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Biogenesis, Gene expression Pattern and Manipulation of Volatile Metabolic Profile by Fragrance Engineering for Ornamental crops: A Review

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ABSTRACT: Floral fragrance plays a significant part in many plants' reproductive processes and has a significant economic value in ensuring crop production and quality in many cases. Cut flowers and decorative plants' aesthetic qualities are also improved. Terpenoid or phenylpropanoid/benzenoid families of chemicals are home to many volatile components of flower scents. Despite the fact that research into the biochemistry of floral fragrance is still in its infancy, in the last ten years experts have started to pinpoint "scent genes" and their expression patterns. A number of these genes, the majority of which, but not all, encode enzymes that catalyze the direct synthesis of volatile terpenoid or phenylpropanoid/benzenoid chemicals, have now been utilized to genetically edit the volatile mixture released from the flowers of various plant species. The results of these investigations, which are presented below, have shown promise for the genetic engineering method of changing flower smells.

Keywords: Economic value, scent genes, enzymes, volatile mixture, biochemistry.

INTRODUCTION

Floriculture is an important sector of the agriculture industry, which is comprised of ornamental plants for cut flowers, home gardening, indoor and outdoor landscaping (Sadhukhan and Huo 2020). Without a dispute, ornamental plants' contribution to the horticultural industry has impacted it. Several aesthetic plants are now regularly adopted in home gardening, commercial landscaping, and cut flowers (Dobres, 2011). A complex blend of low-molecular-weight volatile compounds, including terpenes, phenylpropanoids, and fatty acid derivatives, makes up the composite attribute defined as flower fragrance. (Croteau and Karp 1991; Chappell and Jones, 1995; Dudareva *et al.*, 2004). With more than 40,000 diverse molecules, terpenoids are the most abundant form of external volatiles and are structurally diverse (Buckingham 2004; Muhlemann *et al.*, 2014). Numerous terpenoids, such as mono-, sesqui-, and diterpenes, are recognized as secondary metabolites in plants that play crucial roles in interactions between plants and their environment (Yu and Utsumi 2009; Dudareva *et al.*, 2013). Floral volatiles are lipophilic liquids that, at ambient temperature, have such a high vapor pressure and a low molecular weight. These characteristics enable them unrestricted passage across cellular membranes for discharge into the surrounding environment (Pichersky *et al.*, 2006). The primary function of fragrance chemicals in the interactions between plants and their environment is to entice pollinators, most of whom are insects but not solely (Dudareva and Pichersky 2000). The primary purpose of airborne volatiles include communicating in plant-plant interactions, attracting pollinators, seed dispersers, and other beneficial animals and microorganisms, and protecting plants from herbivores and diseases (Dudareva and Pichersky 2008). It has been widely established over the past 20 years that plants release a variety of volatile mixes in response to herbivore assaults that might contain more than 200 distinct chemicals (Dicke and Van Loon 2000). The evolution of pollinators and bloomingplants depends on communication, especially long-distance communication, which is facilitated by floral fragrance. (Dudareva and Pichersky 2000; Farre-Armengol *et al.*, 2013). Terpenoids released from the air also play a significant role in plant defence against biotic and abiotic stresses (Paschold *et al.*, 2006; Unsicker *et al.*, 2009; Dudareva *et al.*, 2013).

Terpenes are commonly found in flower scents, particularly monoterpenes like linalool, limonene, myrcene, and trans-b-ocimene as well as some sesquiterpenes like farnesene, nerolidol, and

caryophyllene and Pichersky 2000). The study of the control of smell biosynthesis has been made easier by the identification of the genes involved for the production of volatile floral scents (Dudareva and Pichersky 2006). Foral volatiles are produced using increasingly well-characterized biosynthetic routes (Nagegowda *et al.*, 2010), and current research has employed transcriptomics-based methods to find homologous genes from these pathways in non-model plant species (Onda *et al.*, 2015; Magnard *et al.*, 2015; Yue *et al.*, 2015; Hsiao *et. al*., 2006). However, these developments (Amrad *et al.*, 2016; Wong *et al.*, 2017), A significant portion of the biosynthetic variety in fragrances comes from volatile terpenes, particularly during the postharvest period, are the two key issues facing the floriculture industry's scent creation. Both of these objectives may be achieved with the use of genetic engineering, which would raise the price of ornamentals. Additionally, the economic impact of floral smell engineering on crop yields for many agricultural products might be significant (Dudareva and Pichersky 2006). Breeders in this multi-billion dollar industry have focused on creating plants with enhanced vase life, transportation properties, and aesthetic attributes as a result (*i.e.* colour and shape). Many grown flowers have lost their perfume as a result of lack of direct selection or maybe due to a bad link with any of these features (Vainstein *et al.*, 2001). Without losing other crucial commercial qualities, genetic engineering could be able to restore aroma to these types, adding value for the small segment of customers who actually enjoy scented flowers and are ready to pay more for them (Pichersky and Dudareva 2007).

In this review, we put more emphasis on recent developments in our understanding of the molecular mechanisms underlying the biosynthetic pathways, as well as their regulation, functions, patterns of gene expression, and attempts to manipulate specific floral scents using genetic engineering methods as opposed to conventional breeding methods.

Biogenesis of floral scent compounds. Most volatile chemicals found in plants fall into one of three categories: terpenes, phenylpropanoids, or fatty acid derivatives (Pichersky *et al.*, 2006) Different amino acids are the sources of additional volatiles. Plant volatiles are typically regarded as being a component of secondary, or specialized, metabolism because the majority of them are exclusively generated by a small number of plant lineages and serve only those lineages' particular ecological needs. Compared to primary metabolites, which are by definition present in practically all plants, they are less common. Primary and specialized metabolic pathways are not entirely distinct from one another; instead, specialized metabolites are mostly synthesized in the network's terminal branches. As a result, sometimes just one enzyme and one reaction are needed to transform a primary metabolite into a volatile molecule, while other times several steps are necessary (Dudareva *et al.*, 2004). Terpenoids, which consist of 556 fragrance compounds, are the biggest class of plant foral volatiles (Abbas *et al.*, 2017). Terpenes are frequently found in flower scents, particularly monoterpenes like linalool, 10- and 15-carbon sesquiterpenes (Pichersky and Raguso 2016). In angiosperms, a reasonably large gene family encodes the terpene synthase (TPS) enzymes that are involved in the manufacture of these secondary compounds (Chen *et al.*, 2011).

The rising body of research on the role of volatiles in plant defense shows that metabolic engineering can modify the volatile spectrum to boost plant protection in agricultural and forest settings, offering an alternative biological control-based pest management method (Khan *et al.*, 2000). Creating flowers with increased smell quality and/or freshly introduced aromas that customers can appreciate, as well as preserving the fragrant bouquet.

Limonene, myrcene, and trans-b-ocimene as well as some sesquiterpenes like farnesene, nerolidol, and caryophyllene (Dudareva and Pichersky 2000) are responsible for fragrance. The simple five- carbon compound IPP and its allylic isomer DMAPP serve as the beginning substrate for the production of the majority of terpenoids in plants (Chen *et al.*, 2011). The MVA and MEP processes, which are compartmentally segregated and autonomous, produce IPP, the most common precursor of all terpenoids (Chen *et al.*, 2011). The MVA route consists of six enzymatic processes that are carried out by sequentially condensing three molecules of acetyl CoA. After reduction to the MVA pathway and two more steps of phosphorylation and decarboxylation, the final product, IPP, is produced (Lange *et al.*, 2000; Tholl, 2015). Similar to this, the MEP pathway starts with the condensation of pyruvate and glyceraldehydes-3-phosphate and consists of seven enzymatic steps (G3P) (Nagegowda 2010; Muhlemann *et al.*, 2014). IPP either combines with one IPP unit to make geranyl diphosphate (GPP), which is catalyzed by the GPP synthases, or isomerizes to form DMAPP, which acts as a substrate for the production of hemiterpenes (GPS). Farnesyl pyrophosphate (FPP) is created by the condensation of one IPP and one GPP molecule, which is catalysed in the presence of FPP synthases (FPS). Similar to GPP, FPP serves as a precursor for sesquiterpene and monoterpene biosynthesis, respectively (Vranova *et al.*, 2012). It is proven that linalool was produced from GPP in a one-step reaction catalyzed by a monomeric enzyme called linalool synthase. Linalool was produced in copious amounts from the petals, stigma, and style of *Clarkia breweri* flowers, as well as from the stigma and style, which also emit large amounts of linalool oxides (LIS) (Pichersky *et al.*, 1995). The Phenylpropanoids are a sizable family of secondary metabolites in plants that are generated from Phenylalanine. Many are intermediates in the manufacture of defense chemicals, pigments, and structural cell components including lignin and anthocyanins. Usually, they are not explosive. However, a number of phenylpropanoids with decreased carboxyl groups at C9 (to aldehydes, alcohols, alkanes, or alkenes) and/or with alkyl additions to the hydroxyl groups of the benzyl ring or to the carboxyl group (*i.e.*, ethers and esters) are volatiles (Kumari *et al.*, 2017). Phenylalanine is converted by a single enzyme to phenylacetaldehyde, a volatile compound that gives rose, petunia, and many other species their distinctive

floral scent (Kaminga, 2006), While eugenol, a different volatile from the phenylpropanoid family, is produced from coniferyl alcohol, a stage in the overall plant lignin biosynthesis route, in just two steps (Koeduka, 2006; Dexter, 2007). Three enzymes-(iso) methyleugenol, benzylacetate, and methylsalicylate-that catalyse the synthesis of floral volatiles from this group have now been identified and characterised from *Clarkia breweri* flowers. The three enzymes are acetyl-CoA:benzylalcohol acethyltransferase (BEAT), S-adenosyl-l-Met:salicylic acid carboxyl methyltransferase, and S-adenosyl-l- Met:(iso) eugenol O-methyltransferase (IEMT), respectively (SAMT) (Wang, 1997; Dudereva *et al.,* 1998; Duderava *et al.,* 1998; Wang *et al.,* 1998; Ross *et al.,* 1999). The enzyme S-adenosyl-l-Met:benzoic acid carboxyl methyltransferase (BAMT), which catalyses the production of methylbenzoate in snapdragon flowers, has also been found and described (Bushue *et al.,* 1999).

Gene expression pattern and gene identification. Both plant-pollinator and plant-herbivore interactions depend heavily on plant volatiles. Studies that investigate underlying differential gene expression are uncommon, despite the prevalence of intraspecific polymorphisms in the synthesis of volatiles (Bechen *et. al.,* 2022). With over 1700 floral volatiles reported from over 900 angiosperm species, there is significant qualitative and quantitative variety in floral scent that has been observed (Kundsen *et al.,* 2006). Terpene synthase activity was concentrated in three clusters of differentially expressed genes, two of which were characterized by tissue-specific overexpression and one of which was characterised by upregulation exclusively in plants with flowers that generate (R)-()- linalool. Two putative (R)- ()-linalool synthase transcripts were detected in *Oenothera harringtonii*, according to a molecular phylogeny of all terpene synthases. Linalool + plants only have one allele of this gene (Bechen *et. al.,* 2022). The seven to eight subfamilies of TPS genes exhibit either molecule/lineage-specific (e.g., monocot sesquiterpene TPSs) or lineage- specific (e.g., solely gymnosperm TPSs) affinities. Despite the gene family and its products being extensively characterized throughout plant lineages (Van Schie *et al.,* 2011). It is yet unclear what maintains intraspecies heterogeneity in the synthesis of volatile terpenes. An epistatic network of Mendelian loci responsible for volatile chemotypes in *T. vulgaris* was discovered through genetic crosses (Gouyon *et al.,* 1986; vernet *et al.,* 1986). To understand how constraining selection pressures affect chemical polymorphism, it is necessary to grasp the genetic controls of chemotype variation beyond a few of these model systems. There haven't been many opportunities to investigate the genetic roots of foral scent's volatile terpenoid polymorphism. The "10C3-424" had the lowest expression of linalool synthase and TPS, which correlated with the potency of the four cultivars' scents. In "Shiny Gold" TPS 2, TPS 3, TPS 5, TPS 6, and TPS 8 were significantly expressed in the bud and bloom, however TPS 4 expression was reduced compared to that of other TPS genes in both flowering phases. These findings could help with marker-assisted selection to improve smell composition in Freesia cultivars (Shrinivasan *et al.,* 2020). Carotenoids and anthocyanins positively linked with all smell components, according to the correlation study between the pigments and fragrance compounds across floral bud development in the cultivars of rose "Penny Lane" and "Vital" (Yeon and Kim 2020). Genes from model plants that have been functionally described are frequently inserted into ornamental plants to manipulate a variety of properties. Functional genomics has recently been started in ornamental plants for the identification of novel candidate genes that are crucially influencing desired features, notably through transcriptome analysis by next-generation sequencing. We'll talk about a few recent instances of ornamental species' genes being identified (Sadhukhan and Huo 2020). *Beta vulgaris* and *Mirabilis jalapa* were used as sources for a new cytochrome P450 gene, CYP76AD6, which was then expressed in tobacco to produce the red betalain pigment. This gene's enzyme produced red-pigmented tobacco by causing the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine, an early stage in betalain biosynthesis (Polturak *et al.,* 2016). For potential use in genetic engineering, the expression of *Phaius tankervilliae* 9- cis-epoxycarotenoid dioxygenase 1 (PtNCED1), which controls the manufacture of abscisic acid and the rate of seed germination, was characterized (Lee *et al.,* 2018). Using the Illumina technology, the transcriptome of the fragrant tropical ornamental plant *Hedychium coronarium* was examined. This research may help identify genes unique to flowers that regulate petal growth and are involved in the production of floral terpenes and benzoids. Analysis of the aromatic volatiles further characterized the function of several genes (Yue *et al.,* 2015).

Manipulation of volatile metabolic profile. In addition to having practical ramifications, genetically engineering flower scents touches on basic issues regarding the production and control of secondary metabolites (Table 2). It could reveal, for instance, how gene addition or alteration affects steady-state metabolite levels and fluxes in the pathways (Dudareva and Pichersky 2006). There are two different methods for genetically engineering floral scent. One strategy is based on the introduction of foreign genes that encode enzymes with functions that are absent in the target plant; these enzymes enable additional branches of already-existing pathways or the creation of brand-new ones. Modulating floral smell may need more than just the addition of new genes or the amplification of existing genes. One of the primary constraints on the generation of volatiles is the scarcity of substrate. For instance, it has recently been demonstrated that benzoic acid, methyl benzoate's precursor, regulates the amount of methyl benzoate that snapdragon blooms may make (Dudareva *et al.,* 2000). By increasing the activity/level of upstream enzymes, it would be able to overcome the sub strate deficiency (Sandmann, 2001). The second strategy relies on modifying (down- or up- regulating) a native gene's expression (s). Through this process, one

may either inhibit the synthesis of an undesired volatile or promote the production of the volatile by up-regulating a gene in the pathway. The native genes' activity may also be inhibited, allowing metabolic flux to be diverted and changing the composition of thescent spectrum (Vainstein *et al.,* 2001). However, several more attempts to alter the fragrance bouquet fell short for a variety of reasons, such as the lack of adequate substrates for the new reaction (Beekwilder *et al.,* 2004; Aranovich *et al.,* 2007), the scent ingredient is changed to a non-volatile form (Lucker *et al.*, 2001), inadequate volatile emission levels for human olfactory detection or volatiles obscuring the injected compound(s) (Lavy *et al.*, 2002).

Another strategy that has lately been employed for smell modification is the removal of certain volatile components from the flower bouquet. Petunia transgenics devoid of methylbenzoate (Underwood *et al.*, 2005), phenylacetaldehyde (Kaminaga *et al.*, 2006), benzylbenzoate and phenylethylbenzoate (Orlova *et al.*, 2006), and isoeugenol (Dexter *et al.,* 2007) achieved by RNA interference-mediated posttranscriptional gene silencing. Zuker *et al.* (2002) noted that transgenic plants of carnation exhibited flower colour modifications ranging from attenuation (F3H-10 and F3H-33) to complete loss of their original orange/reddish colour (F3H-11 and F3H-14) and accumulated only very low levels of pelargonidin in carnation. Southern blot analysis of E*co*RI- digested DNA confirmed the presence of anti-*f3h* DNA fragment in selected transgenic lines (1.2 kb as predicted) but not in the non-transformed plants. Petals of F3H-11 transgene had not accumulated detectable levels of sense*f3h* transcript and in contrast, sense transcript was detected in control flowers only. GC-MS headspace analysis was performed during the 4th month of flowering and found that the level of methyl benzoate was higher in flower of F3H-11 relative to control however, β-caryophyllene was not affected in transgenic carnation flowers.

The *C. breweri* BEAT gene (benzyl alcohol acetyl transferase for benzyl acetate synthesis) was inserted in Lisianthus to induce scent in the petals (Aranovich *et al.,* 2007). After being fed an alcoholic substrate, recorded observations showed that transgenic leaves and flowers produced volatile chemicals, including benzyl acetate (Noman *et. al.*, 2017). Recent years have seen much research on the processes involved in transcriptional regulation of scent biosynthesis (Muhlemann *et al.,* 2012) evidence suggests that many transcriptional variables play important roles in regulating fragrance emission (Colquhoun and Clark, employ various substrates (Pichersky *et al.,* 2006; Schwab, 2003). Endogenous genes also fall within this category. For instance, the limited internal pool of free salicylic acid in petunia flowers prevents the release of methylsalicylate despite the endogenous benzoic acid/salicylic acid carboxyl methyltransferase (PhBSMT) having higher catalytic efficiency with salicylic acid than benzoic acid. Consequently, the enzyme is in charge of producing methylbenzoate from the cells' large amount of benzoic acid (Negre, 200; 2011). Despite their tremendous usefulness, only a small number of TFs involved in regulating scent release have been found. Exclusively expressed *ODORNT1 (ODO1*) from petunia petals has been discovered to control the shikimate pathway (Verdonk *et al.,* 2005). *ODO1* was allegedly also implicated in the promoter activation of an unidentified ABC transporter that is based on the plasma membrane (Van Moerkercke *et al.*, 2012). Petunia *EOBI* (emission of benezoids 1) is an R2-R3 type transcription factor that specifically regulates flowering time and functions upstream of *ODO1* and downstream of EOBII. The suppression of this EOBI expression resulted in the down regulation of numerous genes associated to the shikimate pathway and scent (Spitzer-Rimon *et al.*, 2012). Spitzer *et al.,* (2007) induced silencing of *CHS* marker and target gene exemplified with *BSMT, PAAS* and *ODO 1* in petunia and they observed that *PTRV2-CHS* inoculated plants were visually evidenced by the appearance of white flowers due to reduction in anthocyanin and flavonoid contents, *PAAS* silencing led to strong reduction of phenylacetaldehyde and phenyl alcohol level and silencing of *ODO 1* reduced the level of several benzoid compounds emitted by flowers while *BSMT* silencing caused reduction in MeBA and MeSA. The transcriptional regulation of the terpenoids route is still unknown, despite the discovery and characterization of TFs controlling the phenyl propanoid/benezoid pathways. A few years ago, the expression of two sequiterpene synthase genes, TPS11 and TPS21, was found in the Arabidopsis inflorescence (Hong *et al.*, 2012).

Linalool synthase (LIS) from the blooms of *Clarkia breweri*, an annual native to California, was the gene that was most frequently employed in these first attempts (Dudareva *et al.*, 1996). Geranyl diphosphate (GPP), a monoterpene alcohol with a sweet, agreeable scent that is present in the flowers of many species, is transformed by TLIS into (3S)-linalool. *Petunia hybrida* LIS overexpression regulated by constitutive 35S promoter (petunia) (Lucker, 2004) and *Dianthus caryophyllus* (carnation) (Lavy *et al.,* 2002). In more recent investigations, three lemon monoterpene synthases were introduced into *Nicotiana tobacum* (tobacco) plants under the control of the constitutive 35S promoter to successfully alter the terpenoid volatile profilem (Lucker *et al.,* 2004). The specific volatiles generated in the flowers of transgenic plants will depend on the substrates available in the floral cells in which the trans gene is expressed because many of the enzymes for volatile biosynthesis may

Underwood *et al.,* 2005).

The removal of some volatile substances from the fragrance bouquet has also changed the phenylpropanoid/benzenoid floral aroma characteristics. Up till now, only petunia has been used for this work. With very minor alterations in the emission of other volatiles, transgenic petunia plants lacking the key fragrance component methylbenzoate were produced through RNAi-mediated silencing of the PhBSMT gene (Underwood *et al.,* 2005). Zvi *et al.*

(2008) studied co-engineering of scent and colour biosynthesis in flowers of petunia. They observed that transgenic plants expressing *Pap 1* exhibited increased level of anthocyanin upto nine fold relative to control and emission of benzaldehyde was increased three to five fold. However, during night time, *Phe* level was five to seven folds lower in transgenic as compared to control flower which was a result of increased utilization of *Phe*. $^{2}H_5$- Phe was more rapidly converted to benzaldehyde in transgenic flowers, resulting in 57 % labelling of total benzaldehyde compared with 30 % in limb of normal flowers. Semi-quantitative (RT-PCR) analysis indicated, higher expression of *C4H, F3h, DFR and* PAAS in transgenic lines. They also observed increased volatile emission by seven and nine folds by increasing benzaldehyde and methyl benzoate in Phe-fed *Pap-1* transgenic flowers during day. When the petunia benzylalcohol/ phenylethanol benzoyl transferase (PhBPBT) was silenced by RNAi, plants were produced whose flowers did not emit benzyl benzoate or phenylethyl benzoate, but all other volatiles were still released in the same amounts (Orlova *et al.*, 2006).

Table 1: Gene used in the metabolic engineering of [volatile] compounds.

Gene	Origin	Engineered species	Change in volatile spectrum	References
Linalool synthase	*Clarkia breweri*	Petunia plastid	linalool glycocoside ↑	Lucker *et al.*, (2001)
		Carnation plastid	(S)- linalool ↑, linalool oxide ↑	Lavy *et al.* (2002)
Linalool/nerolidol synthase	*Fragaria xananassa*	Araidopsisplastid	(S)- linalool ↑, hydroxylated andglycosylated linalool ↑, nerolidol ↑	Aharoni *et al.* (2003)
Limone synthase	*Perilla frutescens*	Tobacco plastid	limonene ↑	Ohara *et al*. (2003)
Geraniol synthase	*Ocimum basilicum*	Tomatoplastid	geraniol ↑ and its derivatise ↑	Davidovich- Rikanati *et al.* (2007)
Patchoulol synthase	*Pogostemoncablin*	Tobacco cytosol and plastid	Patchoulol ↑ and 13 sequiterpenes ↑	Wu SQ *et al.* (2006)
Terpene synthase TPS10	*Zea mays*	Arabidopsiscytosol	(E)-α-bergamotene ↑, (E)-β-farnesene ↑ and other herbivore – induced sesquiterpenes	Sachnee *et al.* (2006)
Germacrene A synthase	*Cichorium intybus*	Arabidopsis cytosol	germacrene A ↑	Aharoni *et al.* (2003)
Limonene-3-hydroxylase	*Mentha xpiperica*	Mentha *x* piperica ER	limonene ↑, menthone ↓, menthol ↓, menthofuran ↓, isomenthone ↓	Mahmoud *et al*. (2004)
	Mentha spicata	Tobacco ER	(+)-trans-isopiperitenol ↑ and its derivatives ↑	Lucker *et al.* (2004)
Menthofuransynthase	*Mentha xpiperica*	Mentha *x* piperica ER	menthofuran ↓, pulegone ↑, menthol ↑	Mahmoud and Croteau (2003)
BSMT	*Petunia hybrida*	Petunia	methylbenzoate ↓	Underwood *et al.* (2005)
PAAS	*Petunia hybrida*	Petunia	phenylacetaldehyde ↓, 2-phenylethanol ↓	Kaminaga *et al.* (2006)
BPBT	*Petuniahybrida*	Petunia	benzylbenzoate ↓ phenylethylbenzoate ↓, benzylalcohol ↑,benzylaldehyde ↑	Orlova *et al.* (2006)
CFAT	*Petunia hybrida*	Petunia	isoeugenol ↓	Dexter *et al.* (2007)
ODO1	*Petunia hybrida*	Petunia	volatile benzenoids ↓	Verdonk *et al.* (2005)
AAT	*Rosa hybrida*	Petunia	benzyl acetate ↑, phenylethyl acetate ↑	Guterman *et al.* (2006)
	Fragaria x anassa	Petunia	no change	Beekwilder *et al.* (2004)
BEAT	*Clarkia breweri*	Lisianthus	no change	Aranovich *et al.* (2007)
HPL	*Arabidopsis thaliana*	Arabidopsis	(E)-2-hexenol ↓, hexyl acetate ↓, C5 volatiles ↑	Salas *et al.* (2006)

AAT, alcohol acetyltransferases; BPBT, benzylalcohol/phenylethanol benzoyltranferase; BEAT, Acetyl- CoA:benzylalcohol acetyltransferase; CFART, coniferyl alcohol acetyltransferase; ODO1, ODORANT1; PAAS, phnyacetaldehyde synthase.

Table 2: Approaches used for metabolic engineering of floral scent.

Approach	Engineered species	Gene used	Result achieved	Olfactory effect	References
Introduction of a single gene	Petunia	CbLIS	Linalyl glucoside	No	Luker *et al.* (2001)
	Carnation	CbLIS	Linalyl oxides	No	Lavy *et al.* (2002)
	Petunia	RhAAT	Benzyl acetate and phenylethyl acetate	ND	Guterman *et al.* (2006)
Introduction of multiple genes	Tobacco	ClTER,ClLIM, ClPIN	γ-terpinene, limonene, and b-pinene and side products	Yes	Luker *et al.* (2004); El Tamar *et al.* (2003)
Introduction of multiple steps	Tobacco TERLIMPIN	MsLIM3H	Isopiperitenol andderivatives	ND	Lucker (2004)
Elimination of some compounds	Petunia	PhBSMT RNAi	Lacks methylbenzoate	Yes	Underwood *et al.* (2005)
		PhBPBT RNAi	Lacks benzylbenzoate and phenylethylbenzoate	ND	Orlova *et al.* (2006)
		PhPAASRNAi	Lacks phenylacetaldehydeand phenylethanol	ND	Kaminaga *et al.* (2006)
		PhCFAT RNAi	Lack of isoeugenol	ND	Dexter *et al.* (2006)
Blocking of competitive pathways	Carnation	Anti- DcF30 H	Increased methylbenzoate emission	Yes	Zuker *et al.* (2002)
Down-regulation of transcription factor	Petunia	PhODO1	Reduced levels of volatile benzenoids	ND	Verdonk *et al.* (2005)

Abbreviations: ClLIM, limonene synthase; ClPIN, b-pinene synthase; ClTER, Citrus limon g-terpinene synthase; CbLIS, Clarkia breweri linalool synthase; DcF30 H, Dianthus caryophyllus flavanoid 30 -hydroxylase; MsLIM3H, Mentha spicata limonene-3-hydroxylase; ND, not determined; PhBPBT, benzylalcohol/phenylethanol benzoyltransferase; PhBSMT, petunia benzoic acid/salicylic acid carboxyl methyltransferase; PhCFAT, coniferyl alcohol acyltransferase; PhODO1, ODORANT1 transcription factor; PhPAAS, phenylacetaldehyde synthase; RhAAT, Rosa hybrida alcohol acetyltransferase. Tobacco TERLIMPIN is a tobacco transgenic line expressing ClTERM, ClLIMand ClPIN.

CONCLUSION

We have made considerable advancements in the past several years in both the capacity to control the volatile spectrum in plants and the identification of the genes and enzymes involved in the manufacture of volatile chemicals (Table 1). A lack of a thorough understanding of plant metabolic networks and their regulation, as well as our limited understanding of network organization, the subcellular localization of the involved enzyme, competing pathways, metabolic channeling, flux-controlling steps, and potential feedback control, are highlighted by the fact that metabolic manipulations frequently produce unpredictable results. The discovery of essential chemicals involved in volatile-induced plant defenses, insect attraction, and their impacts on insect behavior in field experiments will also significantly aid in target selection. There is no doubt that it is now feasible to modify plants such that they can produce and release more volatiles from their blooms.

FUTURE SCOPE

The aforementioned examples demonstrate that metabolic engineering of flower smells is currently possible. However, the unique plant-animal air interactions will determine whether newly introduced "scent enzymes" can find suitable substrates and if the desired products will be generated and released at levels that can be detected by humans and other animals, including insects. Because of our limited knowledge of animal olfactorysystems and plant metabolic pathways, these factors cannot yet be anticipated. It is now necessary to be able to grow plants from callus tissue in order to genetically modify plants in general. Several cut flowers, notably commercially significant roses, chrysanthemums, carnations, and gerbera, have seen successful modifications to date, however for the majority of kinds it is still a "art form". Overall, it is evident that floral scent can be altered genetically, but this will require a more thoughtful design based on the right species selection, prior understanding of the involved pathways, including their cellular and subcellular localization, prudent use of promoters, and empirical testing.

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