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Metabolic engineering of plant volatiles

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Metabolic engineering of the volatile spectrum offers enormous potential for plant improvement because of the great contribution of volatile secondary metabolites to reproduction, defense and food quality. Recent advances in the identification of the genes and enzymes responsible for the biosynthesis of volatile compounds have made this metabolic engineering highly feasible. Notable successes have been reported in enhancing plant defenses and improving scent and aroma quality of flowers and fruits. These studies have also revealed challenges and limitations which will be likely surmounted as our understanding of plant volatile network improves.

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Introduction

Plants produce an amazing diversity of low molecular weight organic compounds known as secondary or specialized metabolites [1]. More than 1% of these metabolites are lipophilic molecules with low boiling points and high vapor pressures at ambient temperature. They are mainly represented by terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives and amino acid derivatives. These volatile compounds are released from leaves, flowers and fruits into the atmosphere and from roots into the soil. The primary functions of airborne volatiles are to defend plants against herbivores and pathogens, to attract pollinators, seed dispersers, and other beneficial animals and microorganisms, and to serve as signals in plant–plant interaction. The contribution of volatiles to plant survival and overall reproductive success in natural ecosystems, and their impact on agronomic and other commercial traits, including yield and food quality, suggest that modification of volatile production via genetic engineering has the potential to improve cultivated plant species.

Metabolic engineering requires a basic understanding of the biochemical pathways and the identification of the genes and enzymes involved in the synthesis of volatile compounds. In the last decade a renewed interest in these questions combined with technical advances have led to both a large increase in the number of plant volatiles identified as well as remarkable progress in discovering the genes and enzymes of volatile biosynthesis. Numerous attempts have been made to modulate volatile profiles in plants via metabolic engineering to enhance direct and indirect plant defense and to improve scent and aroma quality of flowers and fruits [2–5]. While a few projects have been successful in achieving the desired goals, many other attempts have resulted in meager enhancement of volatiles or in other unpredicted metabolic consequences such as further metabolism of the intended end products or deleterious effects on plant growth and development. In this review we highlight the latest advances in plant volatile research and discuss recent efforts to modify volatile traits, emphasizing the challenges and limitations of metabolic engineering of volatile profiles.

Improvement of plant defense via metabolic engineering

In the past two decades it has been well documented that in response to herbivore attack plants emit diverse volatile blends that may be composed of more than 200 different compounds [6]. These emitted volatiles can directly intoxicate, repel or deter herbivorous insects [7–12], or they may attract natural predators and parasitoids of the offending herbivores, thus indirectly protecting the signaling plant from further damage (e.g., tritrophic interactions) [13–15]. The growing number of reports on the involvement of volatiles in plant defense suggests that plant protection in agricultural and forest ecosystems can be enhanced via the modulation of the volatile spectrum by metabolic engineering, thereby providing an alternative pest-management strategy based on biological control [16]. However, several requirements must be fulfilled for successful improvement of plant defense [4]. First, the herbivore enemies capable of sufficiently controlling the herbivore population must be present in the locality where the crop is grown. Second, the introduced or enhanced plant volatile blends must provide key attractants for herbivore enemies, and volatile release must be synchronized with herbivore activity. Finally, the released volatiles should not increase the attractiveness of the plant to non-specific herbivores [17].

In addition to direct and indirect induced defenses, herbivore-induced volatiles can act as airborne signals

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that warn neighboring plants about the pathogen attack and prime them to respond more strongly against future insect attack [18••,19,20••] or serve as signals within a plant and prime systemic defenses [21,22••,23]. Although the molecular mechanisms underlying priming are unknown, priming prepares the plant or its undamaged parts for accelerated defense but delays the response until the actual herbivore attack. Since the defense network remains mostly dormant until actual herbivore attack priming-related costs are substantially lower than those of the induced direct defense, and the benefits of priming outweigh its costs when disease occurs [24•]. Priming crops by planting a few transgenic plants that constantly emit defense volatiles in the field (Figure 1) may offer an efficient form of plant protection and provide an advantage to non-transgenic receiver plants. However, a full use of this approach will only become possible with a comprehensive understanding of the molecular mechanisms of volatile-induced priming, the determination of the major volatile signal components that trigger it and their species specificity, and the identification of reliable molecular markers for the primed state.

Although it is likely that the array of volatiles emitted from a given species evolved as adaptations to specific challenges, it is not surprising that certain plant enemies have in turn evolved to take advantage of such emissions to locate their plant 'prey' for food, egg deposition, and the raising of their young. In addition, it was recently shown that volatiles released from plants can also provide chemical cues to parasitic weeds, the most damaging agricultural pests, for host location and discrimination [25]. An understanding of the parasite–host interactions and, in particular, the role of volatiles in these plant–plant interactions, will aid in the development of new tactics for the non-herbicidal control of weed populations via the metabolic alteration of the hosts' volatile spectrum.

Although to date very little is known about the attractive and repelling properties of specific plant volatiles, the key compounds involved in plant–insect and plant–plant interactions and the molecular mechanisms of their action, the modulation of the volatile spectrum has already been proven to be a useful strategy for enhancing volatile-based plant defenses. The overexpression of strawberry linalool/nerolidol synthase (*FaNES1*) targeted to chloroplasts resulted in transgenic Arabidopsis producing high levels of linalool which repelled the aphid *Mysus persicae* in dual-choice assays [11]. The ectopic expression of the same *FaNES1* gene in transgenic potato increased

Figure 1

Priming with plant beacons. A few transgenic plants engineered for continuous synthesis and emission of airborne signals can 'prime' a field of non-transgenics and thereby increase their ability to resist attacking herbivores and pests.

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the level of linalool and affected tritrophic interactions, creating transgenic plants that were more attractive to predatory mites than the uninfested wild-type plants [2,26]. Improvement of Arabidopsis indirect defense was also achieved by overexpressing *FaNES1* in mitochondria, which contains the sesquiterpene precursor farnesyl diphosphate (FPP). This manipulation led to the synthesis and emission of (*3S*)-(*E*)-nerolidol as well as the C_{11} homoterpene 4,8-dimethyl-1,3(*E*),7-nonatriene [(*E*)-DMNT], believed to be a degradation product of nerolidol, and rendered the plants attractive to the carnivorous predatory mites *Phytoseiulus persimilis*, natural enemies of spider mites [27•].

In another successful example, the overexpression of a maize terpene synthase gene (*TPS10*) in Arabidopsis resulted in transgenic plants with strong emission of several sesquiterpenes that are typically released (in maize) after herbivory by lepidopteran larvae. These transgenic plants were more attractive to the female parasitic wasp *Cotesia marginiventris*, which had had a previous oviposition experience with larvae of the potential host [28•]. Moreover, production of the volatile patchoulol and 13 additional sesquiterpene products in transgenic tobacco overexpressing patchoulol synthase (*PTS*) deterred tobacco hornworms, a majority of which had migrated away from leaves of the transgenic plants to the leaves of wild-type plants and consumed 20–50% more of the wild-type plants within six hours [29••].

Attempts at metabolic engineering of volatile signals involved in direct and indirect defenses have not been restricted to terpenoids. An increase in (*Z*)-3-hexenal, a major green leaf volatile, was achieved in transgenic tobacco plants overexpressing either the yeast acyl-CoA Δ9 desaturase or the insect acyl-CoA Δ11 desaturase. The expression of these transgenes resulted in elevated levels of 16:1 fatty acids and increased 13-lipoxygenase activity, which catalyzes the first step to hexenal production from α-linolenic acid [30]. While the effect of elevated levels of (*Z*)-3-hexenal on insect behavior was not investigated in this study, the negative effect of this compound on aphid performance was demonstrated in transgenic potato plants with reduced levels of the hydroperoxide lyase enzyme, which is responsible for the cleavage of fatty acid hydroperoxides to C_6 aldehydes [10].

These studies have demonstrated both the potential of genetic engineering for the improvement of plant defense as well as the role of some volatile compounds in plant–insect interactions. Despite this progress, they have also revealed the effect of genetic perturbations on plant growth and development, and uncovered some challenges to achieving efficient production of the desired volatile terpenoid compounds. In fact, the diversion of carbon to linalool production in Arabisopsis via *FaNES1* overexpression, while not effecting the levels of plastid-derived isoprenoids such as chlorophylls, lutein and β-carotene, led to a growth-retardation phenotype that was inherited through several generations of transgenic plants [11]. Emission of linalool in transgenic potato resulted in a more severe phenotype; in addition to growth retardation, plants had bleached leaves after their transfer from *in vitro* to the greenhouse [26]. Leaf chlorosis, vein clearing, and reduced stature were also observed in transgenic tobacco producing high levels of patchoulol as a result of the expression of *PTS* coupled with FPP synthase, both targeted to the plastids [29••]. These observed phenotypes could be the consequences of the depletion of isoprenoid precursors for other metabolites essential for plant growth and development, or possibly the toxic effects of the newly introduced terpenoids in plant cells.

For successful metabolic engineering of the volatile spectrum, it is important to produce and emit sufficient amounts of the desired compounds. However, the metabolic fate of newly synthesized compounds will be determined by the entire biochemical repertoire of the plant used. Since a complete understanding of the biochemical repertoire in any plant species is not available, it is difficult to predict how much of the desired compound will actually remain in the desired form. For example, the newly synthesized compounds may be affected by enzymes that are normally present in the cell and have broad substrate specificity, such as dehydrogenases, glucosyl transferases, and others [31]. Presently there is little knowledge of such enzymes in general and their specific distribution in different plant species. In fact, in transgenic Arabidopsis constitutively expressing *FaNES1*, part of the free linalool was subjected to hydroxylation and glycosylation by endogenous enzymes (and, as mentioned previously, when nerolidol was produced, some of it was degraded to the C_{11} homoterpene (*E*)-DMNT). Moreover, the total levels of glycosides of linalool and its derivatives were at least 10-fold higher than those of the free alcohols [11]. Interestingly, this glycosylation profile was different from that detected in linalool-producing transgenic potato, while the 8-hydroxy derivatives of linalool ((*E*)-8-hydroxy linalool, (*Z*)-8-hydroxy linalool and (*E*)-8-hydroxy 6,7-dihydrolinalool) were identical in both species [11,26].

The initial attempts to increase terpenoid production in transgenic plants showed that metabolic engineering of sesquiterpenes is a more challenging task and is not as straightforward as the generation of monoterpenes, which are formed exclusively or at least predominantly via the methylerythritol phosphate (MEP) pathway in the plastids. In many cases, FPP, which is expected to be produced in relatively large amounts for sterol biosynthesis, is not readily available for catalysis by introduced sesquiterpene synthases (reviewed in [5]). In addition, the contribution of the cytosolic mevalonic acid (MVA) and plastidic MEP pathways to sesquiterpene formation and

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thus trafficking of isoprenoid intermediates between organelles depends on the plant species, tissue and physiological state of the plant [32–35]. To date, the overexpression of *TPS10* and *PTS* in Arabidopsis and tobacco, respectively, represent the two most successful attempts at producing high levels of volatile sesquiterpenes by enzymes targeted to the cytosol [28•,29••]. However, to achieve emission of (*3S*)-(*E*)-nerolidol and (*E*)-DMNT in Arabidopsis, *FaNES1* had to be directed to the mitochondria [27•]. In addition, targeting *PTS* along with FPP synthase to the plastids increased the amount of produced patchoulol up to 100 times compared with its cytosolic formation [29••].

It has only recently been appreciated that plants emit volatile compounds from their roots into the rhizosphere [36–38]. Such volatiles may help the plant attract beneficial microorganisms and ward off harmful ones. They may also be useful in competition between plant species [39]. However, it has been shown that some parasitic plants use belowground volatile compounds to locate their hosts [38]. At present there are no reports of genetic engineering attempts to change root volatile emission in order to improve plant fitness; this is clearly a very fertile area for future work.

Metabolic engineering of floral volatiles

In contrast to metabolic engineering of vegetative volatiles where the effect of altered emission profiles on insect behavior was investigated, the impact of changes in floral scent on insect attraction has not yet been studied. Moreover, perception assessments have generally been limited to sensory evaluations by humans, whose odor threshold perception is much lower than that of insects [40,41]. In such experiments, metabolic engineering of floral volatiles was considered successful when the changes in scent profiles were significant enough for human detection. For example, the olfactorily detectable enhancement of volatiles emitted from flowers and leaves was achieved in transgenic tobacco via the introduction of three citrus monoterpene synthases [42•,43]. In another experiment, the redirection of the metabolic flux from the anthocyanin pathway towards benzoic acid in transgenic carnations resulted in an increase of methylbenzoate production which was sufficient for olfactory detection by humans [44]. However, many more attempts to modify the scent bouquet were less successful for different reasons including the absence of suitable substrates for the introduced reaction [45,46], modification of the scent compound into a non-volatile form [47], insufficient levels of emitted volatiles for olfactory detection by humans, or masking of introduced compound(s) by other volatiles [48].

The elimination of some volatile compounds from the floral bouquet is another approach which has recently been used for scent modifications. Transgenic petunias lacking methylbenzoate [49], phenylacetaldehyde [50], benzylbenzoate and phenylethylbenzoate [51••], and isoeugenol [52] were obtained via RNAi-mediated posttranscriptional gene silencing. The effect of these changes on human perception has not yet been tested with the exception of the plants with lower levels of methylbenzoate emission. In this case, the panelists reacted negatively by complaining that flowers were less fragrant [49].

Improvement of aroma quality of fruits, vegetables and herbs

Volatiles are important determinants of the overall aroma properties and taste of fruits [53]. In nature, volatiles contribute to seed dispersion by increasing fruit attractiveness. Volatiles released from vegetative parts of plants may also be attractive to some animals or insects as foodstuff, even though they are unpalatable to most other herbivores.

The presence of volatiles in fruits, vegetables and herbs has important influence on the cultivation of many plant species. As extensive breeding programs are undertaken to maximize certain attributes of foodstuff – for example, overall yield (i.e. size), total solids, sugar content, or pigmentation – less attention is devoted to enhancing or even maintaining volatile production. As a result many current cultivars of domesticated plant species produce less volatiles than their wild relatives or earlier cultivars [54].

Reintroduction of aroma volatiles can be achieved by classical breeding, as was done in tomato by crossing it with its relative *L. peruvianum* [55]. However, this is a laborious and time-consuming process which requires the monitoring of a complex trait. For example, volatile collections and analyses must initially be done with expensive gas chromatography–mass spectrometry (GC–MS) instruments and subsequently human evaluations must also be performed by subjective test panels as it is not yet possible to predict how humans will react to a given mixture of volatile compounds. Human evaluation of smells is particularly subjective because of interspecific variation in the ability to detect specific compounds and the lack of shared vocabulary to describe specific smells. These complications have indeed contributed to the lack of emphasis in most breeding programs on the aroma of produce.

Genetic engineering can ameliorate some drawbacks of classical plant breeding and enhance aroma of fruits. One advantage of this approach is that it is less complex – introducing a single trait at a time. Another is that it allows the introduction of genes whose coding information may not be present in the cultivar. Several recent reviews have enumerated general problems and pitfalls of genetic engineering for biochemical traits (e.g. [56,57]), therefore we will not repeat these caveats here, with the exception of two worth mentioning again. First is that the addition

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Table 1

Genes used in the metabolic engineering of [volatile] compounds

Gene	Origin	Engineered species	Changes in volatile spectrum	Reference
Linalool synthase	*Clarkia breweri*	tomato plastid	(*S*)-linalool ↑, 8-hydroxylinalool ↑	[61]
		petunia plastid	linalool glycoside ↑	[47]
		carnation plastid	(*S*)-linalool ↑, linalool oxide ↑	[48]
Linalool/nerolidol synthase	*Fragaria* x *ananassa*	Arabidopsis plastid	(*S*)-linalool ↑, hydroxylated and glycosylated linalool ↑, nerolidol ↑	[11]
		potato plastid	linalool ↑, hydroxylated and glycosylated linalool ↑	[26]
		Arabidopsis mitochondria	(3*S*)-(*E*)-nerolidol ↑ (*E*)-DMNT ↑	[27]
Limonene synthase	*Perilla frutescens*	tobacco plastid	limonene ↑	[66]
		tobacco cytosol	limonene ↑	[66]
		tobacco ER	no changes	[66]
	Mentha spicata	peppermint plastid	no changes	[67]
		Mentha arvensis and *M.* x *piperita* plastids	no significant changes	[65, 68]
γ-Terpinene synthase β-pinene synthase	*Citrus limon* *Citrus limon* *Citrus limon*	tobacco plastid	γ-terpinene ↑, limonene ↑, β-pinene ↑ and side products ↑	[42]
Geraniol synthase	*Ocimum basilicum*	tomato plastid	geraniol ↑ and its derivatives ↑	[62]
Patchoulol synthase	*Pogostemon cablin*	tobacco cytosol and plastid	patchoulol ↑ and 13 sesquiterpenes ↑	[29]
Terpene synthase TPS10	*Zea mays*	Arabidopsis cytosol	(*E*)-α-bergamotene ↑, (*E*)-β-farnesene ↑, other herbivore-induced sesquiterpenes ↑	[28]
Germacrene A synthase	*Cichorium intybus*	Arabidopsis cytosol	germacrene A ↑	[11]
Limonene-3-hydroxylase	*Mentha x piperita*	*Mentha x piperita* ER	limonene ↑, menthone ↓, menthol ↓, menthofuran ↓, isomenthone ↓	[65]
	Mentha spicata	tobacco ER	(+)-*trans*-isopiperitenol ↑ and its derivatives ↑	[69]
Menthofuran synthase	*Mentha x piperita*	*Mentha x piperita* ER	menthofuran ↓, pulegone ↓, menthol ↑	[64]
BSMT	*Petunia hybrida*	petunia	methylbenzoate ↓	[49]
PAAS	*Petunia hybrida*	petunia	phenylacetaldehyde ↓, 2-phenylethanol ↓	[50]
BPBT	*Petunia hybrida*	petunia	benzylbenzoate↓, phenylethylbenzoate ↓, benzylalcohol ↑, benzylaldehyde ↑	[51]
CFAT	*Petunia hybrida*	petunia	isoeugenol ↓	[52]
AADC	*Solanum lycopersicum*	tomato	phenylacetaldehyde ↑, 2-phenylethanol ↑, 1-nitro-2-phenylathane ↑	[70]
ODO1	*Petunia hybrida*	petunia	volatile benzenoids ↓	[71]
PAR	*Solanum lycopersicum*	petunia	2-phenylethanol ↑, phenylacetaldehyde ↓	[72]
AAT	*Rosa hybrida*	petunia	benzyl acetate ↑, phenylethyl acetate ↑	[73]
	Fragaria x *ananassa*	petunia	no changes	[45]
BEAT	*Clarkia breweri*	lisianthus	no changes	[46]
CCD	*Solanum lycopersicum*	tomato	β-ionone ↓, pseudoionone ↓, geranylacetone ↓	[74]
		petunia	β-ionone ↓	[75]
ADH	*Solanum lycopersicum*	tomato	hexanol ↑, (*Z*)-3-hexenol ↑	[59]
acyl-CoA Δ9 desaturase	*Saccharomyces cerevisiae*	tobacco	*cis*-3-hexenal ↑, *trans*-2, 4-hexadienal ↑	[30]
		tomato	*cis*-3-hexenol ↑, 1-hexanol ↑, hexanal ↑, *cis*-3-hexenal ↑, 6-methyl-5-hepten-2-one ↑, 2-isobutylthiazole ↑	[58]
acyl-CoA Δ11 desaturase	*Trichoplusia ni*	tobacco	*cis*-3-hexenal ↑, *trans*-2, 4-hexadienal ↑	[30]
β-glucosidase	*Aspergillus niger*	tobacco	2-ethylhexanol ↑, β-caryophyllene ↑, cembrene ↑, linalool ↑, nerolidol ↑, furanoid *cis*-linalool oxide ↑, 4-methyl-1-pentanol ↑, 6-methyl-hept-5-en-2-ol ↑	[76]
LOX (TomloxC)	*Solanum lycopersicum*	tomato plastid	hexanal ↓, hexenal ↓, hexenol ↓	[77]
(NaLOX3)	*Nicotiana attenuata*	tobacco	no changes in the wound-induced GLVs	[78]
(LOXH1)	*Solanum tuberosum*	potato	hexanal ↓, (*E*)-2-hexenal ↓, hexanol ↓, (*Z*)-3-hexenol ↓, (*E*)-2-hexenol ↓	[79]
HPL	*Solanum tuberosum*	potato	hexanal ↓, 3-hexenal ↓, C5 volatiles ↑	[10, 79]
	Nicotiana attenuata	tobacco	GLVs ↓	[80]
	Arabidopsis thaliana	Arabidopsis	(*E*)-2-hexenol ↓, hexyl acetate ↓, C5 volatiles ↑	[81]
AOS	*Nicotiana attenuata*	tobacco	GLVs ↓, the wound-induced (*Z*)-3-hexenal ↑	[80]

AADC, aromatic L-amino acid decarboxylase; AAT, alcohol acetyltransferases; ADH, alcohol dehydrogenase; AOS, allene oxide synthase; BPBT, benzylalcohol/phenylethanol benzoyltransferase; BSMT, benzoic acid/salicylic acid carboxyl methyltransferase; CCD, carotenoid cleavage dioxygenase; CFAT, coniferyl alcohol acetyltransferase; GLV, green leaf volatiles; HPL, hydroperoxide lyase; ODO1, ODORANT1; PAAS, phenylacetaldehyde synthase; PAR, 2-phenylacetaldehyde reductase; LOX, lipoxygenase.

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of a single gene is unlikely to result in a substantial production of the desired volatile if the formation of this compound is the end result of a long metabolic pathway. Second, and perhaps even more important, is that a single new volatile is generally unlikely to change the consumers' overall perception regarding the flavor and aroma quality of produce.

The overexpression of a yeast Δ9-desaturase [58] and a non-specific alcohol dehydrogenease (ADH) [59,60] in tomato fruit were early attempts to circumvent these problems. In these cases, the concentrations of various aroma compounds derived from fatty acids such as (*Z*)-3-hexenol, (*Z*)-3-hexenal and/or the ratios of the aldehydes to alcohols changed. The higher levels of alcohols in transgenic fruits were associated with more intense ripe flavor by taste panelists [59]. However, neither of these manipulations introduced new aroma compounds.

The introduction of the *Clarkia breweri* linalool synthase (*LIS*) gene into tomato under the control of the fruit-specific *E8* promoter was the first attempt at adding a new compound to fruit flavor. It resulted in the accumulation in the fruit of small amounts of linalool and its oxidation product, 8-hydroxylinalool, which were detectable by both GC–MS and the human nose [61]. This metabolic manipulation was accomplished because linalool is produced from geranyl diphosphate (GPP) by LIS in a single step and GPP is an intermediate in the synthesis of carotenoids, a pathway that is highly active in ripening tomato fruits. The synthesis of linalool and 8-hydroxylinalool did not affect the total amounts of carotenoids produced by the fruit; however, the amounts of these monoterpenes were also not sufficient to substantially change the overall flavor perception by humans (Lewinsohn and Pichersky, unpublished data).

A much stronger effect on flavor perception was recently achieved by Davidovich-Rikanati *et al.* [62••] by expressing geraniol synthase (*GES*) in tomato under the polygalacturonase promoter, another fruit ripening-specific promoter. Geraniol is also an acyclic monoterpene alcohol that is synthesized in one step from GPP. Unlike linalool, which is a tertiary alcohol whose hydroxyl group cannot be further oxidized, geraniol is a primary alcohol that can easily be oxidized to geranial by non-specific alcohol dehydrogenases [62••]. *GES*-transgenic tomato fruits synthesized large amounts of geraniol, which led to a noticeable decrease in pigmentation. Moreover, transgenic fruits further metabolized geraniol to geranial, which underwent spontaneous tautomerization to neral. Neral and geranial together make a mixture called citral, which imparts a strong lemon flavor. Geranial and neral were also further metabolized to geranic and neric acids, respectively. Additional modifications of geranial and neral resulted in the formation of nerol, citronellol, citronellal, citronelic acid, citronellyl acetate, and rose oxide [62••]. When these transgenic fruits were evaluated by a test panel of 34 people, the majority of participants (80%) indicated that the fruits had stronger aroma, and more than 60% of the panel members preferred the transgenic fruits over the non-transgenic ones.

Attempts to modify vegetative plant volatile production for human consumption have lagged behind the efforts made with tomato fruit. However, recent work in peppermint on boosting the production of terpenes favored by humans (e.g. menthol) and decreasing the synthesis of unfavored compounds (e.g. menthofuran) were partially successful [63,64,65]. Using antisense technology, transgenic plants were obtained that had a 50% reduction in menthofuran concentration, while other transgenic plants showed some increase in the concentrations of limonene, a cyclic monoterpene. However, an assessment of consumer responses to the aroma properties of these transgenic mint plants has not yet been reported.

Conclusions

In the past several years we have witnessed significant progress in both identifying genes and enzymes involved in the biosynthesis of volatiles compounds and our ability to manipulate the volatile spectrum in plants (Table 1). However, metabolic manipulations often yield unpredictable results, highlighting our lack of a comprehensive understanding of plant metabolic networks and their regulation, including our rudimentary knowledge concerning network organization, the subcellular localization of the enzyme involved, competing pathways, metabolic channeling, flux-controlling steps and possible feedback control. Additional molecular and biochemical characterization in combination with metabolic flux analysis and computer assisted modeling [51••] must be carried out to provide the theoretical foundation for successful manipulation of the volatile spectrum and to identify targets for future metabolic engineering. The identification of key compounds involved in volatile-induced plant defenses, as well as insect attraction, and their effects on insect behavior in field studies, will also greatly contribute to target selection.

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