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Role of Biotechnology and Combinatorial Chemistry Approaches in Molecular-Assisted Engineering of Plant Volatile Compounds

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Abstract

Plants produce a wide range of volatile compounds, which plays a crucial role in their growth, development, and interactions with the environment. In recent years, biotechnology and combinatorial chemistry approaches have enabled the efficient and precise manipulation of metabolic pathways, leading to the production of new or improved plant-based products with desired properties. Biotechnology approaches, such as metabolic engineering and synthetic biology, have emerged as powerful tool for genetic modification of plant metabolic pathways to produce targeted volatiles. Combinatorial chemistry approaches, on the other hand, involve the screening and optimization of large chemical libraries to identify novel compounds with desirable properties. The combination of these two approaches provides a powerful platform for the molecular engineering of plant volatile compounds, enabling the development of new fragrances, flavors, and functional ingredients for use in a wide range of applications, including food, cosmetic, and pharmaceutical industries. Additionally, the molecular engineering of plant volatile compounds has the potential to address global challenges, such as food security and sustainability, by providing new and improved plant-based products with enhanced nutritional and functional properties. In conclusion, the use of biotechnology and combinatorial chemistry approaches in the molecular engineering of plant volatile compounds is a rapidly growing area with significant potential to revolutionize the production of plant-based products and address global challenges.

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11.1 Introduction

Plants being sessile develop diverse strategies to compensate for this and protect themselves against challenges. One of these is the synthesis of several metabolic compounds that act as the language for plants to communicate with surrounding environments and with themselves. These compounds are grouped into two major categories: primary and secondary (Theis and Lerdau 2003). Only a small number of the incredible diversity of metabolites that plants create constitute the 'primary' metabolic pathways. These are commonly present in all species. Others, referred to as 'secondary' metabolites, are exclusive to smaller groups of plants (Pichersky and Gang 2000). These secondary metabolites are the results of many plant responses or certain demands. Volatile organic compounds (VOCs) have a starring role among these secondary metabolites (Dicke and Loreto 2010). VOCs are lipophilic low molecular weight compounds having high vapor pressure and a low boiling point at ambient temperatures. These properties make them amenable to cross the cellular membrane and being released into the environment (Pichersky et al. 2006). Plant VOCs can be released constitutively or in response to a range of stimuli and are produced by every type of tissue and vegetation as nitrogen-containing chemicals, green leaf volatiles, and aromatic compounds (Peñuelas and Llusià 2001; Dudareva et al. 2006; Holopainen and Gershenzon 2010; Holopainen et al. 2010). Intriguingly, VOC synthesis depends on the plant species as different plants utilize different metabolites to tackle similar problems. For instance, different flowers produce different odorous volatiles for attracting the same type of pollinator. The VOCs perform multifaceted functions in the plant system, namely, attract the pollinators, plant-to-plant communication, defense against predators and insects, and adaptability to environmental stress and heat (Dudareva and Pichersky 2000; Mumm et al. 2003; Baldwin et al. 2006; Heil and Karban 2010; War et al. 2012). They specifically contribute to food quality, biomass output, reproductive success, and many other significant agronomic aspects (Dudareva and Pichersky 2008).

The current breeding and crop improvement programs for enhanced growth and yield had a negative impact on secondary metabolism, leading to a deficiency in VOC production in many current crop cultivars. For instance, *Theobroma cacao* blooms are tiny, hardly scented that only draw opportunistic flies and ineffective pollinators. On the other hand, wild *Theobroma* sp. flowers are huge, aromatic, and attract bees for their pollination (Young & Severson, 1994). The same patterns were seen for vegetal VOCs. The corn domestication resulted in the loss of (E)-b-caryophyllene which attracts pest-eliminating parasitic wasps and entomopathogenic nematodes thus, makes the plants more susceptible to insect pests (Tamiru et al., 2011). Similarly, growth-defense trade-offs are also seen in even more recent cultivated crops. For instance, cranberries are derived from *Vaccinium* sp. wild progenitor. However, compared to their lower-yielding relatives, high-yielding cranberry genotypes exhibit suppressed herbivore-induced sesquiterpene emissions (Rodriguez-Saona et al., 2011). Together, these demonstrate how poorly crop VOCs are adapted to support beneficial insects in agricultural settings. Additionally, despite their increased size and intensity, agricultural monocultures are becoming a bigger part of modern agriculture. Because of this decline in plant variety, the ecosystem services supplied by the arthropod community such as pest control by beneficial insects and crop pollination are negatively impacted (Gardiner et al., 2010). Therefore, methods must be developed to address these issues and engineering VOCs is a viable technique for enhancing plant chemical variety as well as beneficial insect diversification.

These crucial functions of VOCs and their loss during crop domestication and improvement showed a great potential for biotechnological exploitation (Aharoni et al. 2005). Understanding the fundamental biochemical processes and identifying the genes and enzymes involved in the creation of volatile chemicals are prerequisites for engineering the VOCs using biotechnological approaches. Additionally, the significant rise in the discovery of the genes and enzymes of volatile biosynthesis have resulted from a resurgence of interest in these subjects during the past 10 years. To date, numerous attempts have been undertaken to modify the volatile profiles of plants – guarding against infections and abiotic stress, discouraging herbivores, affecting tritrophic relationships, and enhancing fruit and floral fragrances. The most evident biotechnological modulation application on these VOCs is modulation of their emissions in plants to enhance or repress their activities in order to accomplish specified outcomes. While some initiatives have succeeded in reaching their objectives, many others have only modestly improved volatiles or had unanticipated metabolic impacts, such as increased metabolism of the desired end products or harmful effects on plant growth and development.

Here, in this chapter, we will briefly go over the production of VOCs, talk about bioengineering strategies and targets, and use case studies to demonstrate recent developments in these fields. We also offer an outlook on where these innovative and intriguing technologies might take us in the future.

11.2 Classes and Biosynthetic Pathways of VOCs

The biosynthesis of VOCs depends on the amount of building blocks present in the cells that is, carbon, nitrogen, and sulfur. On the basis of biosynthetic origin and chemical constitution, VOCs are divided into several classes: isoprenoids and terpenoids. In some cases, oxygenated VOCs (OVOCs), such as acetaldehyde (C_2H_4O), acetone (C_3H_6O), methyl-ethyl-ketone (MEK, C_4H_8O), methanol (CH_4O), and methyl-vinyl-ketone (MVK, C_4H_6O), sulfur compounds (Brassicales), and furanocoumarins (Asterales, Apiales, Fabales, and Rosales), are also reported (Berenbaum and Zanger 2008; Agrawal 2011). Herein, we will discuss the classes of VOCs:

11.2.1 Terpenoids

Terpenoids constitute the largest and most diverse class of VOCs. In plants, terpenoids biosynthesis was conducted through two pathways: the mevalonic acid (MVA) and methylerythritol phosphate (MEP). Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) serve as the main precursor for this biosynthesis pathway (McGarvey and Croteau 1995). Further, the MVA produces precursor for volatile sesquiterpenes and MEP pathway for monoterpenes (C10), diterpenes (C20), and hemiterpenes (C5). The MEP pathway occurs in plastid (C15) (Hsieh et al. 2008). On the other side, site for MVA pathway is unclear as previously it was shown to happen in cytosol later evidences suggest its location in endoplasmic reticulum and peroxisome (Simkin et al. 2011; Pulido et al. 2012). According to Winterhalter and Rouseff (2001), plants also produce erratic volatile terpenoids with carbon skeletons ranging from C8 to C18 that are derived from carotenoids through a three-step modification process that starts with dioxygenase cleavage followed by enzymatic transformation, and is finally converted to volatile compounds by acid. In rare instances, such as in *Arabidopsis*, tomato, petunia, and melon, the dioxygenase cleavage process itself can produce a volatile product from a variety of carotenoid pigments, such as a- and b-ionone, geranylacetone, and pseudoionone. The acyclic C11- and C16-homoterpenes 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) and 4,8,12-dimethylnona-1,3,7-triene (DMNT) are irregular and are produced from GGPP and FPP, respectively. A cytochrome P450 monooxygenase-catalyzed oxidative breakdown follows the creation of the tertiary C15- and C20-alcohol precursors (E)-nerolidol and (E, E)-geranyl linalool, respectively, in the first of two enzymatic steps that make up their biosynthesis.

11.2.2 Phenylpropanoid and Benzenoid Compounds

The second largest VOCs are phenylpropanoid and benzenoid. They are synthesized from the phenylalanine (Phe) (Knudsen et al. 2006). Central carbon metabolism and Phe are linked via seven enzyme processes of the shikimate pathway and three of the arogenate pathway (Maeda & Dudareva, 2012). Phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate (E4P), which are the direct precursors of the shikimate pathway, are produced by glycolysis and the PPP, respectively. Since the MEP system depends on the same pathways for its precursors, it must compete with the shikimate/phenylpropanoid pathway for carbon allocation, especially since 30% of photosynthetically fixed carbon is diverted to Phe, mostly for the production of lignin (Razal et al., 1996). The 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHP synthase), the first gene in the shikimate pathway, is crucial for regulating the carbon input into the process. The molecular processes behind this control in plants are, however, still largely unexplored.

11.2.3 Fatty Acid Derivatives

Next class of VOCs are fatty acid derivative compounds such as cis-3-hexenol, 1-hexanal, methyl jasmonate, and nonanal. These are synthesized from linoleic and linolenic, that is, the C_{18} unsaturated fatty acids. A plastidic pool of acetyl-CoA produced from pyruvate (Pyr), the end result of glycolysis, is necessary for the biosynthesis of these fatty acids. Unsaturated fatty acids undergo stereospecific oxygenation once they enter the lipoxygenase (LOX) route, resulting in the intermediates 9-hydroperoxy and 13-hydroperoxy, which are then further metabolized via the two branches of the LOX pathway, producing volatile chemicals. Only the 13-hydroperoxy intermediate is used by the allene oxide synthase branch, which results in the synthesis of jasmonic acid (JA), which is then changed into methyl jasmonate by JA carboxyl methyl transferase. The hydroperoxide lyase branch, on the other hand, transforms both varieties of hydroperoxide fatty acid derivatives into C6 and C9 aldehydes, which are frequently reduced to alcohols by alcohol dehydrogenases, and then further transformed into their esters. These saturated and unsaturated C6/C9 aldehydes and alcohols, often known as green leaf volatiles, are typically produced by plants' green organs in reaction to injury but also give fruits and vegetables their distinctive fresh green scent.

11.2.4 Branched Chain Amino Acid Derivative

Numerous amino acids, including alanine, valine, leucine, isoleucine, and methionine, as well as intermediates in their biosynthesis, are the source of many volatile compounds, particularly those that are highly abundant in floral scents and fruit aromas. These compounds also contain sulfur and nitrogen. These amino acid-derived volatiles production in plants is thought to follow a similar path to that in yeast or bacteria, where these pathways have been more thoroughly investigated. Aminotransferases, much like in microorganisms, catalyze the first deamination or transamination of the amino acids, which produces the α-ketoacid. Decarboxylation, reduction, oxidation, and/or esterification can be applied to these α-ketoacids in order to produce aldehydes, acids, alcohols, and esters. Further, alcohol acyltransferases (AATs) catalyze alcohol esterification processes, and amino acids can serve as the precursors of these acyl-CoAs.

11.3 VOCs Engineering: The Prerequisite Criteria

To modify the VOCs for beneficial purposes can be achieved by four ways: (1) extract the VOCs from naturally occurring species, (2) create it using synthetic chemistry, (3) breed or engineer desired features in the target organism, and (4) engineer excessive VOCs production in a model organism. For a particular need, one or all of these methods may be appropriate.

Where the desired VOC is abundant, extraction from natural sources may be feasible; nevertheless, natural sources infrequently provide enough for widespread industrial applications, and the volatility of these chemicals makes harvesting potentially impractical. Since almost two centuries, chemical synthesis has been used to create chemicals that are useful for agriculture; however, in the case of VOCs, these compounds are either overly complex or too expensive to synthesize at the required yields. Also, no synthetic pathway has been developed thus, making the production impossible. Furthermore, accurate chirality of these compounds is frequently needed for biological activity and the synthetic synthesis is frequently not enantiospecific. In these circumstances, utilizing living organisms for production might be the only practical option. Since harvesting, transport, and application are not required, the production of the desired VOCs in the host/target organism can provide the benefit in most effective way. This process involves breeding and genetic engineering approach. However, this approach is also very complicated and requires a detailed understanding of the biology and biochemistry in a specific system. If the desired feature is present in the germplasm of interest, desirable qualities can be introduced through selective breeding and backcrossing. Despite the fact that some crop species, like maize, have genotypes that can contribute desirable volatile components (Schnee et al. 2006), backcrossing to introduce these components into elite agricultural cultivars can be a time-consuming operation. Genetic manipulation can be employed to acquire genes from unrelated species when such germplasm is not present within the species. Even when germplasm is available, the latter method may be faster for cultivar development; however, the testing and regulatory requirements for genetically modified organisms (GMOs) may mean that the final product takes just as long as possible to reach the market.

Bioproduction in microbial platforms like *Saccharomyces cerevisiae* or *Escherichia coli* may offer a solution in cases where natural sources are unable to produce the desired traits or yield, chemical synthesis is impossible, and/or breeding and genetic engineering are impractical for biological, technological, or sociopolitical reasons. Nowadays, for a wide range of different biochemicals, production in tightly regulated bioprocesses using engineered microorganisms is emerging as the preferred synthesis route (Vickers et al. 2012).

11.4 Molecular-Assisted Engineering of VOCs

Today, the engineering of VOCs was performed in **(i)** plants and **(ii)** microbes. In plants, this involves breeding and genetic engineering approaches.

11.4.1 Engineering the Floral Volatiles

The impact of changes in floral aroma on insect attractiveness has not yet been addressed, in contrast to metabolic engineering of vegetative volatiles where the effect of changing emission profiles on insect behavior was investigated.

Furthermore, as humans have a much lower odor threshold than insects, perception tests have often only included sensory evaluations (Vosshall 2000; Stockhorst and Pietrowsky 2004). The metabolic engineering of floral volatiles in these trials was deemed successful when the alterations in scent profiles were detectable by humans. For instance, the introduction of three citrus monoterpene synthases to transgenic tobacco allowed for the olfactorily perceptible augmentation of volatiles released from flowers and leaves (El Tamer et al. 2003; Lücker et al. 2004). In a different experiment, transgenic carnations with metabolic flux redirected from the anthocyanin route to benzoic acid produced more methylbenzoate, which was enough for human olfactory detection (Zuker et al. 2002). However, many more attempts to alter the scent bouquet failed for a variety of reasons, such as the lack of appropriate substrates for the introduced reaction (Aranovich et al. 2007), transformation of the scent compound into a nonvolatile form (Lücker et al. 2001), insufficient levels of emitted volatiles for human olfactory detection (Lavy et al. 2002), or masking of the introduced compound(s) by other volatiles.

Another strategy that has lately been employed for smell modification is the removal of some volatile components from the floral bouquet. Using RNA interference (RNAi)-mediated posttranscriptional gene silencing, it was possible to create transgenic petunias 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). With the exception of the plants that emit less methylbenzoate, the impact of these modifications on human perception has not yet been investigated. The panelists have unfavorable response in this cases as according to them the modified flowers were not able to produce desired level fragrance (Dexter et al. 2007).

11.4.2 Improvement of Aroma Quality of Fruits, Vegetables, and Herbs

The olfactory characteristics and flavor of fruits are significantly influenced by volatiles (Goff and Klee 2006). In the natural world, volatiles make fruits more alluring thus helping in the spreading of the seeds. Several efforts have been made to enhance the aroma of fruits by utilizing breeding strategies. For instance, in tomato, crossing with its relative *L. peruvianum* reintroduced the desired volatile compounds thus, enhancing the aroma (Kamal et al. 2001). However, the monitoring of the complicated aroma trait is necessary for this process which is time-consuming and tedious process. Since it is still not possible to forecast how people would react to a certain mixture of volatile substances and volatile collections an analysis must first be carried out with pricey GC-MS instruments, and human evaluations must then additionally be carried out by subjective test panels. Further, due to interspecific diversity in the capacity to identify particular molecules and the lack of a common vocabulary to characterize particular odors, human perception of smells is very subjective. In fact, these issues have made it less common for breeding initiatives to prioritize the aroma trait.

Some of the shortcomings of traditional plant breeding can be abridged by genetic engineering and aroma trait of the fruits can be improved. Genetic engineering has the benefit of being less complicated because it introduces one attribute at a time (McCaskill and Croteau 1998; Pichersky and Dudareva 2007). The insertion of genes whose coding information may not be present in the crop is another benefit. However, the general issues and traps of genetic engineering for biochemical properties are as follows: (1) If the generation of the desired volatile is the end result of a lengthy metabolic pathway, the addition of a single gene is unlikely to result in a significant production of the desired volatile. (2) A single new volatile is often improbable to modify the consumers' perceptions about the aroma and flavor quality of the produce.

Early attempts to solve these issues involved overexpression of the yeast D9-desaturase (Wang et al. 1996) and a nonspecific alcohol dehydrogenase (ADH) (Speirs et al. 1998; Prestage et al. 1999) in tomato fruit. The amounts of different aroma compounds such as (Z)-3- hexenol and (Z)-3- hexenal as well as the proportions of aldehydes to alcohols were altered in these transgenics. As per taste panelists, the higher alcohol content in these transgenic fruits provided more potent ripe flavors (Speirs et al. 1998); however, none of these changes added any novel scent components.

The first attempt to introduce a new compound to fruit flavor was the introduction of the *Clarkia breweri* linalool synthase (LIS) gene into tomato under the control of the fruit specific E8 promoter. As a result, small amount of linalool and its oxidation product, 8-hydroxylinalool, were accumulated in the fruit, and both substances could be detected by GC-MS as well as human nose (Lewinsohn et al. 2001). This metabolic modification was successful because linalool is produced by LIS from geranyl diphosphate (GPP) in a single step and GPP is a precursor in the synthesis of carotenoids, a pathway that is very active in ripening tomato fruits.

Davidovich-Rikanati et al. (2007) expressed geraniol synthase (GES) in tomato under the promoter of polygalacturonase, another fruit ripening-specific promoter, to produce a considerably higher influence on flavor perception. Geraniol is another acyclic monoterpene alcohol that can be made from GPP and can easily be converted to geranial by nonspecific alcohol dehydrogenases, in contrast to linalool, which is a tertiary alcohol and its hydroxyl group cannot be further oxidized (Davidovich-Rikanati et al. 2007). Large levels of geraniol were produced by GES-transgenic tomato fruit, which significantly reduced pigmentation. Additionally, geraniol was further metabolized by transgenic fruits into geranial, which naturally tautomerized into neral. Citral, a concoction made from neral and geranial, has a powerful lemon flavor. Additionally, geranial and neral underwent metabolism to form geranic and neric acids, respectively. Nerol, citronellol, citronellal, citronelic acid, citronellyl acetate, and rose oxide were created by further modifications of geranial and neral (Davidovich-Rikanati et al. 2007). A test panel of 34 persons evaluated these transgenic fruits, and the majority of the participants (80%) said that the fruits had a stronger aroma and that they preferred the transgenic fruits over the non-transgenic ones.

The development of volatile plant production in vegetative plants for human consumption has lagged behind that of tomato fruit. However, recent efforts to increase the production of terpenes that humans prefer (such as menthol) and decrease the synthesis of unfavorable substances (such as menthofuran) in peppermint have only partially been effective (Mahmoud and Croteau 2001, 2003; Mahmoud et al. 2004). While other transgenic plants showed a slight increase in the amounts of limonene, a cyclic monoterpene, antisense technology was used to create transgenic plants with a 50% drop in the concentration of menthofuran. However, a review of consumer reactions to these transgenic mint plants fragrance qualities has not yet been conducted.

11.4.3 Engineering the VOCs for Plant Defense

It has been widely established over the past decades that plants release more than 200 distinct volatile chemical compounds in response to herbivore attacks (Dicke and Van Loon 2000). These released volatiles may directly inebriate, repel, or discourage herbivorous insects (Bernasconi et al. 1998; Kessler and Baldwin 2001; Vancanneyt et al. 2001; Aharoni et al. 2003; Seybold et al. 2006) or they may draw predators and parasitoids that would ordinarily prey on the offending herbivores, thereby indirectly protecting the signaling plant from further harm (such as through tritrophic interactions) (Arimura et al. 2004; Degen et al. 2004; Mercke et al. 2004). The progression in biotechnology approaches and growing research indicating the role of VOCs in plant defense attracts scientists to modulate the volatiles in order to improve defense of agricultural and forest ecosystem. This provides an alternate biological pest management strategy. However, in order to successfully strengthen plant defense, a number of conditions must be fulfilled (Degenhardt et al. 2003). First, the area where the crop is planted must have herbivore enemies that can effectively manage the herbivore population. Second, the newly introduced or improved plant volatile blends must include significant herbivore adversary's principal attractants, and volatile emission must coincide with herbivore activity. Finally, the volatiles that have been produced should not make the plant attractive to other non-herbivores.

To date, engineering in several plant species enhance the plant herbivore defense strategies via emitting appropriate VOCs. For instance, the overexpression of strawberry nerolidol/linalool synthase gene (*FaNES1*) in *Arabidopsis* and potato enhances the production of linalool, thus preventing the plants from aphids. However, the protecting strategy differs in these plants – in *Arabidopsis* the *FaNES1* transgenics deter the aphid *Mysus persicae* while in potato it enhances tritrophic interactions by attracting the predatory mites. In another study in *Arabidopsis,* the *FaNES1* was overexpressed in mitochondria where sesquiterpene precursor farnesyl diphosphate (FPP) is present. This results in the production of (3S) (E) nerolidol and C11 homoterpene 4, 8 dimethyl 1, 3 (E), 7 nonatriene [(E) DMNT] compounds which enhances the attractiveness of plants toward *Phytoseiulus persimilis*, a predatory mite that are natural enemy of spider mites (Kappers et al. 2005). Another successful instance was the overexpression of the maize terpene synthase gene, that is, *TPS10* in *Arabidopsis*. The generated transgenic plants strongly emit a number of sesquiterpenes that are generally released in maize during herbivory by lepidopteran larvae. The female parasitic wasp *Cotesia marginiventris*, which had previously experienced oviposition on larvae of the potential host, found these transgenic plants to be more alluring (Schnee et al. 2006). Furthermore, transgenics of tobacco overexpressing patchoulol synthase (PTS) causes production of the volatile patchoulol and 13 additional sesquiterpene which deter the tobacco hornworms. It was also observed that the majority of tobacco hornworms had moved from the leaves of the transgenic plants to the leaves of wild-type plants and consumed 20–50% more of the wild-type plants in 6 h (Wu et al. 2006).

Terpenoids have not been the only volatile signals that have been subject to metabolic engineering in direct and indirect defenses. The fatty acid derived VOCs were also modified by the researchers. Transgenic tobacco plants overexpressing either the yeast acyl-CoAD9 desaturase or the insect acyl-CoA D11 desaturase produced more (Z)-3-hexenal, a significant green leaf volatile. The expression of these transgenes causes increased level of 13-lipoxygenase activity and 16:1 fatty acid, which catalyzes the initial step in the formation of hexenal from α-linolenic acid (Hong et al. 2004). Although the impact of increased levels of (Z)-3-hexenal on insect behavior was not examined in this study, the detrimental impact of this substance on aphid performance was shown in transgenic potato plants with lower levels of the hydroperoxide lyase enzyme, which is in charge of cleaving fatty acid hydroperoxides to C6 aldehydes (Vancanneyt et al. 2001).

11.5 Limitations of Engineering the VOCs in Plants: The Unwanted Side

Plant metabolic engineering is still in its infancy compared to microbials as their metabolic processes are substantially less complex. Though, significant advancement in biotechnology approaches provides us a significantly better understanding of biosynthetic pathways, regulation, compartmentalization, as well as the general biology of plant volatile-mediated compounds and their interactions, however, still our limited knowledge of the plant systems and the unique difficulties posed by engineering volatiles continue to be some of our limitations. The major limitations are:

11.5.1 Detrimental Effect on Growth and Development

The researches have shown the potential of genetic engineering to strengthen plant defense as well as the part that particular volatile molecules play in interactions between plants and insects. Despite these advancements, they have also shown how genetic changes affect plant growth and development and identified several obstacles to obtaining effective production of the necessary volatiles. While the levels of isoprenoids derived from plastids, such as chlorophylls, lutein, and β carotene, were not affected by the divergence of carbon to linalool production in *Arabisopsis* via *FaNES1* overexpression, it did result in a growth-retardation phenotype that was passed down through multiple generations of transgenic plants (Aharoni et al. 2003). A more severe phenotype was produced by the linalool-emitting transgenic potato; plants had bleached leaves after being moved from the in vitro to the greenhouse in addition to growth retardation (Aharoni et al. 2006). Due to the expression of *PTS* combined with *FPP* synthase, both of which are targeted to the plastids, transgenic tobacco that produces high levels of patchoulol also exhibits leaf chlorosis, vein clearing, and shortened stature (Wu et al. 2006). These reported phenotypes might result from the loss of isoprenoid precursors for other metabolites necessary for plant growth and development, or they might be the result of the harmful effects of the newly introduced terpenoids on plant cells.

11.5.2 Product Alteration

For volatile spectrum engineering to succeed it is crucial to create and emit enough of the appropriate molecules. The full biochemical toolkit of the plant employed will determine the metabolic destiny of newly produced molecules. Predicting how much of the intended molecule will actually survive in the desired form is difficult since the biochemical repertoire of each plant species is not completely understood. For instance, enzymes with broad substrate specificity that are typically found in cells, such as glutathione transferases, hydroxylases, dehydrogenases, glycosyl transferases, and others, may have an impact on freshly generated molecules (Dudareva et al. 2004). Currently, little is known about these enzymes in general and their precise distribution in various plant species. Research suggests that these broad substrate specificity enzymes work as competing enzymes and drain the intermediate/product of the pathway, often generating the unwanted products. This issue is more problematic when pathway involve multiple steps. In *FaNES1 Arabidopsis* transgenic plant, the generated linalool was exposed to hydroxylation and glycosylation by endogenous enzymes (and some of the nerolidol generated was degraded to the C11 homoterpene (E)-DMNT). Thus decreasing the overall concentrations of linalool produced (Aharoni et al. 2003).

11.5.3 Nonspecificity of the Enzymes

Nonspecific catalytic activity of the enzymes makes the outcome of metabolic engineering unpredictable. For instance, peppermints (E)-β-farnesene synthase enzyme can produce terpinolene, myrcene, and limonene from GPP; however, it can also produce δ-cadinene and (E)- and (Z)-β farnesene from FPP (Crock et al. 1997). This can be overcome by targeting specific subcellular organelle; however, it may affect the catalytic efficiency of the enzyme. Targeting of FaNES to the mitochondria mainly produces nerolidol while the plastid resulted in linalool production (Aharoni et al. 2003; Kappers et al. 2005). Moreover, the enzymes may also behave differently in vitro and in vivo due to difference in biochemical conditions and cofactors (Ginglinger et al. 2013).

11.5.4 Complex Interspecies Interaction

Plants frequently emit volatiles to draw in advantageous interactions. The equilibrium is delicate and these interactions are often distraught. For instance, in the *Cucurbita pepo* var. *texana* (wild Texas gourd), boosting volatile production increased the attraction of florivores and decreased the production of seeds. However, it did not improve the attraction of pollinators (Theis & Adler 2012). (E)-caryophyllene an antibacterial and mutualist attractant volatile component in Huang et al. (2012) is currently the focus of various engineering initiatives for its advantageous properties. But it competes with the aphid alarm pheromone (E)-farnesene (Vet & Dicke 1992). Tritrophic interactions can also be hampered by the production of isoprene (Loivamäki et al. 2008) and even (E)-farnesene at large concentrations. With these examples, we can say that appropriate gene expression control may be able to reduce potential cost-benefit scenarios.

11.6 Generating Desired VOCs Via Microbial Engineering

Although engineering plants is undoubtedly the most direct way to change how plants and the environment interact, but doing so comes with a number of drawbacks. An alternate strategy is to engineer microbes to produce certain products, which can then be applied as needed. For instance, a volatile attractant or repellant may be released from a reservoir into a field of crops at the right moment. The relatively straightforward metabolism, ease of genetic modification, well-developed engineering tools, and speed of engineering in microorganisms over plants are important advantages of engineering. Furthermore, it is much more practical to harvest volatiles in a controlled fermentation setting than it is to harvest them from plants for large-scale industrial production. Additionally, because microbial bioprocesses based on fermentation are less susceptible to the whims of seasonality, climate, and pest or disease attack, the circumstances of the bioprocess, including production yields (and thus, market pricing) may be more constant.

The first stage in creating a microbial cell factory to create volatile compounds is to select an appropriate production host. Here, it is important to take into account factors (1) native ability to produce VOCs, (2) genetic engineerability, (3) competition with essential requirements, (4) potential toxicity of the end product, and (5) potential to carry out desired biotransformation. The wide range of microbes may be utilized to produce VOCs; however, up to now majority of engineering has been done in the widely used model microorganisms, that is, *E. coli* and *S. cerevisiae*. One should also note that the production of VOCs also varies upon the strain of the host used. Next the genetic capability must be imported after the host organism has been chosen. Plant genes are frequently employed for this. The introns need to be removed in yeast and *E. coli* to maintain a continuous protein coding sequence. The signal peptides or other targeting sequences (e.g., chloroplast targeting region in case of plant chloroplast protein) must also be eliminated. As a result, eliminating the targeting peptide causes microorganisms to produce more of the desired chemical (Burke & Croteau 2002; Vickers et al. 2011; Bott et al. 2012). Also, the precise truncation site is crucial since it might have a big impact. Sequence alignment with microbial homologs that lack targeting sequences and closely related genes that have already been characterized can be used to help choose truncation sites in these cases; however, for a completely uncharacterized gene, it is wise to test several truncations. Codon-optimizing plant isoprenoid genes to alter codon usage toward the host's preference can also improve translation (Anthony et al. 2009; Bott et al. 2012; Calabria et al. 2013). Finally, selection of an appropriate expression system [promoter and terminator sequences, plasmid expression systems or chromosomal integration, variable copy number (plasmid/chromosome), etc.] is required. Balancing all of these elements appropriately may have a substantial effect on the overall success of the project. For biotechnological applications, strict product and enantiomeric specificity is often required. As discussed above, many isoprenoid synthases have relaxed substrate/product specificities, and catalytic activities of different terpene synthases can vary widely (Schomburg et al. 2013).

11.7 Conclusion and Future Direction

The cultivation of many plant species is significantly influenced by the volatile compounds found in fruits, vegetables, and herbs. However, very less focus is given on boosting or even maintaining volatile production as significant breeding programs are undertaken to improve certain food qualities such as overall yield, total solids, sugar content, or pigmentation. Therefore, compared to their wild cultivars, many modern cultivars of domesticated plant species produce fewer volatiles (Gutterson 1993). Progression in breeding programs can enhance this loss of genetic diversity in volatile compounds. Plants release volatile substances into the rhizosphere from their roots, a fact that has only lately been recognized (Chen et al. 2004; Rasmann et al. 2005; Bouwmeester et al. 2007). These volatiles may support the plant's ability to both draw in and repel helpful microbes. They might also be helpful in interspecies plant competition (Horiuchi et al. 2007). However, it has been demonstrated that some parasitic plants detect their hosts by using underground volatile chemicals (Bouwmeester et al. 2007). There have not been any reports of attempts at genetic engineering to alter root volatile emission in order to increase plant fitness, but this is obviously a very promising field for future research.

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