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# Rose floral scent

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## Abstract

**The rose is the queen of flowers and is widely used as a garden plant and for the cut flower market. Roses are also used for the production of essential oil for the cosmetic and perfume industries. A lot of botanical roses are scented and use their volatiles to attract pollinators. Fragrances in garden roses are very diverse and scent has always been an important character in the selection process. Breeders have recently tried to introduce new fragrances, for instance reminiscent of fruit or spice odors. But despite their efforts, some roses on the market are not very fragrant, especially the ones bred for the cut flower market. The cause of this lack of scent is not known. In modern roses or Hybrid Tea roses, scent is mainly produced by petals, although stamens can also contribute to the emission. Scent compounds are concentrated in rose petal epidermis, probably in small lipid droplets. In spite of numerous chemical studies, the biosynthetic pathways of many rose scent compounds are unknown. We are studying several genes involved in the biosynthesis of scent in rose. For example, we recently characterized the enzymes responsible for the so-called “tea scent” emitted by Chinese roses. Apart from “tea scent”, terpenoids, especially monoterpenes, are also major constituents of rose flowers, mostly responsible for the “typical rose scent”. Generally, terpenoids biosynthesis in plants is achieved by various terpene synthases. However, with a combination of transcriptomic and genetic approaches, our group recently discovered a terpene synthase-independent pathway. A key enzyme of this pathway is RhNUDX1, belonging to the Nudix protein family. It has geranyl diphosphate diphosphohydrolase activity *in vitro*. A positive correlation was found between the expression levels of RhNUDX1 and the production of the monoterpene geraniol, indicating the essential role of this protein in scent production in roses.**

**Keywords:** terpenoids, phenylpropanoids, specialized metabolism, nudix hydrolase

## INTRODUCTION

Roses have been cultivated for centuries for a range of different purposes and their fragrance enchants man since the Greek antiquity. Roses are widely used as garden plants and for the cut flower market. Essential oil and solvent extracts (called concrete and absolute) from rose petals, contain hundreds of volatile molecules and are produced for the cosmetic and perfume industries (Lawrence, 1997; Liu et al., 2015). Total production of rose oil and concrete is around 10 tons a year and its price is above 5000 €/kg but can be much higher. The quality of ornamental roses depends on several major traits such as color and flower shape, resistance to pathogens and long vase life for cut flower roses. One character that is being taken increasingly into account is scent, for which there is a clear demand from consumers worldwide. The scents of roses in our gardens originate from those of wild roses, which are their ancestors. The genus *Rosa* has about 150 species distributed in the Northern Hemisphere. The thousands of rose cultivars come from a long process of hybridization, involving a dozen of wild roses as well as domesticated species of European and Middle Eastern origins (*R. gallica*, *R. phoenicia* and *R. moschata*, for example), and Chinese species (*R. chinensis* and *R. gigantea*). During the breeding of modern roses, scent features of wild roses were mixed and combined to achieve an extraordinary range of scent diversity, which sometimes did not even exist in wild roses. Since ancient times, gardeners and breeders have therefore exploited the natural variability of the scent of roses by recovering and acclimatizing spontaneous mutants. The presence of particular fragrances also emerged by chance, along with the intentional selection of other characters. In modern roses or Hybrid Tea group, perfumes sometimes deviate from what is considered as the “typical rose scent”. Breeders are actively working to find original fragrances in cultivars like 'Paul Ricard' (anise scent), 'Constance Spry' (myrrh scent), 'Gloire de Dijon' (“tea scent”), 'Prestige de Lyon' (raspberry) and 'Souvenir de Marcel Proust' (lemongrass), among others. Also, given the value of rose essential oil, the perfume industry would be very interested in high-yield cultivars with valuable scent profiles (Rusanov et al., 2009). However, rose breeders find it difficult to select scent traits and not all roses arriving in the market nowadays are heavily scented, despite their efforts. In particular, roses bred for cut flowers often lack scent and the cause of this lack is not fully understood. Some authors assume the existence of a genetic link between lack of scent and some agronomic traits such as resistance to Botrytis or to black spot disease, and vase life. However, an anise scent is present in some varieties for cut flowers. Moreover, recent experiments failed to demonstrate such relationship between the amounts of volatile compounds emitted and vase life, when fragrant and non-fragrant rose cultivars were compared (Borda et al., 2010). The cause of the absence of typical rose scent in these varieties remains to be determined. Several genes encoding enzymes involved in the biosynthesis of rose scent have been identified in the past few years, shedding light on the genetic determinism of this major trait.

## PRODUCTION OF SCENT IN THE ROSE FLOWER: WHY, WHEN AND WHERE

### **Scent compounds are emitted throughout development of the flower and are used for pollination and defense**

In most plants, odors are used to communicate and are crucial for reproduction and survival. Pollination often requires the help of insects and scent is widely used by plants to attract their pollinators. It may indicate to insects the presence of food, nectar or pollen, or even a breeding site. As other plants, roses emit scent to seduce insects. In the wild, botanical roses are pollinated by insects like bees and bumblebees, which are attracted by petal and pollen scents (Dobson et al., 1999). Research on olfactory memories in bees has shown that they perceive and recognize different rose scent compounds, which allow them to identify the plant. However, insect pests may also be attracted to certain scent compounds (Kessler et al., 2015). This is the case of the *Hoplia communis* scarabs attracted by the smell of 2-phenylethanol (2-PE), a compound which is very often present in roses (Imai et al., 1998). Thus, the fragrance of roses indicates their presence to insect pollinators, but also to undesirable hosts like pests. Our research, and those of other laboratories, showed that the emission of scent varies during the development of the flower (Bergougnoux et al., 2007). Generally, the amount of volatile compounds increases as the flower

blooms and then decreases when the flower approaches senescence. This phenomenon is related to the biological role of the fragrance: the flower emits its attractive scent when it is ready to be pollinated and decreases its production when it is no longer necessary. Researches have also shown that there is a diurnal oscillation of scent emission with a peak during the day, when the flower is fully opened and ready to be pollinated (Helsper et al., 1998; Picone et al., 2004). The rhythmic emission of most rose-flower volatiles is under regulation of the endogenous circadian clock. However, some results demonstrate the complexity of this diurnal regulation: although the daily emission of most scent compounds is synchronized, various independently evolved mechanisms control the production, accumulation and release of different volatiles (Hendel-Rahmanim et al., 2007).

### **The chemistry behind rose fragrance**

Like all fragrances, those of the roses are due to volatile chemicals which, dispersed in the air we inhale, excite the olfactory nerve cells in the upper part of our nasal cavity. The excitation of olfactory neurons by these odor molecules generates nerve signals transmitted to the olfactory cortex and then to different areas of the brain to give rise to feelings associated with the perception of a smell. The scent of roses is extraordinarily complex, with hundreds of different molecules in some species and varieties. For over 30 years, these molecules have been isolated, purified and studied by chemists. The techniques that capture the fragrance of roses are somewhat different from those used to extract essential oil by perfumers. Scent compounds are mainly recovered by the technique of headspace. In this technique, a pump sends a cleaned air flow into an enclosed chamber containing a rose flower. The air is loaded with the floral volatile molecules and then filtered on a resin capable of retaining these molecules. The resin is then washed with an organic solvent which is analyzed by gas chromatography coupled to a mass spectrometer. There are other semi-quantitative techniques without air flow, for example the Solid Phase Micro Extraction (Tholl et al., 2006).

The emitted molecules may be organized into several main families associated with their biosynthesis pathways: terpenoids, phenylpropanoids and benzenoids and fatty acid derivatives.

Terpenoids constitute a class of very important fragrant molecules, responsible for the scent of many flowers and aromatic plants (Tholl, 2015). In many roses, terpenoids represent the most abundant scent compounds, in particular the monoterpene alcohols like geraniol, citronellol, nerol and their derivatives. These terpenoids are generally responsible for the floral and fruity notes typical of the perfume of roses. In addition to these major terpenoids, trace compounds, like the norisoprenoid ionones, also make a great contribution to the scent of these roses because of their low odor threshold in humans (Ohloff, 1978).

Phenylpropanoids and benzenoids, which have a benzene ring in their skeleton, are found in many flower odors (Knudsen et al., 2006). Some of these compounds are major constituents of the perfume of roses, such as 2-PE, with the typical odor of rose water. Other more minor compounds such as eugenol, with a pronounced odor of clove, add spicy notes to the fragrance of certain varieties. These compounds are derived from aromatic amino acids. Despite their benzene skeleton, phenolic methyl ethers are not derived from aromatic amino acids, thus can be classified in a specific family of biosynthetic compounds. Phenolic methyl ethers, such as 3,5-dimethoxytoluene (DMT), are responsible for the characteristic "Tea scent" scent of some rose cultivars.

Finally, the fatty acid derivatives or oxylipins, small molecules resulting from the degradation of the long carbon chains of the lipids (Hassan ul et al., 2015), are responsible for the herbaceous notes present in the scent of roses. This is the case for (Z)-3-hexenol, with the characteristic odor of freshly cut grass.

Among the major compounds of the scent of roses, none is really specific to this flower. For example, geraniol is found in the fragrance of many flowers and eugenol in cloves. However, the combination of these different molecules gives the roses their characteristic fragrances. It is, for example, possible to evoke a typical odor of rose with geraniol, 2-PE or  $\beta$ -damascenone. However, humans cannot smell the various volatile compounds with the same sensitivity. For instance, a trained nose is able to perceive the odor of  $\beta$ -damascenone with a very low threshold of 0.009  $\mu\text{g}$

per liter of water. The odor threshold of geraniol, for example, is much higher (75 µg per liter) (Ohloff, 1978).

### **Petals are the main scent producing organs**

Different rose floral organs (sepals, petals and stamens) are involved in the biosynthesis and emission of scent. Sepals produce numerous volatile defense compounds in secretory glands or trichomes distributed over the epidermis. These glands are particularly abundant in the so-called moss roses. In a previous study, we showed that the moss resulted from repetitive development of trichomes on pre-existing trichomes, which contained many terpenoids, very different from those present in the petals of the same cultivars (Caissard et al., 2006). These secretions, to which insects attacking the flower bud are glued, are very rich in cyclic monoterpenes, therefore giving a resinous note to the scent.

Pollen grains in the stamens produce volatile compounds attracting pollinating insects (Dobson et al., 1990). They sometimes contain compounds different from the ones emitted by the rest of the flower like eugenol (Dubois et al., 2010).

Finally, petals of most of the fragrant varieties produce major compounds such as 2-PE and geraniol. Thus, in the same flower, the sepals secrete compounds that repel insects when the flower is closed. When the flower is blooming, the petals take over and secrete other scent molecules of the same family, which attract pollinating insects. In modern cultivars, the mass of the petals (more than 200 petals for certain varieties) is such that they are responsible for producing most of the scent molecules.

These petals have a simple structure composed of three tissues: a parenchymatous tissue delimited by an upper epidermis with conical cells and a lower epidermis with flat cells. Using two different techniques, solid/liquid phase extraction and headspace collection of volatiles, we showed that, in roses, both epidermal layers are capable of producing and emitting scent volatiles, despite the different morphologies of the cells of these two tissues (Bergougnoux et al., 2007). An objective of the research is to understand how the scent compounds, synthesized in the petal, are transported outside. These processes are very poorly understood and it is not known with certainty in which cell organelles the scent molecules are found. In *Petunia*, it was recently shown that an ABC transporter was facilitating the emission of volatile compounds (Adebesin et al., 2017). We also identified lipid vesicles containing terpenes in the cells of the rose petals. These vesicles could be used for storage or transport of volatile compounds (Bergougnoux et al., 2007).

### **USE OF ROSE SCENT FOR THE PRODUCTION OF ESSENTIAL OIL**

Roses are used for their perfumes since antiquity. Romans, for example, were great rose lovers, which they used to perfume the air from their thermal baths. The distillation of rose flowers for essential oil production probably started in the late sixteenth and early seventeenth centuries, perhaps independently in Europe and the Middle East. Today, world production of rose essential oil and concrete is estimated at around 15 tons per year, of which about 5 tons of essential oil. Turkey and Bulgaria are the two main producers (80% of total production). Although the quantities produced are smaller, Iran, China, Egypt, France, India, and Morocco are also major contributors in this perfume sector.

The damask rose (*R. x damascena*) is the most used species for the production of essential oil. It is not a botanical species, but a garden variety, probably cultivated for a long time and including many forms and cultivars. Its culture in Bulgaria for the production of essential oil was well established in the Kazanlik region at the end of the seventeenth century. This place gave the name to the famous cultivar used today for the production of one of the best essential oils: *R. x damascena* cv 'Kazanlik'. *R. x damascena* is not the only species cultivated for the perfume industry. *R. x centifolia* is another variety grown for an industrial purpose. In France, a few acres of *R. x centifolia* hybrids cultivars (rose of May or rose of Grasse) remain in Grasse for the industry of luxury perfumes. However, since its yield with the hydrodistillation technique is poor, it is used to obtain a concrete by solvent extraction, mainly in France and Morocco. It is a complex horticultural hybrid of poorly known origin, with several botanical ancestors including *R. gallica*, *R. canina* and others. Many other rose cultivars have been evaluated for the production of essential

oil, with varying degrees of success. Currently, several varieties, for example hybrids between *R. rugosa* and *R. sertata*, are grown in China for the production of essential oil and this country aims to become a major player in this industrial sector.

Nowadays, hydrodistillation is the most common process for obtaining essential oils. Petals are immersed in a large volume of water heated to 100 °C. The vapor emitted condenses after cooling in a coil. By decantation, a layer of rose water is obtained, surmounted by essential oil or essence or attar of rose. Production of 1 kg of distilled essential oil requires 3 to 5 tons of roses, the equivalent of about 200 million petals. Consequently, the essential oil price is quite high, from 5000 to 7000 €/kg depending on the sources. A field of *R. × damascena* produces about 1.5 tons of flowers per hectare from the third year of planting and then up to 5 tons/ha in subsequent years. The flowers are collected by hand, one by one, between 5 and 9 in the morning, when the concentration of scent is at its maximum. The second most common technique for extracting rose scent is solvent extraction. In this technique, the flowers, are washed several times with a solvent such as hexane. At the end of extraction, the solvent is evaporated by vacuum. Concrete, a semi-solid product, is obtained. Pigments and waxes are eliminated by treating the concrete with alcohol. The alcohol is cooled to 0-10 °C to precipitate the waxes and filtered. The alcohol is then evaporated under vacuum, which gives a highly concentrated and very expensive product, the rose absolute (1200 to 2000 €/kg). Approximately 400 kg of roses are extracted to obtain 1 kg of concrete and 0.5 kg of absolute.

The products obtained by the different methods are mostly used in perfumery and cosmetic industries. The rose has always been used in the creations of the perfumers. A recent example is Trésor, a Lancôme fragrance in which rose note dominates. More than 275 compounds have been identified in the bulgarian rose essential oil. The most abundant are monoterpene alcohols, such as geraniol, citronellol and nerol. The aromatic compound, 2-PE, with a typical rose odor, is mostly found in rose water. Other molecules are present in trace amounts, but have a very low olfactory detection threshold in humans, and give the rose essential oil a unique character. Some of these compounds with high value for the perfumer, such as  $\beta$ -damascenone, are not naturally synthesized by rose, but are degradation products that form during extraction (Huang et al., 2009). Other molecules are suspected of being allergenic, or even carcinogenic (methyleugenol) and could therefore be banned. Various methods are tested to remove them from the essential oil (Bouhleb et al., 2012). Today, the quality of the essential oil of *R. x damascena* must meet the standards AFNOR NF T 75-345: 1986 and ISO 9842: 2003. However, each of the producing countries presents different environmental conditions (humidity, soil, temperature) and cultivation practices (irrigation), which influence the chemical composition and yield of rose oils. In addition to these extremely important agronomic characteristics, a thorough knowledge of the plant and of the biosynthesis of scent compounds is now needed to improve rose essential oil yield.

## **BIOSYNTHESIS OF ROSE SCENT**

“Scentless” roses have not completely lost the ability to produce scent molecules, but emit very small quantities. The currently favored hypothesis to explain the absence of odor in these roses concerns the malfunctioning of biosynthetic pathways of volatile compounds. Since 2002, identifying enzymes responsible for the synthesis of the major scent compounds in rose has thus become a major goal of research. Pioneering research on *Clarkia breweri* and *Antirrhinum majus* has shown that scent production and emission is often regulated at the level of transcription (Dudareva and Pichersky, 2000). High-throughput transcriptomic approaches were thus used to identify genes involved in rose scent. EST databases representing the transcriptomes of petals from different rose varieties were established. The first approach used *R. chinensis* cv ‘Old Blush’, an ancient Chinese rose variety, which is also a progenitor of modern roses (Channeliere et al., 2002). The second approach used two modern rose varieties, *R. x hybrida* cv ‘Golden Gate’ and cv ‘Fragrant Cloud’, which differ in their scent characteristics (Guterman et al., 2002). Analysis of these petal transcriptome data allowed the identification of several genes potentially involved in scent production. Several other transcriptomic databases were later produced to study various roses traits (Dubois et al., 2012; Han et al., 2017; Kim et al., 2012; Koning-Boucoiran et al., 2015;

Yan et al., 2014). The first scent compounds that were investigated were the phenolic methyl ethers, responsible for the “tea scent” of some Chinese roses (Figure 1).

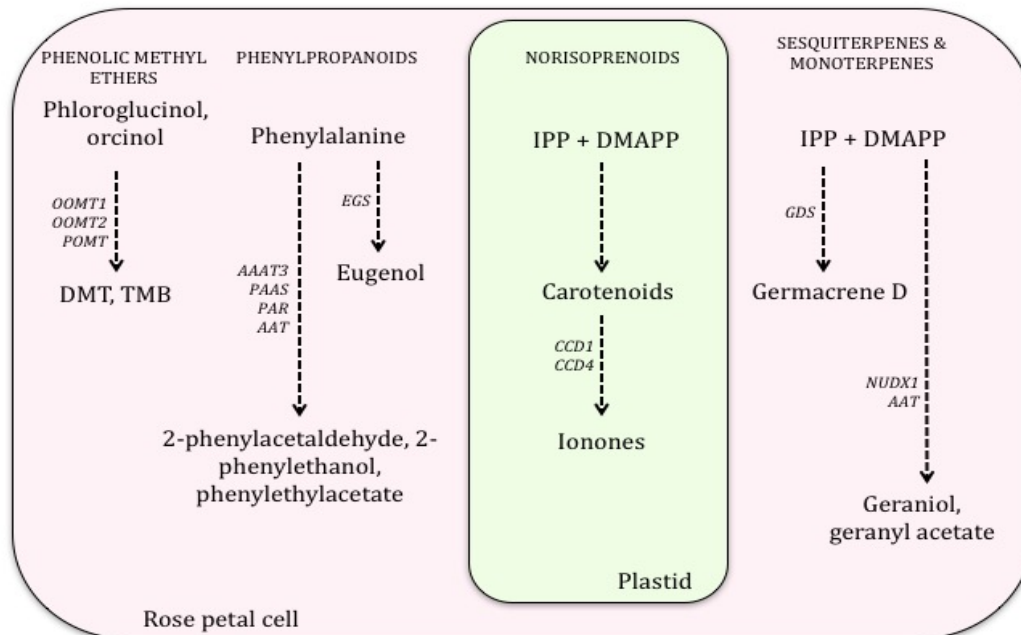


Figure 1. Main biosynthetic pathways and genes coding for enzymes characterized in rose. Dotted arrows indicate multiple steps. AAAT3, aromatic amino acid aminotransferase; AAT, alcohol acetyl transferase; CCD1 and CCD4, carotenoid cleavage (di-) oxygenases 1 and 4; DMT, 3,5-dimethoxytoluene; DMAPP, dimethylallyl diphosphate; EGS, Eugenol synthase; GDS, Germacrene D synthase; IPP, isopentenyl diphosphate; NUDX1, nudix hydrolase 1; OOMT1 and 2, orcinol-*O*-methyltransferases; PAAS, phenylacetaldehyde synthase; PAR, phenylacetaldehyde reductase; POMT, phloroglucinol-*O*-methyltransferase; TMB, 1,3,5-trimethoxybenzene.

### Phenolic methyl ethers

Among the roses that participated in the creation of modern roses, *R. chinensis* is of particular importance. In the 1860s, breeding efforts initiated by the arrival of these Chinese roses have produced the modern rose group named Hybrid Tea roses, possessing recurrent blooming. For some authors, their name comes from their light scent reminiscent of tea. For others, this name has nothing to do with scent and was used because the first Chinese roses came from the Cantonese nursery Fa -Tee. “Tea scent” is a very special sweet smell, which is only found in two wild species of Chinese roses (*R. chinensis* and *R. gigantea*). This unique scent is rich in DMT, which is absent from European roses. TMB is another compound belonging to the same biosynthetic pathway. By introducing the recurrent blooming character, breeders have also introduced this new scent. Most modern Hybrid Tea roses contain only small amounts of DMT, often mixed with compounds responsible for classical rose scent. When these compounds are lacking, the small amount of DMT is generally not perceived by the human nose and these roses are described as not scented. The genetic origin of this fragrance was recently deciphered. DMT is synthesized by two specific enzymes, orcinol-*O*-methyl transferases 1 and 2 (OOMT1 and OOMT2), that catalyze the methylations of orcinol as substrate (Lavid et al., 2002; Scalliet et al., 2002; Scalliet et al., 2006). TMB is synthesized by three successive methylations of phloroglucinol, the first step being catalyzed by a phloroglucinol-*O*-methyl transferase (POMT) (Wu et al., 2004). The next steps of TMB biosynthesis are probably catalyzed by OOMT1 and OOMT2. OOMT1 and OOMT2 are very similar enzymes. They exhibit different substrate specificities that are consistent with their operating sequentially in DMT biosynthesis. We have shown that these substrate specificities are

mostly due to a single amino acid polymorphism in the phenolic substrate binding site of OOMTs. An analysis of *OOMT* gene family in 18 species representing the diversity of the genus *Rosa* indicated that if all wild rose species have *OOMT2* genes, only Chinese roses also have *OOMT1* gene. In addition, we provided evidence that the Chinese-rose-specific *OOMT1* genes most probably evolved from an *OOMT2*-like gene that has homologues in the genomes of all extant roses. We proposed that the emergence of the *OOMT1* gene may have been a critical step in the evolution of scent production in Chinese roses (Scalliet et al., 2008). This gene and the “tea scent”, originally limited to two Chinese wild rose species, were thus transmitted during the process of creation of modern roses and are found today in thousands of cultivars present in gardens all over the world.

## **2-PE and other phenylpropanoids**

In terms of diversity, benzenoids and phenylpropanoids are considered the second largest class of plant volatiles after terpenoids (Dudareva et al., 2013). These compounds are mainly derived from L-phenylalanine (Phe). Among these, phenylacetaldehyde (PAlD) and 2-PE are important rose scent compounds. 2-PE is the major component of rose water and has a typical rosy scent. The genes and enzymes responsible for the biosynthesis of these phenylpropanoids have been identified and characterized in several plants. Interestingly, plants have developed different pathways to convert Phe to PAlD. In rose, two enzymes have been identified. A phenylacetaldehyde synthase (*RhPAAS*) isolated from *R. x hybrida* cv. ‘Fragrant Cloud’ (Kaminaga et al., 2006) is a bifunctional enzyme that catalyzes the efficient coupling of Phe decarboxylation to oxidation, generating phenylacetaldehyde. The second step is performed by a phenylacetaldehyde reductase (*RhPAR*) characterized in *R. x damascena* (Chen et al., 2011b). Surprisingly, another pathway leading to PAlD biosynthesis from Phe also exists in rose. In this alternative pathway, PAlD is first converted into phenylpyruvate by an aromatic amino acid aminotransferase (*RyAAAT3*), which then undergoes decarboxylation to form PAlD by a phenylpyruvic acid decarboxylase (Hirata et al., 2012; Hirata et al., 2016). This alternative pathway identified for the production of 2-PE seems to be activated in response to seasonal changes in temperature.

Other phenylpropanoid compounds are also present in the rose flower, like eugenol and methyleugenol. These phenylpropenes are important constituents of many plants in which they are supposed to play a role in pathogen defense, due to their antibacterial and antifungal effects. Some phenylpropenes are also floral attractants for pollinators (Koeduka, 2014). In some rose cultivars, they are present in high quantities in stamens and, to a lesser extent, in petals. Eugenol is synthesized from coniferyl acetate, by eugenol synthase. In rose, a putative eugenol synthase gene has been isolated, although the functional characterization of the protein is still missing (Yan et al., 2012). An *O*-methyl transferase, highly expressed in the rose flower, was shown to methylate eugenol, to produce the methylated derivative methyleugenol, which is a potential floral attractant for pollination (Wu et al., 2003).

## **Terpenoids**

Terpenoids are one of the largest and most structurally diverse groups of natural products, including more than 40000 compounds (Tholl, 2015). Our laboratory is focused on the genes involved in the biosynthesis of terpenoids, which are crucial molecules for the typical rose scent. Pathways leading to the biosynthesis of terpene compounds are well documented in dozens of plants. Precursors of all terpenes, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are supplied by the well-studied methylerythritol 4-phosphate pathway for the monoterpene synthesis, which generally takes place in plastids (Rodriguez-Concepcion and Boronat, 2002). On the contrary, IPP and DMAPP for the biosynthesis of sesquiterpenes are synthesized in the cytosol, by the mevalonate pathway. These precursors are then transformed to allylic diphosphates, geranyl diphosphate (GPP) for the monoterpenes, and farnesyl diphosphate (FPP) for the sesquiterpene biosynthesis, by prenyltransferases. Finally, GPP and FPP are used as substrates by various terpene synthases, for monoterpene and sesquiterpene production, respectively (Chen et al., 2011a). In rose, information on genes involved in the terpenoid pathway



is very scarce and only few genes have been isolated so far. A sesquiterpene synthase, responsible for the biosynthesis of germacrene D has been characterized (Guterman et al., 2002). Data mining of ESTs databases also led to the characterization of an alcohol acetyltransferase involved in the formation of geranyl acetate (Guterman et al., 2006; Shalit et al., 2003). Although the sequence of a putative linalool synthase has been published (Feng et al., 2014), no functional characterization of a monoterpene synthase has been done in rose.

We have recently identified a key gene, whose expression is strongly correlated with the presence of terpenoids in different rose cultivars (Magnard et al., 2015). In 2005, we used AFLP-DD to compare the transcriptomes of 2 rose cultivars that have very different scents: highly fragrant *R. x hybrida* cv. 'Papa Meiland' and unscented rose *R. x hybrida* cv. 'Rouge Meiland'. The gene with the highest differential expression was found to encode a cytosolic nudix hydrolase. This gene is specifically expressed in petals where scent is produced. In modern rose cultivars, its expression is correlated with the presence of geraniol, a very important monoterpene for the rose scent. The function of RhNUDX1 was finally deciphered using complementary approaches that led to the following conclusions:

Rose flowers use an alternative route to produce geraniol by employing a completely unexpected diphosphohydrolase enzyme of the Nudix family. RhNUDX1 is responsible for the dephosphorylation of GPP, precursor of monoterpenes, into GP (geranyl monophosphate). RhNUDX1 is localized in the cytosol, although in all other plants, monoterpenes are synthesized principally in plastids. Nudix hydrolases belong to a superfamily of pyrophosphatases found in animals, plants and bacteria. They generally catalyze the hydrolysis of nucleoside diphosphates linked to other moieties (X) and contain the Nudix domain defined by Bessman et al. (1996) as G(X5)E(X7)REUXEEXX. In plants, Nudix hydrolases are poorly characterized, although up to 29 genes have been identified in the *Arabidopsis* genome (Kraszewska, 2008). Based on sequence similarities, several functional groups have been defined. It is thought that these enzymes can act as diphosphoinositol polyphosphate phosphohydrolases, coenzyme A pyrophosphatases, ADP-ribose pyrophosphatases, ApnA hydrolases and mRNA decapping enzymes. In *Arabidopsis*, few Nudix hydrolases have been linked to a specific function *in vivo*. AtNUDX7, for example, has been shown to act as negative regulator of plant defense response and has also been implicated in plant abiotic stress responses (Ishikawa et al., 2009). RhNUDX1 shows the closest similarity to AtNUDX1 from *Arabidopsis*. Although AtNUDX1 actual function is still controversial, this protein is proposed to have a similar function to *E. coli* mutator protein MutT, which acts to eliminate harmful compounds, such as 8-oxo-dGTP, which may be misincorporated in DNA during replication (Maki and Sekiguchi, 1992; Yoshimura et al., 2007).

Our discovery that a nudix hydrolase is involved in scent production adds to an increasing line of evidence that shows that although many plants produce similar, or even exactly the same scent compounds, they independently evolved the enzymes needed to do this, pointing at the great importance of having scent compounds in plant life, for pollination or defense (Sun et al., 2016).

Other key terpenoid compounds in rose essential oil are norisoprenoids, such as  $\beta$ -damascenone,  $\beta$ -damascone, and  $\beta$ -ionone, which are derived from carotenoid degradation. Some genes responsible for the cleavage of carotenoids, the carotenoid cleavage (di-) oxygenase (CCD) were isolated from rose tissues and functionally characterized. One particular protein, RdCCD1, which efficiently forms C13-norisoprenoids *in vivo* and is expressed in the rose flower, is a good candidate for the formation of ionones (Huang et al., 2009).

## CONCLUSIONS

All these researches on the biosynthesis of rose fragrance are actually quite recent. They will no doubt lead to a significant increase in the knowledge on rose scent. These investigations will hopefully help rose breeders in their difficult task of selection, to provide a beautiful, fragrant rose with a long vase life.

Understanding the molecular mechanisms that control scent traits may allow the identification of molecular and genetic markers to assist selection. This technique has not been applied to the rose, although it is widely used in animals and crops. Towards this aim, scent volatiles and molecular markers were analyzed in a diploid segregating population (Spiller et al., 2010). QTLs

influencing the amounts of major rose volatiles compounds (geraniol,  $\beta$ -citronellol and 2-PE) were mapped onto a rose genetic map. Genome wide association studies will also help to study fragrance inheritance in rose, as was already done for the pigment contents (Schulz et al., 2016).

Moreover, these studies on rose scent could also be used to draw a link between scent and other parameters of the flower quality, such as color and development. For example, precursors of red to violets pigments (anthocyanins) are also precursors of phenylpropanoids/benzenoid compounds and some yellow to oranges pigments (carotenoids) are terpenoids which are transformed into scent compounds (norisoprenoids). This pigment/scent relationship has already been demonstrated in another fragrant flower, carnation, in which the anthocyanin biosynthesis pathway was blocked by genetic engineering (Zuker et al., 2002). The white flowers obtained accumulated the precursor which was then redirected by the plant to the volatile aromatic compounds. Flowers emitted larger quantities of scent compounds. More surprisingly in rose, overexpression of the *Arabidopsis* transcription factor PAP1, which activates the phenylpropanoid pathway, led to an enhancement of the production of both phenylpropanoid (color and scent) and terpenoid (scent) compounds (Ben Zvi et al., 2012). The role of phytohormones such as gibberellins in the activation of the different branches of the biosynthesis pathways has also recently been demonstrated (Ravid et al., 2017). Improvement of rose cultivars would certainly benefit from a better understanding of these complex relationships between color and scent. In the model plant *Arabidopsis*, key regulators of flower maturation have been recently isolated, and have been shown to synchronize petal expansion and volatile terpenoids emission (Liu et al., 2015). Identifying such regulators in rose is a major goal of future research.

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## Literature cited

- Adebesin, F., Widhalm, J.R., Boachon, B., Lefevre, F., Pierman, B., Lynch, J.H., Alam, I., Junqueira, B., Benke, R., Ray, S., et al. (2017). Emission of volatile organic compounds from petunia flowers is facilitated by an ABC transporter. *Science* 356, 1386-1388.
- Ben Zvi, M.M., Shklarman, E., Masci, T., Kalev, H., Debener, T., Shafir, S., Ovadis, M., and Vainstein, A. (2012). PAP1 transcription factor enhances production of phenylpropanoid and terpenoid scent compounds in rose flowers. *New Phytol* 195, 335-345.
- Bergougnoux, V., Caissard, J.C., Jullien, F., Magnard, J.L., Scalliet, G., Cock, J.M., Huguene, P., and Baudino, S. (2007). Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. *Planta* 226, 853-866.
- Borda, A.M., Clark, D.G., Huber, D.J., Welt, B.A., and Nell, T.A. (2010). Effects of ethylene on volatile emission and fragrance in cut roses: The relationship between fragrance and vase life. *Postharvest Biol Technol* 59, 245-252.
- Bouhleb, C., Dolhem, G., Fernandez, X., and Antoniotti, S. (2012). Model study of the enzymatic modification of natural extracts: peroxidase-based removal of eugenol from rose essential oil. *J Agric Food Chem* 60, 1052-1058.
- Caissard, J.C., Bergougnoux, V., Martin, M., Mauriat, M., and Baudino, S. (2006). Chemical and histochemical analysis of 'Quatre Saisons Blanc Mousseux', a Moss Rose of the *Rosa x damascena* group. *Ann Bot (Lond)* 97, 231-238.
- Channeliere, S., Riviere, S., Scalliet, G., Szecsi, J., Jullien, F., Dolle, C., Vergne, P., Dumas, C., Bendahmane, M., Huguene, P., et al. (2002). Analysis of gene expression in rose petals using expressed sequence tags. *FEBS Lett* 515, 35-38.
- Chen, F., Tholl, D., Bohlmann, J., and Pichersky, E. (2011a). The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant J* 66, 212-229.
- Chen, X.M., Kobayashi, H., Sakai, M., Hirata, H., Asai, T., Ohnishi, T., Baldermann, S., and Watanabe, N. (2011b). Functional characterization of rose phenylacetaldehyde reductase (PAR), an enzyme involved in the biosynthesis of the scent compound 2-phenylethanol. *J Plant Physiol* 168, 88-95.
- Dobson, H.E.M., Bergström, G., and Groth, I. (1990). Differences in fragrance chemistry between flower parts of *Rosa rugosa* Thunb. (*Rosaceae*). *Israel Journal of Botany* 39, 143-156.
- Dobson, H.E.M., Danielson, E.M., and Wesep, I.D.V. (1999). Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (*Rosaceae*). *Plant Species Biol* 14, 153-166.

- Dubois, A., Carrere, S., Raymond, O., Pouvreau, B., Cottret, L., Rocchia, A., Onesto, J.P., Sakr, S., Atanassova, R., Baudino, S., et al. (2012). Transcriptome database resource and gene expression atlas for the rose. *BMC Genomics* *13*, 638-648.
- Dubois, A., Raymond, O., Maene, M., Baudino, S., Langlade, N.B., Boltz, V., Vergne, P., and Bendahmane, M. (2010). Tinkering with the C-Function: A Molecular Frame for the Selection of Double Flowers in Cultivated Roses. *PLoS ONE* *5*, e9288.
- Dudareva, N., Klempien, A., Muhlemann, J.K., and Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol* *198*, 16-32.
- Dudareva, N., and Pichersky, E. (2000). Biochemical and molecular genetic aspects of floral scents. *Plant Physiol* *122*, 627-633.
- Feng, L., Chen, C., Li, T., Wang, M., Tao, J., Zhao, D., and Sheng, L. (2014). Flowery odor formation revealed by differential expression of monoterpene biosynthetic genes and monoterpene accumulation in rose (*Rosa rugosa* Thunb.). *Plant Physiol Biochem* *75*, 80-88.
- Guterman, I., Masci, T., Chen, X., Negre, F., Pichersky, E., Dudareva, N., Weiss, D., and Vainstein, A. (2006). Generation of phenylpropanoid pathway-derived volatiles in transgenic plants: rose alcohol acetyltransferase produces phenylethyl acetate and benzyl acetate in petunia flowers. *Plant Mol Biol* *60*, 555-563.
- Guterman, I., Shalit, M., Menda, N., Piestun, D., Dafny-Yelin, M., Shalev, G., Bar, E., Davydov, O., Ovadis, M., Emanuel, M., et al. (2002). Rose scent: genomics approach to discovering novel floral fragrance-related genes. *Plant Cell* *14*, 2325-2338.
- Han, Y., Wan, H., Cheng, T., Wang, J., Yang, W., Pan, H., and Zhang, Q. (2017). Comparative RNA-seq analysis of transcriptome dynamics during petal development in *Rosa chinensis*. *Sci Rep* *7*, 43382.
- Hassan ul, M.N., Zainal, Z., and Ismail, I. (2015). Green leaf volatiles: biosynthesis, biological functions and their applications in biotechnology. *Plant Biotechnol J* *13*, 727-739.
- Helsper, J.P.F.G., Davies, J.A., Bouwmeester, H.J., Krol, A.F., and van Kampen, M.H. (1998). Circadian rhythmicity in emission of volatile compounds by flowers of *Rosa hybrida* L. cv. Honesty. *Planta* *207*, 88-95.
- Hendel-Rahmanim, K., Masci, T., Vainstein, A., and Weiss, D. (2007). Diurnal regulation of scent emission in rose flowers. *Planta* *226*, 1491-1499.
- Hirata, H., Ohnishi, T., Ishida, H., Tomida, K., Sakai, M., Hara, M., and Watanabe, N. (2012). Functional characterization of aromatic amino acid aminotransferase involved in 2-phenylethanol biosynthesis in isolated rose petal protoplasts. *J Plant Physiol* *169*, 444-451.
- Hirata, H., Ohnishi, T., Tomida, K., Ishida, H., Kanda, M., Sakai, M., Yoshimura, J., Suzuki, H., Ishikawa, T., Dohra, H., et al. (2016). Seasonal induction of alternative principal pathway for rose flower scent. *Scientific Reports* *6*, 20234.
- Huang, F.C., Horvath, G., Molnar, P., Turcsi, E., Deli, J., Schrader, J., Sandmann, G., Schmidt, H., and Schwab, W. (2009). Substrate promiscuity of RdCCD1, a carotenoid cleavage oxygenase from *Rosa damascena*. *Phytochemistry* *70*, 457-464.
- Imai, T., Maekawa, M., Tsuchiya, S., and Fujimori, T. (1998). Field Attraction of *Hoplia communis* to 2-Phenylethanol, a major volatile component from host flowers, *Rosa* spp. *J Chem Ecol* *24*, 1491-1497.
- Ishikawa, K., Ogawa, T., Hirose, E., Nakayama, Y., Harada, K., Fukusaki, E., Yoshimura, K., and Shigeoka, S. (2009). Modulation of the poly(ADP-ribosyl)ation reaction via the Arabidopsis ADP-Ribose/NADH pyrophosphohydrolase, AtNUDX7, is involved in the response to oxidative stress. *Plant Physiol* *151*, 741-754.
- Kaminaga, Y., Schnepf, J., Peel, G., Kish, C.M., Ben-Nissan, G., Weiss, D., Orlova, I., Lavie, O., Rhodes, D., Wood, K., et al. (2006). Plant phenylacetaldehyde synthase is a bifunctional homotetrameric enzyme that catalyzes phenylalanine decarboxylation and oxidation. *J Biol Chem* *281*, 23357-23366.
- Kessler, D., Kallenbach, M., Diezel, C., Rothe, E., Murdock, M., and Baldwin, I.T. (2015). How scent and nectar influence floral antagonists and mutualists. *eLife* *4*, e07641.
- Kim, J., Park, J.H., Lim, C.J., Lim, J.Y., Ryu, J.Y., Lee, B.W., Choi, J.P., Kim, W.B., Lee, H.Y., Choi, Y., et al. (2012). Small RNA and transcriptome deep sequencing proffers insight into floral gene regulation in *Rosa* cultivars. *BMC Genomics* *13*, 657.
- Knudsen, J.T., Eriksson, R., Gershenzon, J., and Ståhl, B. (2006). Diversity and distribution of floral scent. *Bot Rev* *72*, 1-120.
- Koeduka, T. (2014). The phenylpropene synthase pathway and its applications in the engineering of volatile phenylpropanoids in plants. *Plant Biotechnol* *31*, 401-407.
- Koning-Boucoiran, C.F., Esselink, G.D., Vukosavljev, M., van 't Westende, W.P., Gitonga, V.W., Krens, F.A., Voorrips, R.E., van de Weg, W.E., Schulz, D., Debener, T., et al. (2015). Using RNA-Seq to assemble a rose transcriptome with more than 13,000 full-length expressed genes and to develop the WagRhSNP 68k Axiom SNP array for rose (*Rosa* L.). *Frontiers in plant science* *6*, 249.
- Kraszewska, E. (2008). The plant Nudix hydrolase family. *Acta Biochim Pol* *55*, 663-671.

- Lavid, N., Wang, J., Shalit, M., Guterman, I., Bar, E., Beuerle, T., Menda, N., Shafir, S., Zamir, D., Adam, Z., et al. (2002). O-methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. *Plant Physiol* 129, 1899-1907.
- Lawrence, B.M. (1997). Progress in essential oils. Rose oil. *Perfum Flavor* 22, 57-74.
- Liu, Z., Boachon, B., Lugan, R., Tavares, R., Erhardt, M., Mutterer, J., Demais, V., Pateyron, S., Brunaud, V., Ohnishi, T., et al. (2015). A Conserved Cytochrome P450 Evolved in Seed Plants Regulates Flower Maturation. *Molecular plant* 8, 1751-1765.
- Magnard, J.L., Rocchia, A., Caissard, J.C., Vergne, P., Sun, P., Hecquet, R., Dubois, A., Hibrand-Saint Oyant, L., Jullien, F., Nicole, F., et al. (2015). Biosynthesis of monoterpene scent compounds in roses. *Science* 349, 81-83.
- Maki, H., and Sekiguchi, M. (1992). MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature* 355, 273-275.
- Ohloff, G. (1978). Importance of minor components in flavors and fragrance. *Perfum Flavor* 3, 11-22.
- Picone, J.M., Clery, R.A., Watanabe, N., MacTavish, H.S., and Turnbull, C.G. (2004). Rhythmic emission of floral volatiles from *Rosa damascena* semperflorens cv. 'Quatre Saisons'. *Planta* 219, 468-478.
- Ravid, J., Spitzer-Rimon, B., Takebayashi, Y., Seo, M., Cna'ani, A., Aravena-Calvo, J., Masci, T., Farhi, M., and Vainstein, A. (2017). GA as a regulatory link between the showy floral traits color and scent. *New Phytol* 215, 411-422.
- Rodriguez-Concepcion, M., and Boronat, A. (2002). Elucidation of the Methylerythritol Phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. *Plant Physiol* 130, 1079-1089.
- Rusanov, K., Kovacheva, N., Stefanova, K., Atanassov, A., and Atanassov, I. (2009). *Rosa Damascena*-genetic resources and capacity building for molecular breeding. *Biotechnology & Biotechnological Equipment* 23, 1436-1439.
- Scalliet, G., Journot, N., Jullien, F., Baudino, S., Magnard, J.L., Channeliere, S., Vergne, P., Dumas, C., Bendahmane, M., Cock, J.M., et al. (2002). Biosynthesis of the major scent components 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene by novel rose O-methyltransferases. *FEBS Lett* 523, 113-118.
- Scalliet, G., Lionnet, C., Le Behec, M., Dutron, L., Magnard, J.L., Baudino, S., Bergougnoux, V., Jullien, F., Chambrier, P., Vergne, P., et al. (2006). Role of petal-specific orcinol O-methyltransferases in the evolution of rose scent. *Plant Physiol* 140, 18-29.
- Scalliet, G., Piola, F., Douady, C.J., Rety, S., Raymond, O., Baudino, S., Bordji, K., Bendahmane, M., Dumas, C., Cock, J.M., et al. (2008). Scent evolution in Chinese roses. *Proc Natl Acad Sci USA* 105, 5927-5932.
- Schulz, D.F., Schott, R.T., Voorrips, R.E., Smulders, M.J., Linde, M., and Debener, T. (2016). Genome-Wide Association Analysis of the Anthocyanin and Carotenoid Contents of Rose Petals. *Frontiers in plant science* 7, 1798.
- Shalit, M., Guterman, I., Volpin, H., Bar, E., Tamari, T., Menda, N., Adam, Z., Zamir, D., Vainstein, A., Weiss, D., et al. (2003). Volatile ester formation in roses. Identification of an acetyl-coenzyme A. Geraniol/Citronellol acetyltransferase in developing rose petals. *Plant Physiol* 131, 1868-1876.
- Spiller, M., Berger, R.G., and Debener, T. (2010). Genetic dissection of scent metabolic profiles in diploid rose populations. *Theor Appl Genet* 120, 1461-1471.
- Sun, P., Schuurink, R.C., Caissard, J.C., Huguene, P., and Baudino, S. (2016). My Way: Noncanonical Biosynthesis Pathways for Plant Volatiles. *Trends Plant Sci* 21, 884-894.
- Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Adv Biochem Eng Biotechnol* 148, 63-106.
- Tholl, D., Boland, W., Hansel, A., Loreto, F., Rose, U.S., and Schnitzler, J.P. (2006). Practical approaches to plant volatile analysis. *Plant J* 45, 540-560.
- Wu, S., Watanabe, N., Mita, S., Dohra, H., Ueda, Y., Shibuya, M., and Ebizuka, Y. (2004). The key role of phloroglucinol O-methyltransferase in the biosynthesis of *Rosa chinensis* volatile 1,3,5-trimethoxybenzene. *Plant Physiol* 135, 95-102.
- Wu, S., Watanabe, N., Mita, S., Ueda, Y., Shibuya, M., and Ebizuka, Y. (2003). Two O-methyltransferases isolated from flower petals of *Rosa chinensis* var. *spontanea* involved in scent biosynthesis. *J Biosci Bioeng* 96, 119-128.
- Yan, H., Baudino, S., Caissard, J.C., Zhang, H., Jian, H., Wang, Q., Qiu, X., Zhang, T., and Tang, K. (2012). Cloning and expression analysis of eugenol synthase gene (RhEGS1) in cut rose (*Rosa hybrida*). *Scientia Agricultura sinica* 45, 590-597.
- Yan, H., Zhang, H., Chen, M., Jian, H., Baudino, S., Caissard, J.C., Bendahmane, M., Li, S., Zhang, T., Zhou, N., et al. (2014). Transcriptome and gene expression analysis during flower blooming in *Rosa chinensis* 'Pallida'. *Gene* 540, 96-103.
- Yoshimura, K., Ogawa, T., Ueda, Y., and Shigeoka, S. (2007). AtNUDX1, an 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate pyrophosphohydrolase, is responsible for eliminating oxidized nucleotides in Arabidopsis. *Plant Cell Physiol* 48, 1438-1449.

Zuker, A., Tzfira, T., Ben-Meir, H., Ovadis, M., Shklarman, E., Itzhaki, H., Forkmann, G., Martens, S., Neta-Sharir, I., Weiss, D., et al. (2002). Modification of flower color and fragrance by antisense suppression of the flavanone 3-hydroxylase gene. *Mol Breed* 9, 33-41.