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Model Plants and Functional Genomic Approaches Applied to the Characterization of Genes Involved in Floral Scent Biosynthesis

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ABSTRACT

Flower scents have been subjected to extensive chemical characterization for many years, providing detailed analyses of the complex mixture of volatiles emitted by flowers. However, the past ten years have seen a rapid acceleration of progress in flower scent research, with the characterization of numerous genes involved in scent biosynthesis. This review focuses on the evolution of flower scent research, from the early biochemical to the present genomic approaches, illustrating how the use of different plant models, combined with functional genomic approaches, contributed to the present knowledge in this field.

1. INTRODUCTION

Flower scent is determined by a complex mixture of low-molecular weight volatile molecules and, whilst these molecules are highly diverse, most of them belong to three main classes of compounds: terpenoids, phenylpropanoids / benzenoids and fatty acid derivatives (Dudareva *et al.* 2004). Volatile terpenoids (i.e. monoterpenes, sesquiterpenes) are synthesized from the precursor isopentenyl diphosphate (IPP), which is derived either from the cytosolic mevalonic acid (MVA) pathway or from the plastid-localized methylerythritol phosphate (MEP) pathway. Volatile phenylpropanoid and benzenoid compounds are derived from phenylalanine by a series of modifications including shortening of the carbon skeleton, methylation or esterification. Volatile fatty-acid derivatives are produced by lipoxygenase cleavage of membrane lipids and include saturated and unsaturated short-chain alcohols, aldehydes, or esters. For many years, flower scents have been subjected to extensive chemical characterization, providing detailed analyses of the complex mixture of volatiles emitted by flowers (Knudsen *et al.* 1993). In the early 90's, pioneering investigations aiming at the characterization of enzymes and genes involved in flower scent biosynthesis considerably expanded this research field, which experienced rapid progress over the subsequent ten years. The combined use of different plant models and functional genomic approaches has led to the identification of numerous genes encoding enzymes involved in volatile biosynthesis, and to the recent characterization of the first flower scent regulatory gene. This chapter describes the approaches, which led to the characterization of some of these scent-related genes using different plant models, with emphasis on an economically important ornamental plant: the rose.

2. EARLY BIOCHEMICAL APPROACHES USING *CLARKIA BREWERI* AND SNAPDRAGON

Plant volatile biosynthetic pathways were investigated using aromatic plants from the early 70's on. For example, work on the peppermint (*Mentha piperita*) glandular trichome model system has yielded substantial insight into the metabolism of monoterpenes (Mahmoud and Croteau 2002). An important prerequisite for these particular studies was the development of methods for isolating the oil gland secretory cells in which monoterpene biosynthesis occurs (Gershenzon *et al.* 1992). This technical advance allowed the efficient purification of enzymes involved in monoterpene biosynthesis from this enriched source, and led to the cloning of a cDNA encoding (-)-4S-limonene synthase, which represented

the first cloning of a monoterpene cyclase (Colby 1993). Furthermore, a cDNA library constructed using mRNA from isolated peppermint secretory cells was used to sequence randomly selected cDNA clones. Candidate genes putatively involved in monoterpene biosynthesis were identified among these Expressed Sequence Tags (ESTs), and heterologous expression of some of them in *Escherichia coli* allowed functional characterization of further enzymes involved in essential oil biosynthesis (Lange *et al.* 2000). In contrast, research on floral fragrance focused for many years on chemical analysis, and the first attempt to characterize genes involved in flower scent biosynthesis came from the pioneering investigation of scent production in *Clarkia breweri*, an annual plant native to California.

The fragrance of *C. breweri* consists of about 10 compounds, including both benzenoids and monoterpenes, and particularly S-linalool, an acyclic monoterpene derived from geranyl diphosphate (Raguso and Pichersky 1995). *C. breweri* petals and stigmata extracts were shown to possess linalool synthase (LIS) activity when incubated in the presence of GPP and LIS was initially purified from several grams of freshly excised stigmata of *C. breweri* flowers (Pichersky *et al.* 1995). The amino-acid sequence of the N-terminus of the purified protein was determined, as well as the sequence of two internal peptides, and this sequence information allowed the isolation of a LIS cDNA from a *C. breweri* flower cDNA library, representing the first cloning of a gene involved in flower scent biosynthesis (Dudareva *et al.* 1996). Additionally, cloning of the LIS gene allowed an investigation of scent evolution in the genus *Clarkia*. Indeed, the scented *C. breweri* is believed to have evolved recently from the non-scented *C. concinna*. In *C. breweri*, the LIS gene is highly expressed in the stigma and in petals, whereas in the non-scented *C. concinna*, LIS is weakly expressed, exclusively in the stigma (Dudareva *et al.* 1996). Thus, it appears that *C. breweri*'s ability to produce large amounts of S-linalool has evolved by increasing the expression level of LIS in petals, this evolution being correlated with pollination by moths, a mode of reproduction that is novel among *Clarkia* species (Raguso and Pichersky 1995).

Similar strategies, using protein purification, sequencing of internal peptides derived from the purified protein and screening of a flower cDNA library, have been used successfully to identify additional genes involved in the biosynthesis of floral volatiles in *C. breweri*. Heterologous expression in *E. coli* of the corresponding proteins allowed the functional characterization of several enzymes involved in the biosynthesis of phenylpropanoid and benzenoid compounds. These included S-adenosyl-L-methionine:(iso)eugenol O-methyltransferase (IEMT) (Wang *et al.* 1997), Acetyl-CoA:benzylalcohol acetyltransferase (BEAT) (Dudareva *et al.* 1998), and S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase (SAMT) (Ross *et al.* 1999) involved in the biosynthesis of methyl (iso)eugenol, benzylacetate and methylsalicylate, respectively.

More recently, the common garden snapdragon *Antirrhinum majus* was used successfully as a model to investigate the biosynthesis of floral volatiles. Snapdragon flower scent is composed of three major volatile compounds, the benzenoid ester methyl benzoate and the monoterpenes trans- β -ocimene and myrcene (Dudareva *et al.* 2000). The last step of methyl benzoate biosynthesis involves an S-adenosyl-L-methionine (SAM)-dependent methylation reaction of benzoic acid, catalysed by the enzyme benzoic acid carboxyl methyl transferase (BAMT). BAMT was purified from upper and lower petal lobes and two peptide sequences were used to construct degenerate oligonucleotides for polymerase chain reaction (PCR) amplification of a fragment of the BAMT cDNA. This fragment was used to isolate a full-length cDNA, by screening a snapdragon petal cDNA library, and the BAMT protein was subsequently characterized, after heterologous expression in *E. coli* (Dudareva *et al.* 2000). Until very recently, *C. breweri* and snapdragon represented the only plant species for which isolation of enzymes and genes responsible for the formation of flower scent volatiles had been accomplished, mostly using biochemical approaches as indicated above. However, this situation has begun to change over the past 5 years, with the initiation of genomic approaches, the introduction of high throughput tools, and the emergence of new model plants.

3. RECENT FUNCTIONAL GENOMIC APPROACHES AND EMERGENCE OF NEW MODEL PLANTS

Floral scent research using genomic tools has recently been initiated in *C. breweri* and snapdragon, with the establishment of petal EST databases in both model plants. A search of snapdragon EST database for candidate scent-related genes revealed three different cDNAs with homology to terpene synthases (TPS), two of them being very similar. Functional characterization of the corresponding proteins, after heterologous expression in *E. coli*, showed that the two similar proteins were myrcene synthases and that the third protein was a trans- β -ocimene synthase. Phylogenetic analysis revealed that these new snapdragon monoterpene synthases, together with a TPS from *Arabidopsis* (At1g61680), define a new subfamily of the TPS family designated the Tps-g group (Dudareva *et al.* 2003). The same strategy was used to identify and characterize the enzyme benzoyl-coenzyme A: benzyl alcohol benzoyl transferase (BEBT), catalyzing the formation of benzylbenzoate, a minor constituent of the *C. breweri* floral aroma (D'Auria *et al.* 2002). Furthermore, cDNAs with homology to the peppermint small subunit of geranyl diphosphate synthase (GPPS-SSU) (Burke *et al.* 1999) were identified among petal ESTs from both *C. breweri* and snapdragon. Using a homology-based cloning strategy, a large subunit of geranyl diphosphate synthase (GPPS-LSU) cDNA was also isolated from snapdragon. Coexpression in *E. coli* of both GPPS-SSU and GPPS-LSU from snapdragon yielded a functional heterodimer that catalyzed the synthesis of GPP, whereas GPPS-LSU alone exhibited geranylgeranyl diphosphate synthase activity (Tholl *et al.* 2004). These data indicate that, as in peppermint, the formation of monoterpenes in snapdragon and *C. breweri* involves heterodimeric geranyl diphosphate synthases, in contrast to other plant species, such as *Arabidopsis* or grand fir (*Abies grandis*), which have been shown to possess homodimeric GPPS (Bouvier *et al.* 2000, Burke and Croteau 2002).

Early model plants used in flower scent research, such as *C. breweri* or snapdragon, were chosen mainly for their fragrance characteristics and their suitability for biochemical analyses, but not for their suitability for genetic approaches. On the other hand, *Arabidopsis thaliana* did not attract much attention as a possible model plant in flower scent research until very recently, due to the small size of its flowers and their lack of a strong scent. Starting from the fact that *Arabidopsis* genome contains about 40 TPS homologs (Aubourg *et al.* 2002), two studies were launched with the aim of characterizing *Arabidopsis* volatiles, that may have been derived from the activity of some members of this gene family. Indeed, headspace analyses of *Arabidopsis* plants showed that they released a dozen volatile molecules including both monoterpenes and sesquiterpenes, primarily from the flowers (Aharoni *et al.* 2003, Chen *et al.* 2003). Heterologous expression of several candidate TPS sequences in *E. coli* allowed the characterization of both monoterpene and sesquiterpene synthases. The monoterpene synthases, which include S-linalool synthase (At1g61680) and At3g25810, give rise to a mixture of different monoterpenes when incubated with GPP (Chen *et al.* 2003). These

enzymes utilize GPP, presumably produced by the GPP synthase characterized previously (Bouvier *et al.* 2000). The biosynthesis of the complex mixture of sesquiterpenes emitted by Arabidopsis flowers relies on the activity of two sesquiterpene synthases encoded by At5g23960 (trans- β -caryophyllene synthase) and At5g44630 (Tholl *et al.* 2005). Additionally, heterologous expression of a linalool/nerolidol synthase from strawberry (*Fragaria x ananassa*) in transgenic Arabidopsis lines showed that modification of terpenoid metabolism could lead to aphids such as *Mysus persicae* being repelled, underlining the potential of Arabidopsis for studies of the ecological role of terpenoids (Aharoni *et al.* 2003). Furthermore, high throughput analysis methods, combining solid-phase microextraction (SPME) sampling and gas chromatography-mass spectrometry (GC-MS) analysis, allowed the characterization of approximately 40 Arabidopsis lines during a single overnight run (Aharoni *et al.* 2003), opening the perspective of large-scale screening and genetics approaches in flower scent research.

In recent years, petunia (*Petunia x hybrida*) has emerged as a valuable model for flower scent research. Indeed, this important ornamental plant is well suited to biochemical analyses because of its large flowers, but genetic approaches are also possible, with the availability of large sets of insertion mutants and simple transformation procedures. *P. x hybrida* cv Mitchell flowers emit a mixture of benzenoid compounds including benzaldehyde, benzylalcohol, methylbenzoate, benzylbenzoate, phenylacetaldehyde, and phenylethanol, in addition to phenylpropanoids such as eugenol and isoeugenol. Modelling of benzenoid metabolism in petunia petals, using isotope labelling and metabolic flux analyses, indicated that all benzenoids were derived from phenylalanine (Boatright *et al.* 2004). Screening petunia EST database for potential scent-related genes allowed the isolation of cDNAs encoding two enzymes involved in benzenoid metabolism: SAM: benzoic acid/salicylic acid carboxyl methyltransferase (BSMT) involved in the biosynthesis of methylbenzoate, and benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase (BPBT) involved in the biosynthesis of benzylbenzoate and phenylethylbenzoate (Negre *et al.* 2003, Boatright *et al.* 2004). Using a homology-based cloning strategy, Simkin *et al.* (2004) characterized PhCCD1, a carotenoid cleavage dioxygenase catalysing the synthesis of β -ionone, a minor but strongly-scented constituent of petunia scent. Furthermore, Verdonk *et al.* (2003) used petal-specific microarrays to identify genes differentially expressed in the scented *P. hybrida* cv Mitchell and the scentless *P. hybrida* cv W138. cDNAs of genes with increased transcription just before scent production in Mitchell, whose transcription level remains low in W138, were selected. Among these were several cDNAs encoding enzymes involved in benzenoid metabolism, such as 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, phenylalanine ammonia-lyase (PAL), BPBT, and one cDNA encoding a MYB family transcription factor. In *P. hybrida* cv Mitchell, this transcription factor named ODORANT1 (ODO1) is specifically expressed in petals, exhibiting a rhythmic expression that correlates with scent production. Downregulation of *ODO1* in RNAi transgenic petunia lines results in a strong reduction in benzenoid emission, due to a reduced expression of several enzymes such as EPSP synthase and PAL (Verdonk *et al.* 2005). Therefore, this work identified *ODO1* as the first regulator of floral scent biosynthesis, illustrating the potential of targeted transcriptomic approaches in flower scent research.

4. AN ECONOMICALLY IMPORTANT ORNAMENTAL MODEL PLANT FOR FLOWER SCENT RESEARCH: THE ROSE

Among ornamental plants, the rose (*Rosa x hybrida*) is of interest as a model because it is the major species in this economic sector (representing approximately 30% of the market). Furthermore, scent composition in numerous rose varieties has been well documented, with several studies aiming at analysing the volatile compounds emitted by various rose cultivars (Flament *et al.* 1993). Even if the use of the rose as a model plant is hampered by the lack of easy transformation procedures, recent studies have shown that the rose is well suited to functional genomic approaches, and these approaches have led to the isolation of new scent-related genes (Channelière *et al.* 2002, Guterman *et al.* 2002, Lavid *et al.* 2002, Scalliet *et al.* 2002 2006, Shalit *et al.* 2003).

The extraordinary diversity of modern rose varieties has been obtained, by intensive breeding, from a relatively small number of progenitors including both Chinese varieties, such as *R. chinensis* and *R. gigantea*, and European varieties such as *R. gallica* and *R. moschata*, the former contributing recurrent flowering and the latter cold and disease resistance characters (Krüssmann 1982). In addition, the European and Chinese progenitors also each contributed distinctive scent characteristics. The major scent components provided by the European roses included 2-phenylethanol and a number of monoterpenes. The Chinese roses, on the other hand, principally produce fatty acid derivatives (such as hexenol and hexenyl acetate) and phenolic methyl ethers such as 3,5-dimethoxytoluene (DMT) or 1,3,5-trimethoxybenzene (TMB), and hence contributed these scent components to modern rose varieties (Flament *et al.* 1993, Joichi *et al.* 2005). The combination of molecules from these two lineages produced the remarkable diversity of rose scents, which consist of a complex mixture of hundreds of volatile compounds, representing the three main pathways (phenylpropanoids, fatty acid derivatives, and terpenoids).

Genomic approaches have recently been used to identify genes involved in rose scent biosynthesis, starting with the establishment of EST databases representing the transcriptomes of petals from different rose varieties. The first approach used *Rosa chinensis* cv Old Blush, an ancient Chinese rose variety, which is also a progenitor of modern roses (Channelière *et al.* 2002). The second approach used two modern rose varieties, Golden Gate and Fragrant Cloud, which differ in their scent characteristics (Guterman *et al.* 2002). Analysis of these petal EST databases allowed the identification of several genes potentially involved in scent production. Functional characterization of some of these genes led to the identification of two orcinol O-methyltransferases (OOMTs) involved in the biosynthesis of phenolic methyl ethers (Lavid *et al.* 2002, Scalliet *et al.* 2002) and an alcohol acetyltransferase involved in the formation of geranyl acetate (Shalit *et al.* 2003). Furthermore, Guterman *et al.* (2002) established DNA chips, using a selection of the EST collection obtained with petals of the scented (Fragrant Cloud) and non-scented (Golden Gate) rose varieties. Microarray analysis of the genes differentially expressed in these two varieties led to the characterization of a sesquiterpene synthase involved in the production of germacrene D (Guterman *et al.* 2002).

The to date best characterized scent biosynthetic pathway in roses is the pathway leading to the production of phenolic methyl ethers such as DMT or TMB (Fig. 1). Although phenolic methyl ether biosynthesis was originally restricted to Chinese roses, DMT is nowadays a major scent compound of many rose varieties, which have inherited this trait from their Chinese progenitors. Several enzymes belonging to the family of small molecule O-methyltransferases (SMOMT) have recently been characterized in roses, and some of these enzymes have been shown to be involved in the biosynthesis of phenolic methyl ethers (Gang 2005). TMB biosynthesis is specific to *R. chinensis* (Joichi *et al.* 2005) and involves 3 methylation reactions starting from the precursor phloroglucinol. The first methylation step is catalysed by phloroglucinol O-methyltransferase (POMT). This enzyme was purified from petals of *R. chinensis*, providing amino-acid sequence information that allowed the isolation of the

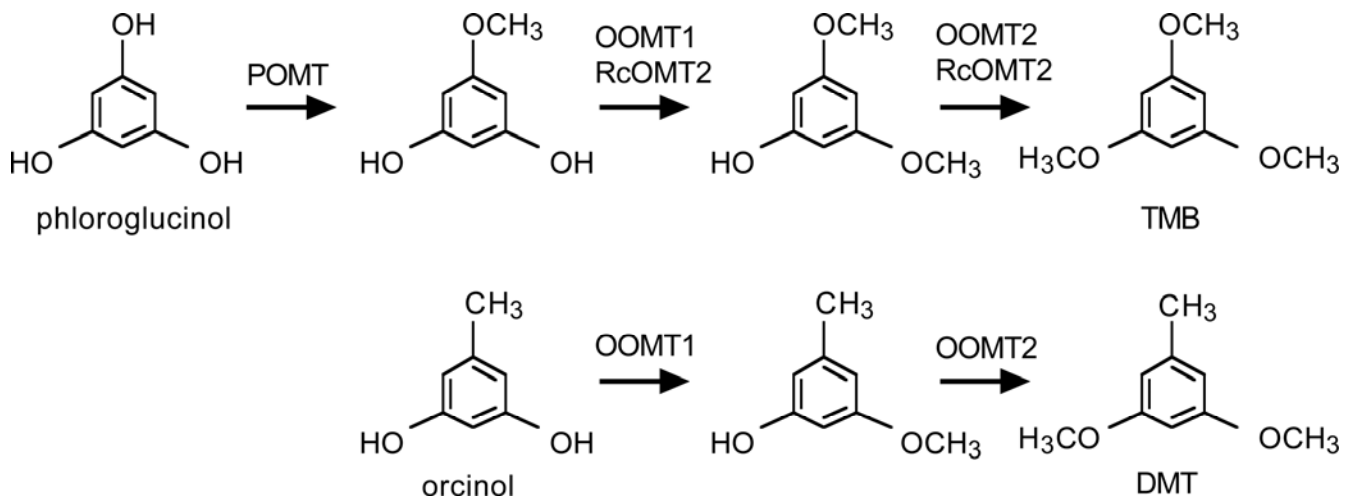


Fig. 1 Biosynthesis of phenolic methyl ethers in roses (adapted from Scalliet *et al.* 2002). The enzymes catalysing the different methylation reactions are indicated.

corresponding cDNA (Wu *et al.* 2004). Additionally, an EST survey of the transcripts present in the petals of *R. chinensis* cv Old Blush allowed the identification of two highly similar OOMT1 and OOMT2 enzymes, involved in the subsequent methylation steps (Channelière *et al.* 2002, Scalliet *et al.* 2002). Interestingly, *OOMT1* and *OOMT2* from *R. chinensis* cv Old Blush are identical to the homologous genes characterized in the modern rose variety Golden Gate, which emits DMT instead of TMB (Lavid *et al.* 2002), indicating that the *OOMT1* and *OOMT2* enzymes, themselves, do not influence whether a rose accumulates DMT or TMB. The tendency to produce DMT or TMB may rather depend on substrate availability and on the presence of enzymes involved in earlier steps of the two pathways, such as POMT. Alternatively, TMB biosynthesis may also rely on RcOMT2, another SMOMT characterized recently in *R. chinensis* (Wu *et al.* 2003).

OOMT1 and *OOMT2* catalyse the two final methylation reactions of DMT and TMB biosynthesis and, although they are very similar (96.5% identity at the amino-acid level), they exhibit different substrate specificities. Based on their different properties, *OOMT1* and *OOMT2* were proposed to operate sequentially to catalyse the first and the second methylation step of orcinol, respectively (Lavid *et al.* 2002). *OOMT1* and *OOMT2* genes are specifically expressed in petals and immunolocalization experiments have shown that OOMT proteins accumulate predominantly in epidermal cells. Following tissue localization of OOMTs, their sub-cellular localization was investigated using *in vivo* localization of Green Fluorescent Protein (GFP) fusions expressed in petal epidermal cells. Confocal microscopy investigation of the localization OOMT-GFP fusion protein indicate that, in these cells, OOMTs become increasingly associated with membranes during petal development, suggesting that these enzymes, involved in the last steps of DMT biosynthesis, may be directly linked to the cells' secretory machinery (Scalliet *et al.* 2006).

The genomes of European roses such as *R. gallica* and *R. damascena* contain *OOMT* genes, although these rose species do not produce phenolic methyl ethers. Heterologous expression of these genes in *E. coli* has shown that they potentially encode active enzymes, which are able to catalyse orcinol methylation *in vitro*. However, *OOMT* transcripts were not detected in floral organs of *R. gallica* and *R. damascena* (Scalliet *et al.* 2006). Chinese roses are thought to have evolved recently within the *Rosa* genus (Wissemann and Ritz 2005) and this would be consistent with new functions having evolved within this group. Hence, Chinese roses may have evolved an ability to produce phenolic methyl ethers as a result of increased *OOMT* gene expression in petals, in a manner similar to the evolution of *LIS* expression in the genus *Clarkia* (Dudareva *et al.* 1996).

5. FUTURE PROSPECTS: PETAL EPIDERMAL CELLS AS A SECRETORY CELL MODEL

To date, little is known about the intracellular trafficking of secondary metabolites in secretory cells, from their sites of synthesis to their site(s) of emission (Kolosova *et al.* 2001). Peppermint glandular trichomes have been exploited as a model cellular system to investigate monoterpene metabolism (Mahmoud and Croteau 2002) but this system has not been used to address the problem of how these metabolites are secreted at the cellular level. One reason for this may be that these secretory structures are scattered across the surface of the leaf making it difficult to conduct analyses of events in individual trichomes. In contrast, in many plant species the adaxial petal epidermis is composed of specialized, cone-shaped cells, which are believed to be the major site of scent compound biosynthesis and secretion (Fig. 2A). *In situ* hybridization or immunolocalization studies performed with *LIS*, *IEMT*, *BAMT* and *OOMTs* have revealed specific expression of these genes in the epidermal cells of *C. breweri*, snapdragon, and rose flowers, respectively (Dudareva and Pichersky 2000, Kolosova *et al.* 2001, Scalliet *et al.* 2006). Because of their organisation as a homogeneous cell layer, petal epidermal cells present an advantage, compared

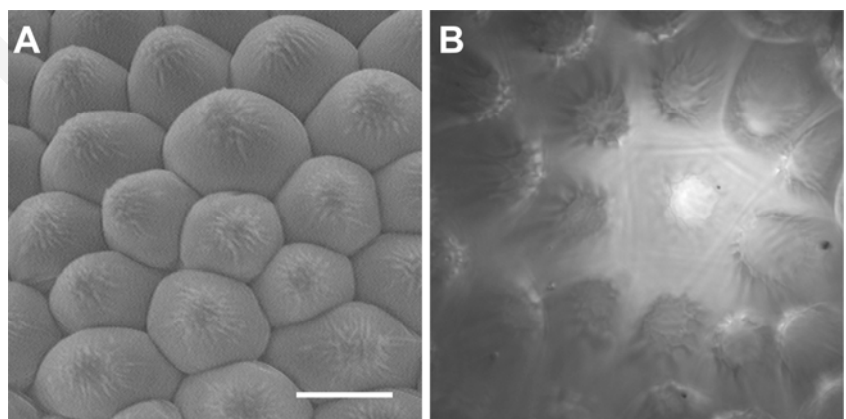


Fig. 2 Biolistic transformation of petal epidermal cells. (A) Environmental scanning electron micrograph of petal adaxial epidermal cells of *Rosa chinensis* cv Old Blush. Bar: 20 μm . (B) Epifluorescence microscopy of a petal epidermal cell expressing GFP, after biolistic transformation.

with the dispersed glandular trichomes, as a target for the biolistic delivery of gene constructs (Fig. 2B; Quattrocchio *et al.* 1999, Scalliet *et al.* 2006). This approach is particularly well adapted to studies using GFP as an *in vivo* reporter, providing access to a methodology that has emerged as an extremely valuable tool in plant cell biology (Hawes *et al.* 2001). Confocal microscopy investigation of GFP fusion proteins localization, following biolistic transformation of petal epidermal cells represents a convenient method to obtain information complementary to that obtained using immunolocalization methods (Scalliet *et al.* 2006). Dynamic monitoring of the subcellular localization of GFP fused to proteins involved in scent biosynthesis may therefore provide valuable information about the mechanisms of secondary metabolite secretion, underlining the potential of petal epidermal cells as a secretory cell model system.

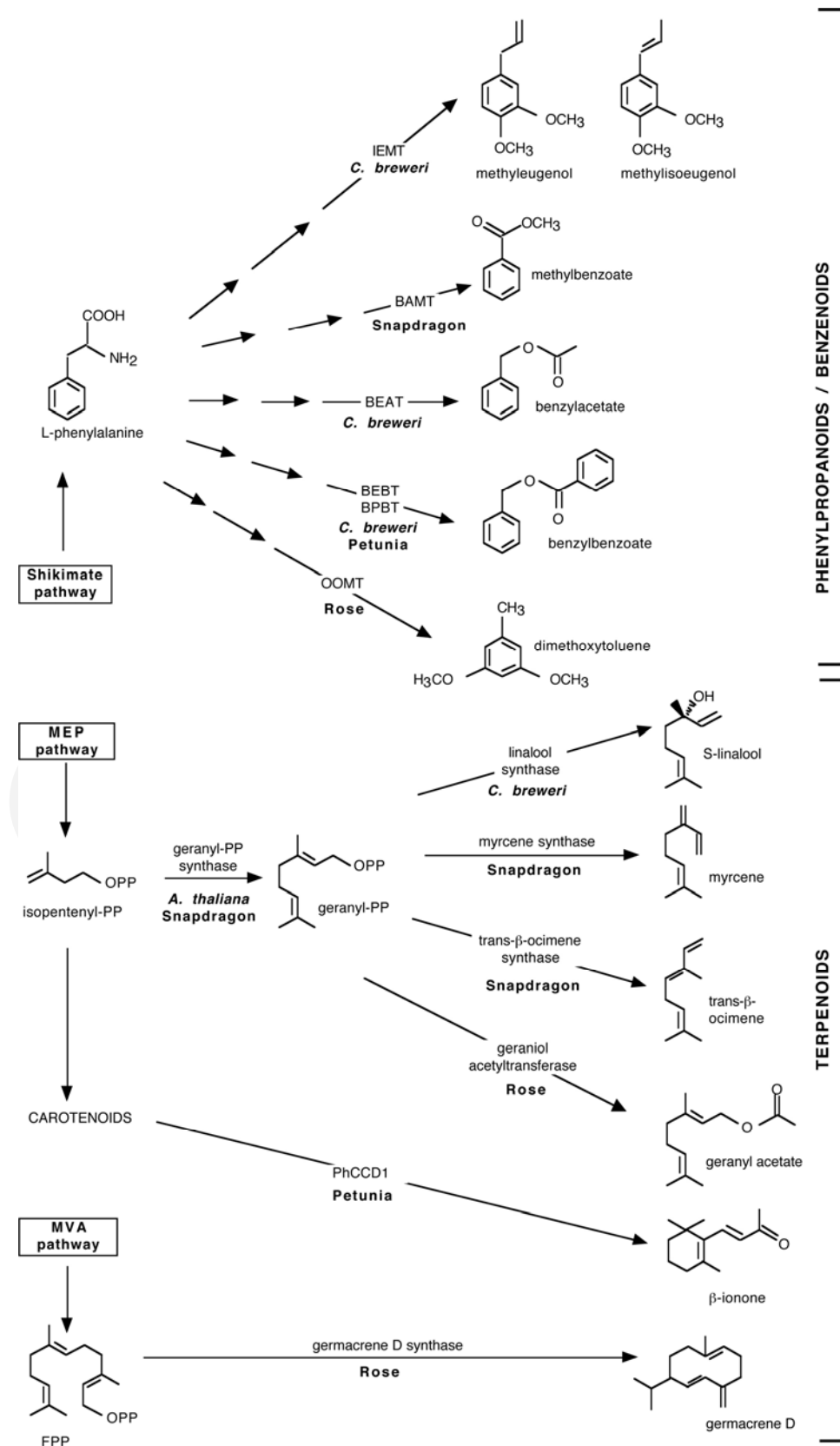


Fig. 3 Biosynthetic origin of some flower scent compounds. Enzymes involved in the biosynthesis of scent compounds are indicated, together with examples of model plants used for their characterization (for references see Table 1).

Table 1 Some scent-related genes characterized using different plant models.

The biochemical approach involves protein purification and sequencing, followed by cDNA library screening. EST-based approaches involve EST database screening for scent-related genes.

Plant model	Gene name / Activity	Main product(s)	Approach	Reference
	linalool synthase (LIS)	S-linalool	biochemical	Dudareva <i>et al.</i> 1996
<i>Clarkia breweri</i>	SAM:(iso)eugenol O-methyltransferase (IEMT)	methyl-(iso)eugenol	biochemical	Wang <i>et al.</i> 1997
	Acetyl-CoA:benzylalcohol acetyltransferase (BEAT)	benzylacetate	biochemical	Dudareva <i>et al.</i> 1998
	SAM:salicylic acid carboxyl methyltransferase (SAMT)	methylsalicylate	biochemical	Ross <i>et al.</i> 1999
	benzoyl-coA: benzyl alcohol benzoyl transferase (BEBT)	benzylbenzoate	EST	D'Auria <i>et al.</i> 2002
Snapdragon	SAM: benzoic acid carboxyl methyl transferase (BAMT)	methylbenzoate	biochemical	Dudareva <i>et al.</i> 2000
	myrcene synthase	myrcene	EST	Dudareva <i>et al.</i> 2003
	trans- β -ocimene synthase	trans- β -ocimene	EST	Dudareva <i>et al.</i> 2003
	geranyl diphosphate synthase (GPPS)	geranyl diphosphate	EST	Tholl <i>et al.</i> 2004
Arabidopsis	geranyl diphosphate synthase (GPPS)	geranyl diphosphate	homology-based cloning	Bouvier <i>et al.</i> 2000
	linalool synthase (At1g61680)	S-linalool	genome analysis	Chen <i>et al.</i> 2003
	At3g25810	α -pinene, sabinene, β -myrcene, limonene	genome analysis	Chen <i>et al.</i> 2003
	trans- β -caryophyllene synthase (At5g23960)	trans- β -caryophyllene	genome analysis	Chen <i>et al.</i> 2003
	At5g44630	(+)- α -barbatene (+)-thujopsene	genome analysis	Tholl <i>et al.</i> 2005
Petunia	SAM:benzoic acid/salicylic acid carboxyl methyltransferase (BSMT)	methylbenzoate	EST	Negre <i>et al.</i> 2003
	benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase (BPBT)	benzylbenzoate	EST	Boatright <i>et al.</i> 2004
	carotenoid cleavage dioxygenase 1 (PhCCD1)	phenylethylbenzoate	homology-based cloning	Simkin <i>et al.</i> 2004
	ODORANT1 (ODO1); MYB transcription factor	β -ionone	microarrays	Verdonk <i>et al.</i> 2005
Rose	germacrene D synthase	germacrene D	EST / microarrays	Guterman <i>et al.</i> 2002
	Acetyl-CoA: geraniol/ citronellol acetyltransferase	geranyl acetate	EST / microarrays	Shalit <i>et al.</i> 2003
	orcinol O-methyltransferase (OOMT)	DMT / TMB	EST / microarrays	Lavid <i>et al.</i> 2002, Scalliet <i>et al.</i> 2002
	RcOMT2	TMB	biochemical	Wu <i>et al.</i> 2003
	phloroglucinol O-methyltransferase (POMT)	3,5-dihydroxyanisole	biochemical	Wu <i>et al.</i> 2004

6. CONCLUSION

Characterization of genes involved in floral scent biosynthesis was initiated in the 90's, starting with biochemical approaches using *C. breweri* and snapdragon as plant models. In recent years, functional genomic approaches have been initiated in *C. breweri* and snapdragon, but also in new plant models, including Arabidopsis, petunia and the rose. These projects combined detailed fragrance analyses with the creation of petal EST databases, providing both scent-related candidate genes and sequence information, which could be used in high throughput approaches such as microarrays. Using these different approaches, flower scent research has been very successful in the characterization of numerous scent related genes involved in different metabolic pathways, as summarized in Table 1 and Fig. 3. The characterization of new scent regulatory genes, and genetic engineering strategies will provide new tools to investigate the ecological role of the extraordinary diversity of flower scents.

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