyltransferase (COMT) isolated from 'Old Blush', showed high affinity towards caffeic acid (Scalliet *et al.* 2002). Furthermore, three novel homologue *R. chinensis* *O*-methyltransferases (RcOMT1, RcOMT2, and RcOMT3) were isolated from *R. chinensis* var. *spontanea*, a

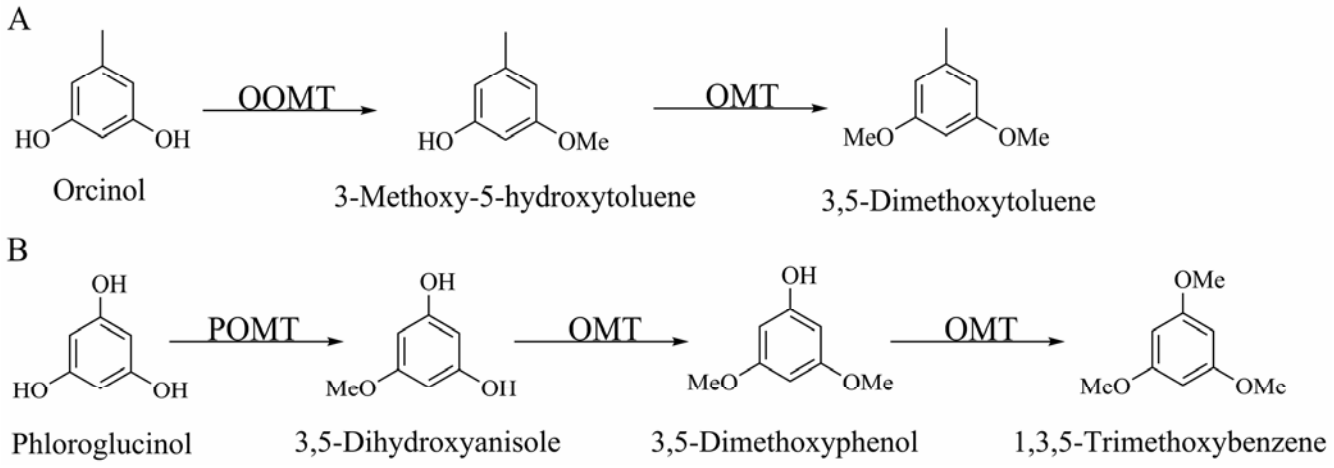

Fig. 4 Pathways of the formation of 3,5-dimethoxy toluene (A) and 1,3,5-trimethoxy benzene (B) in rose flowers.

variety also known for releasing high amounts of phenolic methyl ethers (Wu *et al.* 2003). These three recombinant enzymes showed sequence identity of 33 to 49% in the C-terminal region, but the OOMTs mentioned before cluster into another clade. The RcOMT1 is a highly substrate specific enzyme. The highest activity was found against the two substrates eugenol (**Fig. 2B**) and isoeugenol, which carry the hydroxyl group in para position. In contrast, RcOMT2 showed affinity towards a broad range of phenolic substrates. Even though the preferred substrates were those having a hydroxyl function in meta position, the enzyme can also react with compounds carrying the hydroxyl function in para position. The enzyme could catalyze the methylation of 3,5-dihydroxyanisole, but showed relatively low activity against phloroglucinol and 3,5-dimethoxyphenol (**Fig. 4B**). RcOMT3 showed weak activities towards caffeic acid and catechol derivates. Its physiological function still has to be elucidated. Even after the identification of the OMT mentioned above, the formation of 1,3,5-trimethoxybenzene from phloroglucinol cannot solely be explained by the action of these enzymes. Additionally, the cell free extracts of the rose petals of *R. chinensis* var. *spontanea* and, as discussed earlier, of 'Fragrant Cloud', 'Golden Gate', 'Lady Hillingdon', and 'Old Blush' showed different affinities against the tested substrates. These results suggested the presence of even more OMTs in roses. Wu *et al*. (2004) isolated and purified the first phloroglucinol-*O*-methyltransferase (POMT) from *R. chinensis* var. *spontanea.* This enzyme is involved in the initial step of 1,3,5-trimethoxybenzene formation from phloroglucinol to 3,5-dihydroxyanisole (**Fig. 4B**). With the identification of this POMT, enzymes carrying out each reaction step from phloroglucinol to 1,3,5-trimethoxybenzene were characterized.

Until now, more than 80 genes of OMTs in roses are known, but the number of OMTs identified in various rose species differs dramatically. One gene, for example, is known in *R. phoenicia* Boissier, whereas 8 genes have been identified in *R. chinensis* var. *spontanea.* Recently, Scalliet *et al.* (2008) classified the enzymes involved in the formation of 3,5-dimethoxytoluene into the two families, OOMT1 and OOMT2 (**Fig. 4A**), which are catalyzing the reaction of orcinol to 3-methoxy-5-hydroxytoluene and of 3-methoxy-5-hydroxytoluene to 3,5-dimethoxy toluene, respectively. With over 96.5%, the nucleotide identity of these two genes is high, but the specificity of the related enzymes clearly differs in promoting either the first or second methylation reaction. Molecular modelling and the biochemical activity assays of the enzyme mutants proved that a single amino acid variation (Tyr-127 in OOMT1 or Phe-126 in OOMT2) is responsible for the changing specificities of the active sites (Scalliet *et al.* 2008).

Additionally, the formation of 3,5-dimethoxytoluene was investigated by studies on the expression levels of proteins (OOMT) related to scent formation. The usage of anti-OOMT antibodies revealed that OOMT proteins are present in the stamina of Eurasian roses, but the majority of the examined species did not accumulate them in the petals (species of the sections of the genus *Rosa*: *Banksianae*, *Bracteatae*, *Caninae*, *Gallicanae*, *Rosa*, *Cinnamomeae*, and *Synstylae* were investigated). In contrast, OOMT proteins are accumulated in high levels in petals of Chinese roses such as 'Old Blush', *R. chinensis* var. *spontanea*, *R. gigantea* Collett ex Crépin, *R. odorata* Sweet 'Hume's Blush Tea-Scented China', *R. odorata* 'Park's Yellow Tea-Scented China', indicating that formation of phenolic methyl ethers in Chinese and European roses correlates with OOMT gene expression in petals (Scalliet *et al.* 2008).

Although significant progress has been made in the understanding of OMTs, more detailed investigations, especially regarding their substrate specificity, are essential for a complete elucidation of the function of OMTs in rose scent biosynthesis.

VOLATILE COMPOUNDS DERIVED FROM FATTY ACIDS

In roses of the *R. damascena* group, fatty acid derivatives with "green flavour" like *trans*-2-hexanal and *cis*-3-hexanol are prominent compounds in the leaves and sepals. In the petals the main compounds derived from fatty acids are aliphatic hydrocarbons such as nonadecane or nonadecene (Craissard *et al.* 2006) (**Fig. 2D**). An important class of enzymes involved in the formation of fatty acid derived volatiles are lipoxygenases (LOX), namely P450 enzymes like 9-LOX and 13-LOX. The enzymic oxidation for example of linoleic acid (18:3) by 9-LOX leads to hexanal. Furthermore, the cleavage of linoleic acid at the 12-13 double bond leads to the C12 precursor of jasmonic acid (Dudareva *et al.* 2004). Recent studies revealed that its methylated form, the volatile methyljasmonate, affects the carotenoid content in the yellow rose cultivar *R. hybrida* 'Frisca'. Increased methyl jasmonate levels caused a delayed carotenoid degradation (Glick *et al*. 2007).

In addition to the glycoconjugated forms of volatiles, lipophilic non-volatile terpenyl fatty acyl esters provide stable storage forms of the corresponding alcohols within the epicuticular wax layer. These esters are mainly based on the acyclic monoterpene alcohol geraniol, coupled primarily to fatty acids of chain lengths 16-20. In terms of total mass they represent 14 to 64% of the monoterpenes present in petals (Dunphy 2006). Dunphy proposed in 2006 that in *R. hybrida* 'Lady Seton' flowers the glycosides may principally act as reservoirs of the phenylpropanoids, while the fatty acyl esters could primarily be a source of the terpenols.

FORMATION OF VOLATILE COMPOUNDS FROM CAROTENOIDS

Carotenoids contribute to the yellow colour of flower petals and are important precursors for volatile norisoprenoids with very low odour thresholds. The odour thresholds of damascenone and β-ionone (**Fig. 2C**) are 0.009 and 0.007 µg/kg in water, respectively (Ohloff *et al.* 1990). Although the amounts of damascenone (0.14 %) and β-ionone (0.3%), compared with terpenoids like (-)-citronellol (38%), are low, they strongly affect the scent. In the essential oil of *R. damascena*, damascenone (**Fig. 2C**), β-ionone (**Fig. 2C**), and the rose oxides (**Fig. 2A**) contribute more than 90% to the total aroma impression (Ohloff *et al.* 1990). Hence, trace amounts already have a strong effect on the odour. Formation pathways of volatile norisoprenoids by chemical oxidation, photooxidation and co-oxidation with fatty acids are well established. Up to now, the bio-formation of damascenone could not be completely elucidated, but its chemical formation from neoxanthin is well confirmed (Isoe *et al.* 1973; Bezman *et al.* 2005). Furthermore, an immediate precursor of damascenone has been isolated from rose flowers and two glycopyranosides have been identified as progenitors of damascenone (Straubinger *et al.* 1997; Suzuki *et al.* 2002a).

Carotenoid cleavage enzymes were suggested to be involved in the norisoprenoid formation in yellow and orange flowers, which are rich in carotenoid derived aroma compounds (Kaiser and Kraft 2001). So far more than 10 carotenoid cleavage dioxygenase 1 (CCD1) homologues from various plants (*Arabidopsis thaliana* (L.) Heynh., *Bixa orellana* L., *Citrus limon* (L.) Burm. f., *Citrus x sinensis* (L.) Osbeck, *Citrus unshiu* Marc., *Coffea Arabica* L., *Coffea canephora* Pierre ex Froehner, *Crocus sativus* L., *Cucumis melo* L., *Lycopersicon esculentum* Mill., *Petunia x hybrida*, *Vitis vinifera* L.) are known and further enzymes were isolated from the tissues of *Averrhoa carambola* L., *Camellia sinensis* (L.) Kuntze, *Cydonia oblonga* Mill., and *Prunus persica* (L.) Batsch (Schwartz *et al.* 2001; Fleischmann *et al.* 2002; Bouvier *et al.* 2003a, 2003b; Fleischmann *et al.* 2003; Simkin *et al.* 2004a, 2004b; Baldermann *et al.* 2005; Fleischmann *et al.* 2005; Mathieu *et al.* 2005; Ibdah *et al.* 2006; Kato *et al.* 2006; Simkin *et al.* 2008). These enzymes

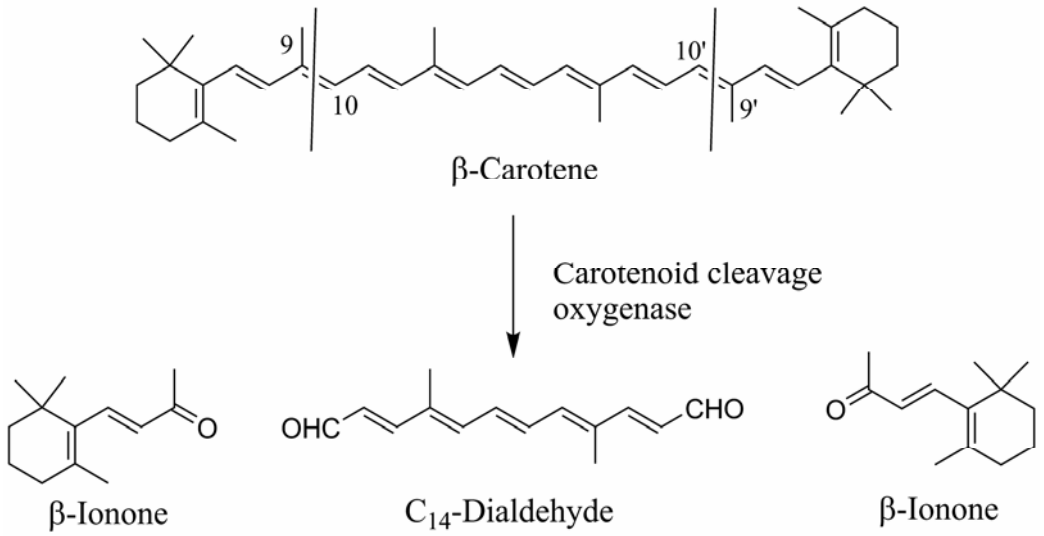

Fig. 5 Primary cleavage reaction by carotenoid cleavage enzymes with the substrate β-carotene leads to the primary reaction products β-ionone and C_{14}-dialdehyde.

exhibit the direct formation of C_{13} norisoprenoids from carotenoids (**Fig. 5**; Huang *et al*. 2009). In *R. damascena*, Suzuki *et al*. (2002b) confirmed the direct cleavage of β-carotene and neoxanthin using a crude enzyme extract. Recently we investigated *R. hybrida* 'Pareo', a yellow tea-rose hybrid, and could prove the presence and action of carotenases in an enzyme extract obtained of the flower petals (Baldermann *et al*. 2008).

Besides the isolation of active enzymes from rose petals, the existence of two genes encoding carotenoid cleavage dioxygenase 1 (RdCCD1) and carotenoid cleavage dioxygenases 4 (RdCCD4) were recently proved in *R. damascena* (Huang *et al*. 2007a, 2007b; http://beta.uniprot.org/uniprot/A9Z0V7; http://beta.uniprot.org/uniprot/B0FLM8). With 552 amino acids (AA) and a calculated molecular size of 62.4 kDa, RdCCD1 is similar in size compared with other recombinant CCD1 enzymes, i.e. *Arabidopsis* (AtCCD1, 501 AA, 56.9 kDa), *Bixa orellana* (BoCCD1 544 AA, 60.8 kDa), *Crocus* (CsCCD1 546 AA, 61.7 kDa), and *Petunia* (PhCCD1 546 AA, 61.3 kDa) (Schwartz *et al*. 2001; Bouvier *et al*. 2003a, 2003b; Simkin *et al*. 2004b). The function of the CCD4 family is still not completely understood, however Ohmiya *et al*. (2006) could show that the CmCCD4a genes obtained from *Chrysanthemum morifolium* Ramat. flower petals contribute to the development of white flower colours.

Despite recent studies in roses offering proof that the potent flavour class of norisoprenoids is formed by carotenoid cleavage enzymes, many interesting aspects still need to be clarified. For example, it is still unclear how norisoprenoid release is regulated.

GLYCOSIDICALLY BOUND VOLATILES AND THEIR RELEASE BY GLYCOSIDASES

Not all volatiles in plants immediately release from their storage- or formation tissues by active or passive transporttation as shown in **Fig. 1**. To allow better storage within the plant, many aroma compounds occur as glycosylated precursors in the plant. From a chemical point of view, glycosylated aroma compounds enhance water solubility and decrease reactivity compared with their free aglycone counterparts (Winterhalter and Skouroumounis 1997). **Fig. 6** summarizes our own work and present knowledge on glycosidically bound volatiles in *Rosa* flowers. In many cases, glycosylated volatiles are found in *Rosa* flowers as β-D-glucosides. The amount of glucoside (e.g. 2-phenylethyl β-D-glucoside) is generally higher in early stages of flower development and decreases after flower opening (Oka *et al*. 1999). In addition, glycosides like 2-phenylethyl β-D-glucoside are stored inside the petals and can act as the primary source of rhythmically emitted volatiles such as 2-phenylethanol, being released by the action of the β-D-glucosidase throughout the photoperiod. They enter the pool of free volatiles prior to emission (Picone *et al*. 2004). However, the relative importance of the glucoside concentration in rhythmic flavour emission remains to be determined.

These glycoconjugated aroma compounds can be hydrolyzed by endogenous glycosidases (e.g. β-glucosidase and β-primeverosidase) to liberate the volatile compounds from the plant. Investigations in *R. damascena* indicated that 2-phenylethanol is released from its precursor 2-phenylethyl β-D-glucoside during or after flower opening (Watanabe *et al*. 2001). During flower development in *R. damascena*, the β-glucosidase activity increases 5 times, whereas β-galactosidase activity does not change remarkably and remains constant at a high level. In contrast, β-xylosidase, β-primeverosidase, and α-arabinosidase showed lower activities (Oka *et al*. 1999). β-Glucosidase was suggested to be partly responsible for controlling the diurnal emission of 2-phe-nylethanol in *R. damascena* (Hayashi *et al*. 2004). The partially purified β-glucosidase from 'Hoh-Jun' petals possesses high affinity for 2-phenylethyl β-D-glucopyranoside, (*S*)-citronellyl β-D-glucopyranoside, and *Z*-3-hexenyl β-D-glucopyranoside, moderate affinity for *p*-nitrophenyl β-D-glucopyranoside, and weak affinity for *p*-nitrophenyl β-D-galactopyranoside and 2-phenylethyl β-D-galactopyranoside. Furthermore, this β-glucosidase consists of two proteins (160 and 155 kDa) and is classified as glycoside hydrolase of family 1, which comprises many glucosidases involved in the hydrolysis of the glycosides of secondary metabolites in plants like *Prunus avium* (L.) L. or *Prunus serotina* Ehrh. (Sakai *et al*. 2008). The results discussed above suggest that β-glucosidases play an important role in the emission of the scent compounds from rose flowers. However, until now, no direct correlation of glucosidase activity and rhythmic cycles of volatile emission from rose flowers could be found.

REGULATION OF VOLATILE COMPOUND EMISSION

The substantial progress in understanding biosynthetic genes and enzymes involved in the formation of floral volatiles facilitates approaches to elucidate the regulation of their emission. Recent studies provide evidence that the rhythmic emission of floral scent is controlled by an endogenous circadian clock and is directly regulated by light. The influence of light on the regulation of rhythmic emission of volatile benzenoids in petunia flowers was demons-

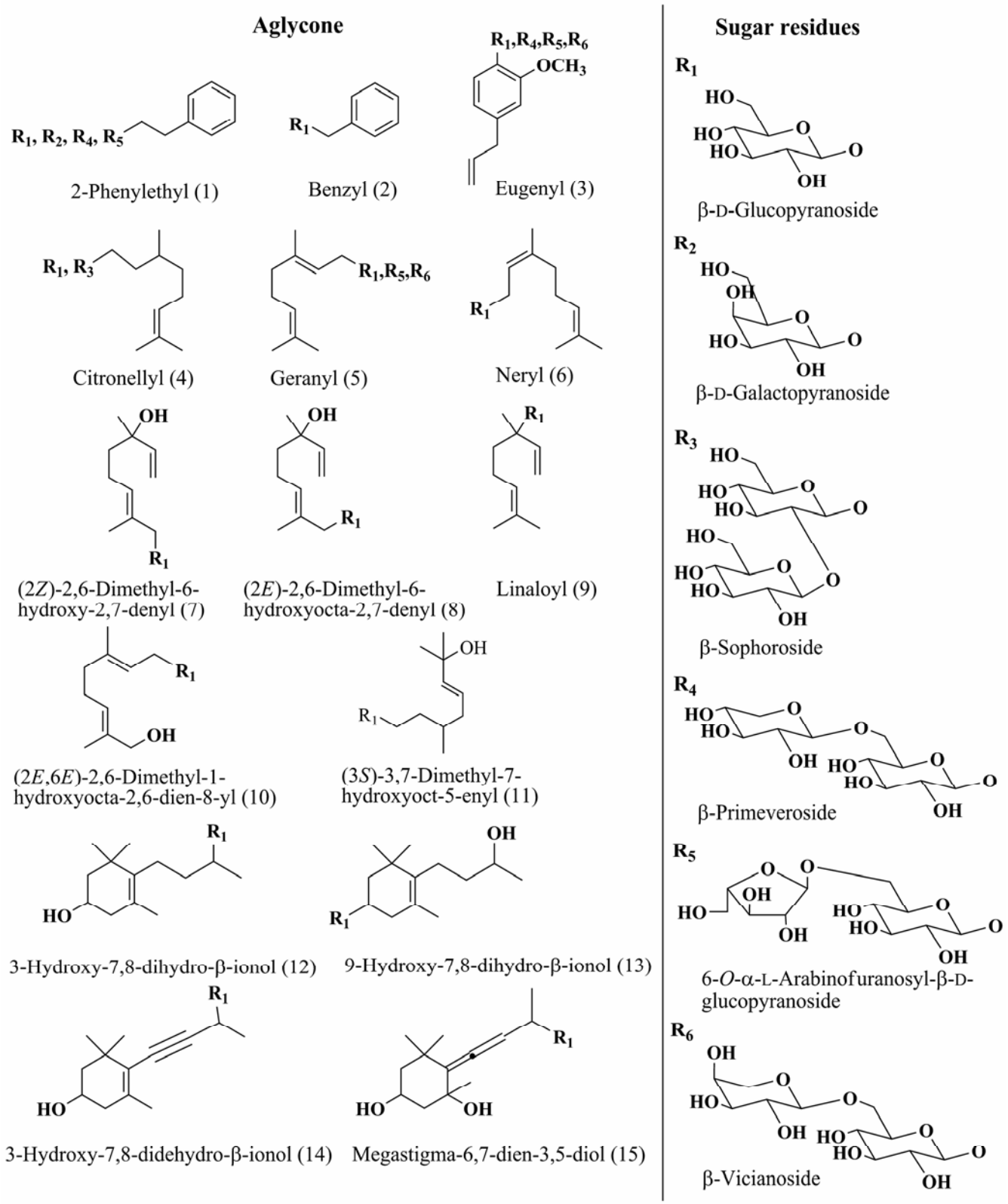

Fig. 6 Structures and occurrence of glycosidically bound volatiles in *Rosa* flowers. From *Rosa damascene* Mill., (**1**), (**3**), (**5**), (**11**), (**15**) (Oka *et al.* 1997; Watanabe *et al.* 1998; Straubinger *et al.* 1999; Watanabe 2001; Watanabe *et al.* 2001; Suzuki *et al.* 2002a); from *R. damascena* 'Trigintipetala', (**2**), (**4**), (**7**), (**8**), (**10**), (**12**), (**13**), (**14**) (Straubinger *et al.* 1997; Oka *et al.* 1998); from *R. hybrida* L. 'Lady Seton' (**5**), (**6**) (Francis and Allcock 1969); from *R. canina* L., (**9**) (Ackermann *et al.* 1989).

trated by Schuurink *et al.* in 2006. Nocturnally pollinated flowers generally tend to have a maximum of scent emission during the dark period, while for flowers pollinated during the daytime, the situation is reversed (Matile and Altenburger 1988; Loughrin *et al.* 1990). Wild species of the genus *Rosa* are pollinated by bees, a day pollinator. Investigations in *R. damascena* and *R. hybrida* revealed that its scent emission has a maximum during the light period (Helsper *et al.* 1998; Picone *et al.* 2004). The rhythmic emission of rose scent was observed not only when 12 h photoperiods were applied to the plants, but also in the absence of environmental cues (under continuous darkness or continuous light), indicating the regulation by an endogenous circadian clock (Picone *et al.* 2004). However, the release of several volatiles such as oxidized monoterpenols, *trans*-caryophyllene, dihydro-β-ionone, geranyl acetate, and germacrence D in hybrid rose flowers is regulated directly by light and for most scent compounds are synchronized

(Helsper *et al.* 1998; Hendel-Rahmanim *et al.* 2007). It is not yet known why some volatiles are rhythmically released and others are not, nor why some scent compounds are released under the control of light while others are emitted under the control of endogenous circadian mechanisms.

The detailed time-course analysis during the daily light/dark cycle of geranyl acetate and its biosynthetic gene, alcohol acetyl transferase (*RhAAT*) in hybrid rose flowers revealed similar daily fluctuations of the endogenous geranyl acetate level and *RhAAT* expression (Hendel-Rahmanim *et al.* 2007). It is noteworthy that the rhythmic expression of *RhAAT* continued in continuous darkness or continuous light, while accumulation and emission of geranyl acetate were inhibited under continuous light conditions. These results suggest that rhythmic emission of geranyl acetate may be regulated at the level of its substrate geraniol, which is suppressed in continuous light (Hendel-Rahmanim *et al.* 2007). Therefore, it can be speculated that in some cases, the availability of substrates for the final step of volatile formation in roses determines the efficiency of their emission, especially when the enzymes responsible for the final reaction process have a broad substrate specificity pattern (Dudareva and Pichersky 2006; Hendel-Rahmanim *et al.* 2007).

In addition to the regulation by the endogenous circadian clock and light, several chemical signals and metabolic processes, i.e. plant hormones, ethylene, carbohydrate- and cell wall metabolism, are thought to be involved in the scent emission of roses (reviewed by Kumar *et al.* 2008).

The results discussed above and the regulatory mechanisms remain to be completely elucidated, and suggest complex options for the control of scent emission by rose flowers.

EVOLUTION OF THE SCENT COMPOUNDS

In many taxa, scented species are closely related to unscented ones. In the case of rose scent compounds, originally only emitted by Chinese Roses or by European Roses, they are now often found in a single modern variety due to extensive breeding. With the identification of genes involved in the biosynthesis of rose scent, new pathways of scent evolution can be investigated. One example is the scent formation in Chinese roses, where based on the results obtained by molecular modelling of *O*-methyltranfsferases and site-directed mutagenesis, the biosynthesis of 3,5-dimethoxytoluene in Chinese roses was analyzed (Scalliet *et al.* 2008).

CONCLUDING REMARKS AND FUTURE RESEARCH

Recently, knowledge about metabolic scent formation pathways in rose flowers dramatically increased, however there are important issues yet to be investigated. Data about transportation, storage, and emission of volatile compounds, for example, are still very limited. The understanding of biosynthesis pathways including localization of scent formation will give access to the production of scent-optimized plants by metabolic engineering. An enormous economical potential can be realized, because plant volatiles are not only useful fragrances for us, but also beneficial because they have antimicrobial, antioxidative, and anticarcinogenic activities (Goff *et al.* 2006).

Moreover, not all evolutionary processes can be explained by modern analytical methods, like the deficiencies in 1,3,5-trimethoxytoluene synthesis by modern hybrids derived from *R. chinensis* still needs to be determined (Lavid *et al.* 2002; Wu *et al.* 2004). Therefore, it will be necessary to characterize the enzymes involved in the scent formation with respect to kinetic aspects and substrate specificity. The importance of activation and inactivation processes on the transcriptional, posttranscriptional, translational, or post translational level will be elucidated. The knowledge about those regulatory factors will lead to a more complete understanding of chemical signalling in roses and will therefore improve the chances to successfully breed new fragrant varieties.

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