Plant Glandular Trichomes: Natural Cell Factories of High Biotechnological Interest[1][OPEN]

Alexandre Huchelmann, Marc Boutry, and Charles Hachez[2]

Life Sciences Institute, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

ORCID IDs: 0000-0001-9302-405X (A.H.); 0000-0002-2315-6900 (M.B.); 0000-0002-3688-7614 (C.H.).

Multicellular glandular trichomes are epidermal outgrowths characterized by the presence of a head made of cells that have the ability to secrete or store large quantities of specialized metabolites. Our understanding of the transcriptional control of glandular trichome initiation and development is still in its infancy. This review points to some central questions that need to be addressed to better understand how such specialized cell structures arise from the plant protodermis. A key and unique feature of glandular trichomes is their ability to synthesize and secrete large amounts, relative to their size, of a limited number of metabolites. As such, they qualify as true cell factories, making them interesting targets for metabolic engineering. In this review, recent advances regarding terpene metabolic engineering are highlighted, with a special focus on tobacco (*Nicotiana tabacum*). In particular, the choice of transcriptional promoters to drive transgene expression and the best ways to sink existing pools of terpene precursors are discussed. The bioavailability of existing pools of natural precursor molecules is a key parameter and is controlled by so-called cross talk between different biosynthetic pathways. As highlighted in this review, the exact nature and extent of such cross talk are only partially understood at present. In the future, awareness of, and detailed knowledge on, the biology of plant glandular trichome development and metabolism will generate new leads to tap the largely unexploited potential of glandular trichomes in plant resistance to pests and lead to the improved production of specialized metabolites with high industrial or pharmacological value.

Trichomes, the epidermal outgrowths covering most aerial plant tissues, are found in a very large number of plant species and are composed of single-cell or multi-cellular structures. These structures are divided into two general categories: they can be glandular or nonglandular, depending on their morphology and secretion ability. Glandular trichomes can be found on approximately 30% of all vascular plant species (Fahn, 2000), and in a single plant species, several types of trichomes (both glandular and nonglandular) can be observed. Glandular trichomes are characterized by the presence of cells that have the ability to secrete or store large quantities of secondary (also called specialized) metabolites, which contribute to increasing the plant fitness to the environment (for details, see Box 1).

Two main types of glandular trichomes stand out: peltate or capitate, which differ according to their head size and stalk length. Capitate trichomes typically possess a stalk whose length is more than half the head height, whereas peltate trichomes are defined as short-stalked (unicellular or bicellular stalk) trichomes with a large secretory head made of four to 18 cells arranged in one or two concentric circles. Capitate trichomes are quite variable in their stalk cell number and length, glandular head morphology, as well as secretion pattern and can be classified into various types (Glas et al., 2012).

A key and unique feature of glandular trichomes is their ability to synthesize and secrete large amounts, relative to their size, of a limited number of specialized metabolites: mainly terpenoids (Gershenzon et al., 1992; Gershenzon and Dudareva, 2007) but also phenylpropanoids (Gang et al., 2001; Deschamps et al., 2006; Xie et al., 2008), flavonoids (Voirin et al., 1993; Tattini et al., 2000), methylketones (Fridman et al., 2005), and acyl sugars (Kroumova and Wagner, 2003; Schilmiller et al., 2010; Weinhold and Baldwin, 2011).

Over the long term, the ability to modulate the density and productivity of such secreting structures in plants would be of great biotechnological interest. This requires the identification and characterization of the genes initiating, regulating, and driving the development of such glandular structures. Awareness of, and detailed knowledge on, the biology of plant glandular trichome development and metabolism will generate new leads to turn trichomes into biochemical factories using metabolic engineering approaches (Tissier, 2012a), tap their largely unexploited potential in plant resistance to pests, and lead to the improved production of important specialized metabolites (Lange and Turner, 2013; Lange et al., 2011).

PLANT GLANDULAR TRICHOMES: AN INTERESTING PARADIGM TO STUDY PLANT CELL DIFFERENTIATION

To modulate the density of glandular trichomes in the epidermis or their productivity for biotechnological

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.17.00727

ADVANCES

- Given the recent progress in "-omics" technology and genome editing (CRISPR/Cas9), the time has come to investigate up-to-now overlooked aspects of glandular trichome biology in non-model species down to a molecular level. For example, given these technical advances, the study of the molecular genetic aspects governing glandular trichome development has now been greatly facilitated in numerous species.

- Advances in mass spectrometry now allow the simultaneous determination of hundreds of metabolites in a small number or even in single cells, with a sensitivity in the attomole (10^{-18} mole) range. Such advances in metabolic profiling are highly interesting for glandular trichomes that, depending on the plant species and trichome type, may not always be easy to isolate for chemical analysis.

- Metabolic engineering of specialized metabolites in glandular trichomes still faces many experimental challenges, including ways of efficiently dealing with the potential cytotoxicity of the synthesized molecules (for example via the expression of some specific ABC transporters), and the necessity to limit the negative impact of such engineering at the whole-plant level (via the use of cell type-specific gene promoters).

- Importantly, the bioavailability of the pool of precursors to be used needs to be addressed, as those precursors are sometimes stored in a different subcellular compartment (e.g., the cytosolic vs. plastidial pools of isoprenoid precursors). This could necessitate the identification/cloning of appropriate transporters between the plastidial envelope and the cytosol to facilitate the exchange of precursor molecules between these compartments.

purposes, a detailed understanding of the molecular genetic framework governing their development and patterning in the plant epidermis would be beneficial. Glandular trichomes have been studied mostly to decipher the biochemical pathways of the compounds they produce and secrete (Champagne and Boutry, 2013; Lange and Turner, 2013) and, thereby, have contributed to advancing our understanding of the secondary metabolism in plants.

A Detailed Understanding of Glandular Trichome Initiation and Development Is Currently Missing

All multicellular organisms face the challenge of co-ordinating cell proliferation with cell differentiation and patterning. Defects in this coordination can lead to incorrect tissue formation, malformed organs, and cancerous growth. Glandular trichomes exemplify this coordination challenge: they are elaborate, highly or-ganized, and polarized cell structures whose morpho-genesis is modulated by an intricate array of molecular processes controlling the different steps of their pat-terning on the leaf epidermis and subsequent differen-tiation. Therefore, glandular trichomes can be used as a paradigm to tackle basic questions about the develop-ment and differentiation of specialized multicellular secretory structures in plants.

A complete circuit for glandular trichome formation and patterning in the leaf epidermis will require a sound understanding of their initiation process in the plant protodermis and of the subsequent develop-mental steps leading to the formation of a polarized and specialized multicellular structure, of the genes that regulate cell division, participate in cell signaling, and promote specialized cell fate (Fig. 1). It also requires an understanding of how this developmental regulatory network affects cellular biological targets such as the core cell cycle machinery. A particularly large gap in our current knowledge is the identification of regula-tors of entry into the glandular trichome cell fate and of progression through the pathway.

Research on nonglandular trichomes has been very fruitful in Arabidopsis (*Arabidopsis thaliana*). It has generated a developmental framework for unicellular trichome formation and identified over 30 genes in-volved in the initiation and development of non-glandular trichomes. We chose not to review the development of trichomes in Arabidopsis, as this has been thoroughly reviewed (Balkunde et al., 2010; An et al., 2011; Tominaga-Wada et al., 2011; Pattanaik et al., 2014; Matías-Hernández et al., 2016). Unlike the situa-tion of Arabidopsis, which contains a single type of unicellular, nonglandular trichome, our understanding of the molecular genetic aspects of glandular trichome development is still in its infancy but currently im-proving due to recent progress in omics and genome-editing technologies as well as to a growing focus from several research teams to characterize this process in different plant species (Li et al., 2004; Dai et al., 2010; Bosch et al., 2014; Bergau et al., 2015; Liu et al., 2016). The (draft) genome sequences of a number of plant species with glandular trichomes are now available. A nonexhaustive list includes different tomato species (*Solanum lycopersicum* and *Solanum pimpinellifolium* [Tomato Genome Consortium, 2012] and *Solanum pen-nellii* [Bolger et al., 2014]), potato (*Solanum tuberosum* [Xu et al., 2011]), cucumber (*Cucumis sativus* [Huang et al., 2009]), tobacco (*Nicotiana tabacum* [Sierro et al., 2014; Edwards et al., 2017]) and its related species (*Nicotiana sylvestris* and *Nicotiana tomentosiformis* [Sierro et al., 2013]), hot pepper (*Capsicum annuum* [Kim et al., 2014]), mint (*Mentha longifolia* [Vining et al., 2017]), cannabis (*Cannabis sativa* [van Bakel et al., 2011]), and hop (*Humulus lupulus* [Natsume et al., 2015]).

Since most of them can be genetically transformed, studying the molecular genetics of trichome develop-ment in these species has become much easier (among

Our understanding of the metabolomic profiles of glandular trichomes is greatly increasing (Aschenbrenner et al., 2015; Bombo et al., 2016; Spring et al., 2015; Wang et al., 2015; Muravnik et al., 2016; Venditti et al., 2016). Recently, technological advances have even made it possible to obtain the metabolic profile of a single trichome cell (Nakashima et al., 2016). However, the exact role of glandular trichomes in mediating plant interactions with their environment has only started to be investigated at a molecular level.

Terpenoids are the most abundant class of metabolites found in trichomes and have important properties in terms of plant defense and interaction with herbivores (Glas et al., 2012). In petunia, the production of insecticidal steroids is important for plant defense, as silencing of a trichome-specific steroid transporter increased the sensitivity to pests (Sasse et al., 2016). Metabolic engineering of terpenoids in tomato trichomes can also increase the resistance of plants to phytopathogens (Bleeker et al., 2012).

Methylketones are a class of medium length carbon chain molecules that are involved in the defense against pests (Williams et al., 1980; Maluf et al., 1997). While *Solanum habrochaites*, a wild tomato, produces a large amount of those metabolites in its trichomes, those molecules are almost absent in their cultivated counterpart, *Solanum lycopersicum*. Engineering the cultivated tomato trichomes to efficiently produce methylketones could lead to an increased resistance to pests. The trichome-specific expression of genes coding for two methylketone synthases from *S. habrochaites*, the enzymes involved in methylketone biosynthesis (Yu et al., 2010), only led to a small increase in the methylketone content in *S. lycopersicum* (Yu and Pichersky, 2014).

Acyl sugars are produced in the glandular trichomes of most species of the Solanaceae family (Kroumova and Wagner, 2003; Luu et al., 2017). In tomato and wild tomato, these molecules render the leaf sticky and, by physically trapping pests, play a role in defense against herbivorous attacks (Puterka et al., 2003; Simmons et al., 2004). In *Nicotiana attenuata*, the role of acyl sugars is totally different as these trichome-derived compounds attract the moth *Manduca sexta*, the larvae of which feed on the wild tobacco. Frass from the larvae contain volatile acyl chains hydrolyzed from the acylsugars that in turn attract *Pogonomyrmex rugosus*, one of their natural predators (Weinhold and Baldwin, 2011). These compounds also protect the plant from pathogenic fungi (Luu et al., 2017).

Flavonoids are metabolites synthesized in the glandular trichomes of various species (Tattini et al., 2000; Valkama et al., 2003; Liakopoulos et al., 2006; Spring et al., 2015) and have been shown to also play a role in plant defense. Rather than a protection against pathogens, these flavonoids are used as a protection against UV irradiation in plants exposed to high solar radiation, such as olive (*Olea europaea*), sunflower (*Helianthus annus*) and the Mediterranean plant *Phillyrea latifolia* (Tattini et al., 2000; Liakopoulos et al., 2006).

The metabolites produced in plant trichomes thus fulfill a variety of roles in plants that all contribute to increasing the fitness of the plant to the environment, thereby increasing plant survival.

others by using genome-editing or RNA interference technologies). Particularly for species where genetics is poorly developed (e.g. mint), the combination of transcriptomics at different stages of trichome development (see below) and of genome-editing or RNA interference technology should be a powerful approach to address the function of candidate genes.

Trichome-Specific Data Exist, But Cell Stage-Specific Data Are Crucially Needed to Advance Our Understanding of Glandular Trichome Development

Extensive glandular trichome-specific EST resources were generated for a variety of (nonmodel) plant species (Dai et al., 2010; Tissier, 2012b; Soetaert et al., 2013; Chen et al., 2014; Jin et al., 2014; Trikka et al., 2015; Akhtar et al., 2017). Dedicated trichome-related open-access resources exist that help researchers mine this vast and increasing amount of trichome-related data. One such resource is the TrichOME database (Dai et al., 2010), which hosts functional omics data, including

transcriptomics (ESTs/unigene sequences), metabolomics (mass spectrometry-based trichome metabolite profiles), as well as trichome-related genes curated from the published literature from various species. These include members of Lamiaceae (*Mentha piperita*, *Salvia fruticosa*, *Cistus creticus*, and *Ocimum basilicum*), Solanaceae (*Nicotiana benthamiana*, *N. tabacum*, *Solanum habrochaites*, *S. lycopersicum*, and *S. pennellii*), Asteraceae (*Artemisia annua*), Fabaceae (*Medicago sativa* and *Medicago truncatula*), and Cannabaceae (*C. sativa* and *H. lupulus*).

These trichome-specific expression data are particularly useful to identify trichome-specific genes involved in particular biosynthetic pathways as well as in other trichome-related processes. A key question is how to extract the most significant data out of this massive and growing amount of bulk information to advance our understanding of specific aspects of glandular trichome biology (Tissier, 2012a). From a developmental perspective, one of the main limitations of such resources is that most of these trichome-specific expression data
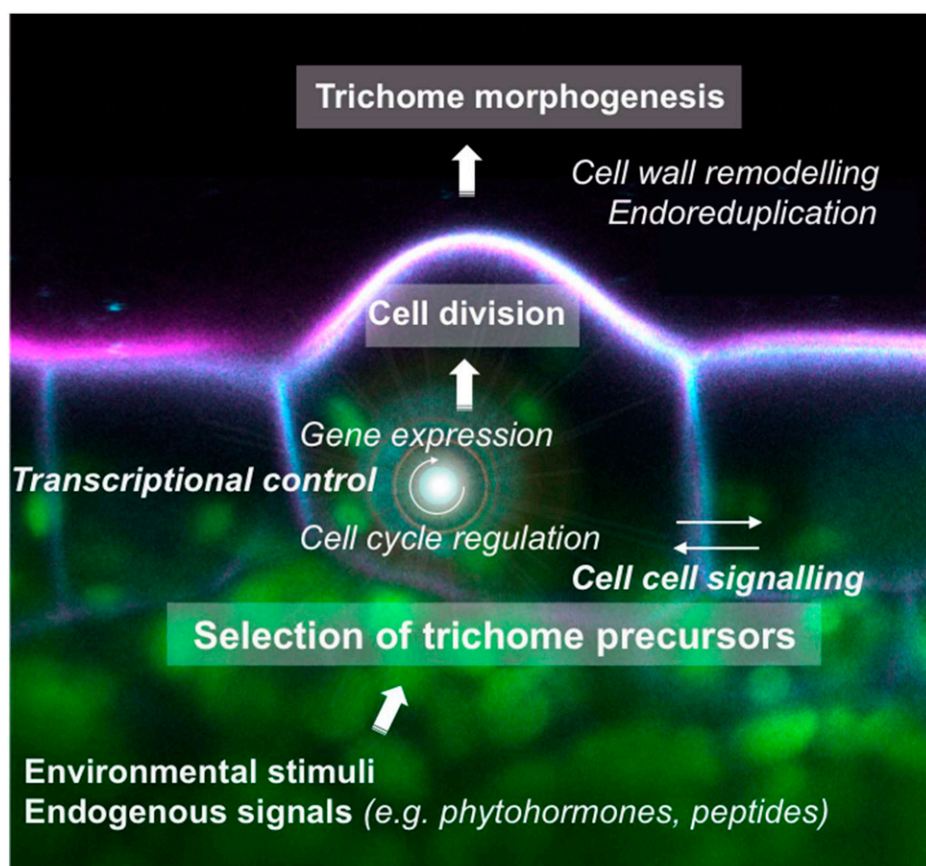
Figure 1. Glandular trichome initiation and development, a process with many unknowns. A differentiating protodermal cell integrates both environmental and endogenous signals. Such signal integration results in the selection of a pool of trichome cell precursors that will initiate a specific developmental program. In these trichome initials, cell-specific transcriptional control of gene expression and cell cycle regulation results in the onset of a controlled cell division and trichome morphogenesis program, most of which is still not so well understood in the case of glandular trichomes. It probably also involves some cell-cell signaling promoting the one cell-spacing rule, which allows a specific patterning of trichomes in the epidermis. Morphogenesis of the trichome glandular head also necessitates extensive remodeling of the cell wall. The extent of endoreduplication in glandular trichomes is still mostly uncharacterized. The illustration shows a modified confocal image of a long glandular trichome initial from *N. tabacum*. Chloroplasts are shown in green, propidium iodide-stained cell walls in magenta, and nuclei in cyan.

were derived from mature glandular trichomes (which are usually easier to isolate than developing structures) or from a population of trichomes at mixed developmental stages. Indeed, glandular trichome initiation and differentiation occur at different times in different locations within a single leaf. Therefore, even very young leaves contain a trichome population of mixed developmental stages (Bergau et al., 2015). Given the way most EST resources were generated up to now, the expression of genes playing a role in early developmental steps may be, in the best case, underestimated or, in the worst case, even not detected. This is particularly the case of genes active in the initiation phase (selection of trichome initials) or early in development.

In addition to trichome-specific EST resources, numerous glandular trichome-specific gene promoters have been reported in the literature for a variety of plants, including (but not restricted to) *Antirrhinum majus*, *A. annua*, *C. sativus*, *H. lupulus*, *Mentha* spp., *N. tabacum*, *S. lycopersicum*, and *S. habrochaites* (Okada and Ito, 2001; Wang et al., 2002, 2011, 2013; Gutiérrez-Alcalá et al., 2005; Liu et al., 2006; Jaffé et al., 2007; Kim et al., 2008; Shangguan et al., 2008; Ennajdaoui et al., 2010; Choi et al., 2012; Sallaud et al., 2012; Spyropoulou et al., 2014; Kortbeek et al., 2016; Laterre et al., 2017; Vining et al., 2017; for review, see Tissier, 2012b). It is worth noting that most of these trichome-specific promoters are active in mature glandular trichomes

(mostly in glandular cells at the tip of the trichome). Their activity during early trichome development has been barely analyzed.

Molecular data pointing to genes playing a specific role in glandular trichome development already exist, especially concerning some transcription factors, cell cycle regulators, as well as receptors involved in phytohormone-induced signaling cascades. Several transcription factors belonging to different protein families and playing a role in glandular trichome development have indeed been identified: AmMIXTA, a MYB transcription factor from *A. majus* whose ectopic expression in tobacco induces the development of additional long glandular trichomes (Glover et al., 1998); GoPGF, a basic helix-loop-helix (bHLH) transcription factor from *Gossypium* spp., acting as a positive regulator of glandular trichome formation, its silencing leading to a completely glandless phenotype (Ma et al., 2016); AaHD1, a homeodomain-Leu zipper transcription factor required for jasmonate-mediated glandular trichome initiation in *A. annua* (Yan et al., 2017); AtGIS, a C2H2 zinc-finger transcription factor from Arabidopsis whose ectopic expression in tobacco regulates glandular trichome development through GA$_3$ signaling (Liu et al., 2017); AaMYB1, a MYB transcription factor from *A. annua* whose overexpression induces the formation of a greater number of trichomes (Matías-Hernández et al., 2017); and CsGL3, an HD-Zip

transcription factor whose mutation leads to a glabrous phenotype in cucumber (Cui et al., 2016).

In tomato, several genes required for the proper development and function of different types of glandular trichomes have been reported. The *woolly* (*Wo*) gene, encoding a class IV homeodomain-Leu zipper protein homolog to the Arabidopsis GL2, and a B-type cyclin gene, *SlCycB2* (possibly regulated by *Wo*), control the initiation and development of type I trichomes. Mutant alleles of *Wo* triggered a hairy phenotype due to the overproduction of type I trichomes, while suppression of *Wo* or *SlCycB2* expression by RNA interference decreased their density in tomato (Yang et al., 2011a, 2011b). Another mutation (hairless) affects the SRA1 (Specifically Rac1-Associated protein) subunit of the WAVE regulatory complex. SRA1 controls the branching of actin filaments and is required for the normal development of all trichome types of tomato, which suggests that proper actin-cytoskeleton dynamics is a basal requirement for normal trichome morphogenesis (Kang et al., 2016). Mutations of some enzymes involved in secondary metabolism also impact trichome density and/or the metabolic activity of glandular trichomes. For example, in the anthocyanin-free mutant, loss of function of the chalcone isomerase (SlCHI1) triggers a reduction of type VI trichome density and metabolic output (Kang et al., 2014), while down-regulation of DXS2, a methylerythritol 4-phosphate (MEP) enzyme, increases their density (Paetzold et al., 2010). The molecular mechanisms through which these genes affect trichome density are currently unknown.

The tomato Wo^v allele was shown to promote abnormal multicellular trichome differentiation when ectopically overexpressed in *N. tabacum* (Yang et al., 2015). This hints at conserved transcriptional networks among Solanaceae species. However, the trichomes in this Wo^v overexpressor line failed to develop glandular heads and appeared as rather aggregated and undifferentiated structures. Whether a WD40-bHLH-MYB regulatory mechanism similar to the one in Arabidopsis also controls glandular trichome development in Solanaceae is still unclear (Serna and Martin, 2006; Yang et al., 2015). A recent RNA-sequencing analysis of *N. tabacum* trichomes showed that orthologs of Arabidopsis genes involved in trichome formation via the WD40-bHLH-MYB regulatory mechanism (such as TTG1, GL2, GL3, and several MYB transcription factors) are expressed in these structures (Yang et al., 2015). However, their transcriptional levels were not altered significantly in response to the overexpression of a Wo^v transgene, which induced a clear trichome proliferation phenotype. On the contrary, homologs of genes (*Wo* and *SlCycB2*) involved in trichome formation in asterids (Yang et al., 2011a, 2011b) were significantly up-regulated in Wo^v transgenic *N. tabacum* plants (Yang et al., 2015).

The differentiation of long-stalked glandular trichomes may be initiated and controlled in *N. tabacum* by the activity of another MYB transcription factor.

Indeed, ectopic expression of *MIXTA* from *A. majus* (Glover et al., 1998) or of its *Gossypium hirsutum* ortholog *CotMYBA* (Payne et al., 1999) resulted in the development of excess long-stalked trichomes. Therefore, it is likely that another unidentified *N. tabacum* *MYB* gene, an ortholog to *AmMIXTA* and *CotMYBA*, plays a role in the development of long-stalked glandular trichomes in this species. Whether this *MYB* gene needs to be part of a regulatory complex to initiate and promote glandular trichome development is not yet known.

Five trichome-related mutants of the genes *CsGL3*, *TRIL*, *MICT*, *TBH*, and *CsGL1*, all of them encoding homeodomain-Leu zipper transcription factors from different subfamilies, have been reported in *C. sativus* (Chen et al., 2014; Li et al., 2015; Pan et al., 2015; Zhao et al., 2015; Cui et al., 2016; Wang et al., 2016b). Based on the observed phenotypes, a molecular mechanism underlying the development of multicellular trichomes in this species has been proposed in a recent review (Liu et al., 2016) and seems to confirm that the transcriptional control of multicellular trichomes in *C. sativus* differs from the one observed in Arabidopsis.

The development of glandular trichomes is tightly regulated by the integration of diverse environmental and endogenous signals. In this respect, some phytohormones, especially jasmonate (JA) and possibly GAs, elicit glandular trichome development via signaling cascades and the activation of trichome-specific transcriptional regulators (Li et al., 2004; Koo and Howe, 2009; Bose et al., 2013; Bosch et al., 2014; Tian et al., 2014; Liu et al., 2017; Yan et al., 2017). In *Mentha arvensis*, exogenous application of GA resulted in a moderate increase in trichome density and diameter of the gland, suggesting a positive, although moderate, effect of GA on trichome initiation and development in mint (Bose et al., 2013). For example, reduced JA levels (through silencing of OPR3, a key enzyme in the biosynthesis of the precursor of JA) led to impaired glandular trichome development in tomato: the density of type VI trichomes was reduced drastically, and their metabolite content was different from that of the wild type (Bosch et al., 2014). The JA receptor JASMONIC ACID INSENSITIVE1, the tomato ortholog of the ubiquitin ligase CORONATINE INSENSITIVE1 in Arabidopsis, is involved in this JA-mediated signaling cascade (Li et al., 2004; Katsir et al., 2008; Kang et al., 2010; Bosch et al., 2014). It remains to be investigated whether a synergistic effect between GA and JA signaling, similar to that observed in Arabidopsis (Qi et al., 2014), also promotes glandular trichome development.

Time-Course Analysis of Glandular Trichome Development Coupled to Cell Type- and Stage-Specific Expression Data as a Way to Advance Our Understanding of Glandular Trichome Development

The development of multicellular glandular trichomes proceeds through the enlargement of single

epidermal cells, followed by several cell divisions to generate a structure perpendicular to the epidermal surface (Fig. 2), and specific types of glandular trichomes seem to have a well-defined developmental plan (Tissier, 2012a; Bergau et al., 2015). This highly regulated differentiation program also includes a polarized and localized cell wall lysis and remodeling (Bergau et al., 2015).

Within a given plant species, it would be interesting to dissect the developmental sequence of glandular trichome formation (including a time-course analysis) and to sort the cells at specific stages using marker-assisted cell sorting. The difficulty resides in the specific isolation of cells at early developmental stages. Recently, flow cytometry was used to specifically separate young and mature type VI trichomes from the wild tomato species *S. habrochaites* based on their distinct autofluorescence signals. This allowed the analysis of their transcriptomic and metabolomic profiles in a cell stage-specific way (Bergau et al., 2016).

Such systematic dissection of the development of glandular trichomes seems very promising but could be refined. Ideally, instead of autofluorescence (which may span various developmental stages), a series of trichome-specific transcriptional promoters driving the expression of a fluorescent marker (used as a cell stage marker) and closely associated with well-defined developmental stages should be used. Such genetic resources are not yet available in the field of glandular trichome development. Focus should be set on identifying markers of entry into the glandular trichome pathway as well as those labeling subsequent early differentiation steps. Some published gene promoters, like the one of *AmMYBML3* (Jaffé et al., 2007), are already known to specifically label developing trichomes and could be used to drive the expression of a fluorescent reporter protein. Flow cytometry-assisted cell (or nucleus) sorting would then permit us to characterize the transcriptomic changes in a stage-specific way, in a manner similar to what was done to characterize stomatal development (Adrian et al., 2015). In an iterative fashion, the data could be mined to identify additional marker genes, so that only a few markers are necessary to initiate such transcriptomic studies. As an alternative approach, ectopic overexpression of some transcription factors is known to induce glandular trichome development (Payne et al., 1999; Yang et al., 2015). Inducible overexpression of such transcription factors could be used in RNA-sequencing assays as a molecular switch to identify genes acting during early developmental stages (Yang et al., 2015) that, in turn, could be used as cell stage markers. Further research is definitely needed to identify key genes driving the postembryonic development of glandular trichomes and to generate a molecular toolbox facilitating more applied genetic engineering approaches.
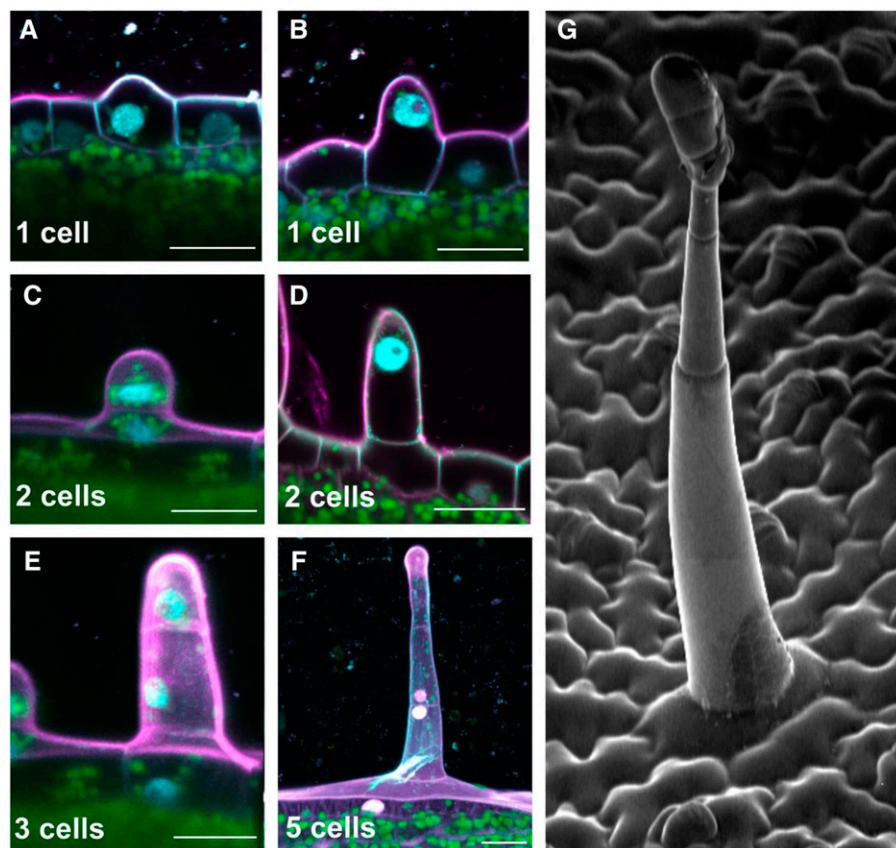

Figure 2. Glandular trichome initiation and development in *N. tabacum*. A to F, Confocal microscopy images showing the early steps of glandular trichome development. The number of cells forming the developing glandular trichome is shown at the bottom of each frame. A differentiating protodermal cell enlarges and forms a protuberance (A), the cell nucleus migrates to the tip of the protuberance (B), and cell division takes place (C), forming a structure made of two cells (D). The upper cell protruding from the epidermis then undergoes an asymmetric division, forming one large cell (which will form the multicellular stalk after several rounds of controlled cell division) and one small cell (which will give rise to the multicellular glandular head; E). A developing trichome made of five cells is shown in F. Scale bars, 20 μm. Magenta represents cell wall (propidium iodide staining), cyan represents nuclei (4′,6-Diamidine-2′-phenylindole staining), and green represents chloroplasts (chlorophyll *a* autofluorescence). G, Scanning electron micrograph showing the typical cell architecture of a mature long glandular trichome.

Glandular Trichome Development: A Diversity of Model Species

The field of glandular trichome development lacks a unique and robust model system. This is partly due to the difficulty of finding an appropriate model system. Glandular trichomes are extremely diverse in terms of shape, cell number, and type of secreted compounds and may not be the result of a single evolutionary event (Serna and Martin, 2006). This implies that their development may not be under similar transcriptional control in different plant families or even within a single plant species between different trichome types (Serna and Martin, 2006). The current view is that no single species can serve as a unique model to study the biology of glandular trichomes but that certain species or families of species progressively emerge as references for certain types of trichomes, such as Lamiaceae for peltate trichomes or Solanaceae for capitate trichomes, as suggested by Tissier (2012a). So far, published data suggest that multicellular trichome formation probably occurs through different transcriptional regulatory networks from those regulating trichome formation in Arabidopsis, so mere orthologous relationships may not be inferred (Payne et al., 1999; Serna and Martin, 2006; Yang et al., 2015; Liu et al., 2016).

TURNING GLANDULAR TRICHOMES INTO CHEMICAL FACTORIES

Plant specialized metabolites have been used for centuries as a source for fragrances and medicine. Since the discovery of their molecular structures and the elucidation of their biosynthesis pathways, breeders and chemists have been trying to select the best compounds by crossing species and varieties. For small molecules, chemical synthesis is another option once the structure of the molecule has been determined. However, the size and complexity of the stereochemistry of some plant metabolites make their chemical synthesis extremely complicated and expensive.

The rise of molecular genetics and a better understanding of the genomes has changed the way breeders work: they now use molecular genetic screening approaches to help them select the best breeding candidates and descendants. This speeds up the selection process and optimizes the breeding program.

Among plant specialized metabolites, terpenoids are the most abundant in term of quantity and diversity (for review, see Croteau et al., 2000; Bouvier et al., 2005; Gershenzon and Dudareva, 2007). Some of them are renowned not only for their economic value but also for their molecular complexity.

Metabolic engineering of terpenoids emerged as a new method to produce naturally occurring products. Microorganisms have been heavily used for the heterologous expression of plant metabolites (for review, see Kirby and Keasling, 2009; Keasling, 2010; Marienhagen and Bott, 2013), and at present, plants also have become a host of interest for the heterologous or homologous production of some plant specialized metabolites (for review, see Aharoni et al., 2005; Dixon, 2005; Wu and Chappell, 2008).

The biosynthesis of isoprenoids in plants is unique, and many reviews have already covered the different aspects of their production and regulation (Bouvier et al., 2005; Hemmerlin et al., 2012; Lipko and Swiezewska, 2016). Plants synthesize the common precursor for isoprenoids, isopentenyl diphosphate (IPP), and its allylic isoform dimethylallyl diphosphate (DMAPP) by two distinct and compartmentalized pathways, the cytosolic mevalonate (MVA) pathway and the plastidial MEP pathway (Fig. 3). Specialized terpenoids are generally synthesized by either the MEP or the MVA pathway depending on their length: sesquiterpenes (C15) and triterpenes (C30) mainly derive from the MVA pathway, while monoterpenes (C10) and diterpenes (C20) derive from the MEP pathway.

Metabolic Engineering in *N. tabacum*

N. tabacum is an interesting model system for metabolic engineering of terpenoid compounds because it synthesizes an important pool of natural precursors (IPP/DMAPP) and, besides the essential metabolites derived from the isoprenoid biosynthesis pathways, it produces a very high amount of a limited range of specialized metabolites. These consist of two types of terpenoids, namely sesquiterpenes (Back and Chappell, 1996; Starks et al., 1997; Ralston et al., 2001), as phytoalexins specifically produced in response to a pathogen attack (Stoessl et al., 1976), and diterpenes, constitutively synthesized and secreted by the head of glandular trichomes (Keene and Wagner, 1985; Kandra and Wagner, 1988; Guo and Wagner, 1995; Wang and Wagner, 2003; Fig. 3). Capsidiol, one of the main phytoalexin sesquiterpenes produced in response to a fungal attack, and cembratriendiol, one of the main diterpenes found in the cuticle, derive from the MVA and the MEP pathway, respectively (Huchelmann et al., 2014). Both types of metabolites are not required for the growth and development of the plants and do not involve a complex metabolic pathway. Changing the fate of the metabolic fluxes normally used for sesquiterpene and diterpene production looks, in theory, quite simple. As the phytoalexin sesquiterpenes are only synthesized in response to a pathogen attack, the main pool of terpenoid precursors available under normal conditions is that involved in diterpene synthesis.

Terpenoid Engineering with Constitutive and Ubiquist Promoters

The metabolic engineering of tobacco plants to produce various terpenoids is widely described, and reviews already covered the methods and the final metabolic profiles (for review, see Verpoorte and Memelink, 2002; Lange and Ahkami, 2013; Moses et al., 2013). Most of the engineered metabolites were
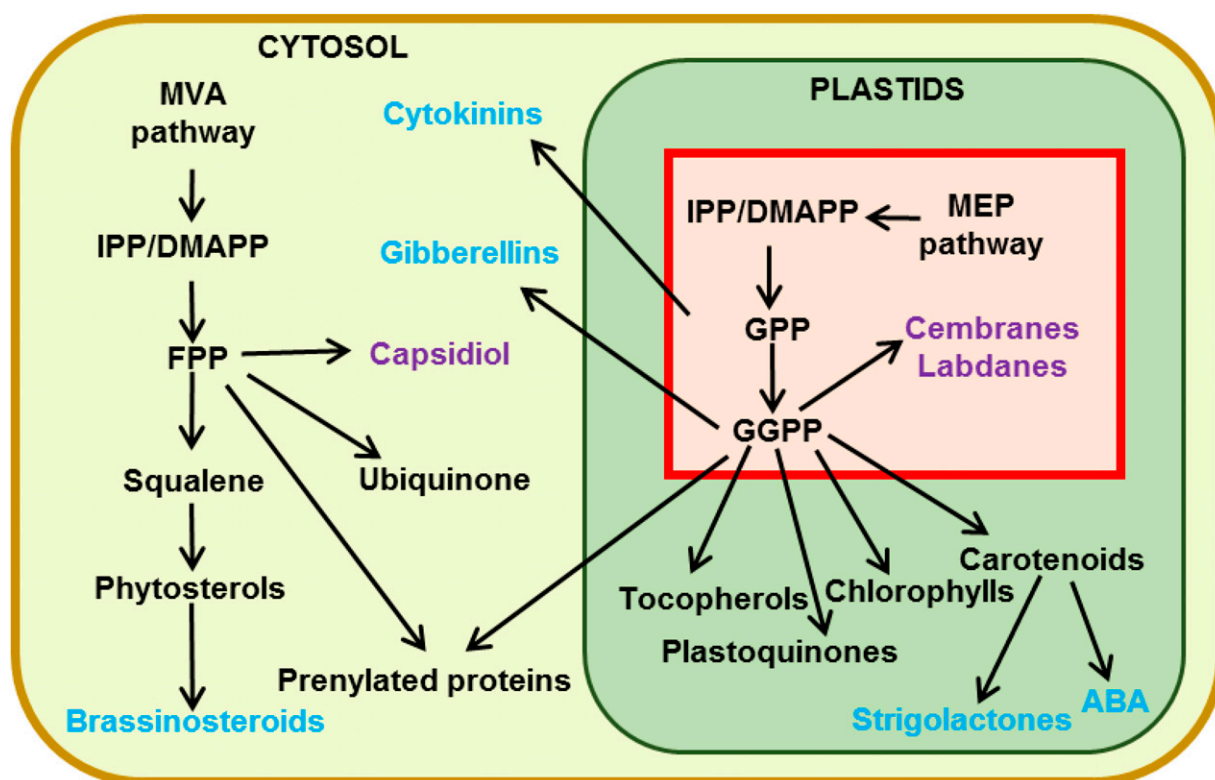
Figure 3. Isoprenoid metabolism in *N. tabacum* cells. The red square represents the major MEP isoprenoid metabolism in plastids of developed trichomes. Phytohormones are indicated in blue and specialized metabolites in violet. ABA, Abscisic acid; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate.

synthesized using the original precursor pools, derived from the MEP pathway for monoterpenes and diterpenes and from the MVA pathway for sesquiterpenes and triterpenes. Promoters used to drive expression of the transgenes were ubiquist and constitutive. Although successful, the amount of metabolites produced in transgenic tobacco lines is usually relatively low (in the range of a few ng g^{-1} fresh weight; Lücker et al., 2004; Wei et al., 2004; Farhi et al., 2011) compared with the endogenous production of capsidiol (up to 100 μg g^{-1} fresh weight; Dokládal et al., 2012) or of diterpenes (up to 75 μg cm^{-2} depending on the variety; Severson et al., 1984). To be economically viable, the engineering of terpenoids in plants should reach yields comparable to those occurring naturally.

As mentioned previously, sesquiterpenes (C$_{15}$) and triterpenes (C$_{30}$) are considered to derive from the (cytosolic) MVA pathway, while monoterpenes (C$_{10}$) and diterpenes (C$_{20}$) are derived from the (plastidial) MEP pathway. However, such a distinction is actually not so strict, given the existence of cross talk between the MVA and MEP pathways, which consists of an exchange of prenyl diphosphates between the cytosol and the plastids (for review, see Hemmerlin et al., 2012). More and more metabolites have been shown not to derive from a strictly cytosolic or plastidial pool of precursors, but the exact way the cross talk works is still unclear (Box 2). As an example, sesquiterpenes can be synthesized using a plastidial pool of IPP (Dudareva et al., 2005; Bartram et al., 2006). Monoterpenes and diterpenes also can have mixed origins (Itoh et al., 2003; Wungsintaweekul and De-Eknamkul, 2005; Hampel et al., 2007).

New strategies emerged for the engineering of terpenoids in plants, which consisted of targeting the overexpressed enzymes to different subcellular localizations to take advantage of either the MVA- or the MEP-derived pool of precursors (Wu et al., 2006). Using such a strategy, the engineering of sesquiterpenes and monoterpenes was monitored in tobacco (Wu et al., 2006). To produce sesquiterpenes (patchoulol or amorpha-4,11-diene), those authors expressed in tobacco the corresponding terpene synthase and a farnesyl diphosphate synthase fused (or not) to a chloroplast-targeting sequence to exploit either the MEP- or the MVA-derived pool of precursors, respectively. In this pioneering study, expression of the transgenes was driven by ubiquist promoters (a different one for each transgene). Addressing the enzymes to the cytosol only led to a low yield of sesquiterpenes (a few ng g^{-1} fresh weight, as in previous studies), while addressing the enzyme to the plastids (to take advantage of the MEP-derived pool of IPP/DMAPP) led to a much higher yield (up to 25 μg g^{-1} fresh

BOX 2. The Crosstalk between Isoprenoid Biosynthesis Pathways

Isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are the common precursors of the two isoprenoid biosynthetic pathways (the mevalonate [MVA] and the methylerythritol phosphate [MEP] pathways) in plants. Several chemical and biochemical analyses already demonstrated that the crosstalk involves the exchange of IPP/DMAPP (Hemmerlin et al., 2003; Bick and Lange, 2003; Flügge and Gao, 2005; Jozwiak et al., 2017). Also, geranylgeranyl diphosphate synthesized in the plastids is used for protein geranylgeranylation in the cytosol by the protein geranylgeranyltransferase type-I (Gerber et al., 2009). Geranyl diphosphate synthesized in the plastids can exit in the cytosol where it is converted into farnesyl diphosphate (FPP) by FPP synthase (Adam et al., 1999) or is directly used by terpene synthases to produce monoterpenes (Gutensohn et al., 2014). Farnesol or one of its derivatives has been shown to inhibit the MEP pathway and is so expected to cross the plastid envelope (Huchelmann et al., 2016). Even though the MEP pathway was discovered in plants in the early 1990s (Arigoni et al., 1997; Schwarz 1994; reviewed by Rohmer, 2003), and the crosstalk roughly ten years later (Hemmerlin et al. 2003, 2012), the precursor exchange mechanism has not been discovered yet. To fully understand the importance of the crosstalk and use its features for metabolic engineering purposes, the identification and characterization of proteins involved in transport is needed.
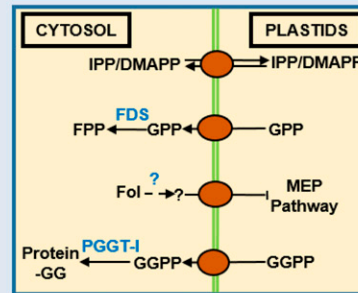
Box 2 Figure. Transport of terpenoid precursors through the plastid envelope. Exchange of isopentenyl diphosphate (IPP) and/or dimethylallyl diphosphate (DMAPP) between the cytosolic and plastid pathways is possible via an unknown mechanism. Other exchanges have been shown to take place, although the transporters have not been identified. The C10 geranyl diphosphate (GPP) and the C20 geranylgeranyl diphosphate (GGPP) are known to exit the plastids. Farnesol (Fol), a C15 molecule, or one of its metabolites (symbolized by a question mark) is known to enter the plastids. FPP, Farnesyl diphosphate; FDS, FPP synthase; MEP, methylerythritol phosphate; PGGT-I, protein geranylgeranyltransferase type I; Protein-GG, geranylgeranylated protein.

weight). Those impressive results are also quite surprising, as sesquiterpenes are synthesized naturally in the cytosol, while the engineered production is higher when the enzymes are localized in plastids. Monoterpene production in the cytosol was achieved using the same strategy to express the monoterpene synthase and the geranyl diphosphate synthase. However, in this case, the localization of the enzymes seemed to be less important, as both engineering strategies (using the MVA- and the MEP-derived precursors) led to roughly the same amount of R-(+)-limonene (400–500 ng g^{-1} fresh weight; Wu et al., 2006).

Modifying the fate of isoprenoid precursors can lead to severe phenotypes, which might be expected given the importance of isoprenoids in plant growth and development and the fact that expression was ubiquist. Tobacco lines producing the highest quantity of sesquiterpenes were indeed severely affected: they exhibited chlorosis and dwarfism. This phenotype also was observed in metabolic engineering of the ginsenoside saponin in tobacco (Gwak et al., 2017). In this case, production of the saponin led to a severe phenotype, including dwarfism, change in flower and pollen morphology, and impaired seed production (Gwak

et al., 2017). Similarly, dwarfism was observed upon metabolic engineering of tobacco chloroplast to produce artemisinic acid (Saxena et al., 2014). All these approaches used ubiquist promoters to drive expression of the transgenes. Because of the similarity of the phenotypes observed (dwarfism, chlorosis, and decreased seed production) between the different reports, cytotoxicity of the new metabolites is probably not the only cause. The problem could lay in the constitutive expression of transgenes, which might result in plant depletion of its essential terpenoid precursors (IPP/DMAPP). For plant metabolic engineering to be efficient, there is a necessity to better control the spatiotemporal expression of the transgenes. One strategy to limit the effect of sinking essential IPP pools consists of specifically targeting the expression of the genes in a cell type-specific way. In this respect, glandular trichomes are an ideal expression system.

Terpenoid Engineering Specifically in Trichomes

From a metabolic point of view, glandular trichomes are of particular interest, as they are involved in the

synthesis, storage, and/or excretion of specialized metabolites, making these compounds easily available. The carbon metabolism of glandular trichomes in Solanaceae has evolved to support high metabolite production (Balcke et al., 2017). Some genera, such as *Nicotiana*, produce up to 15% of their leaf biomass in the trichomes (Wagner et al., 2004). The C_{20} terpenoids are exported to diffuse within the cuticle and mediate resistance to insects and fungi (Chang and Grunwald, 1976; Severson et al., 1984; Wang and Wagner, 2003; Sallaud et al., 2012). Rerouting the diterpene production from cembrane and labdane types (naturally produced in tobacco) to other diterpenes has been performed by addressing the terpene synthase directly to the plastids with a trichome-specific promoter.

Diterpene Engineering

One of the best examples is the heterologous production of taxadiene in trichomes of the wild tobacco *N. sylvestris* (Rontein et al., 2008; Tissier et al., 2012). Taxadiene, particularly taxa-4(5),11(12)-diene, is the precursor of paclitaxel, a potent anticancer diterpene usually extracted from the bark of *Taxus* spp. (Hezari et al., 1995; Koepp et al., 1995). Several groups have already tried to synthesize this precursor ubiquitously in plants (for review, see Soliman and Tang, 2015), but the production of the diterpene triggered growth defects in tomato (Kovacs et al., 2007) and a lethal phenotype in Arabidopsis, most probably because of an overused geranylgeranyl diphosphate pool for diterpene synthesis (Besumbes et al., 2004). As a result, the MEP-derived primary metabolites were not synthesized in sufficient amounts to sustain normal growth. Only a few attempts led to the production of taxadiene in plants without any defect, as in ginseng (*Panax ginseng*; Cha et al., 2012) and *N. benthamiana* (Hasan et al., 2014) by the ubiquitous overexpression of the taxadiene synthase, but the amount produced was quite low.

Expression of taxadiene synthase specifically in *N. tabacum* trichomes, using the transcriptional promoter of cembratrienol synthase (CBTS), led to the production in the exudate of 5 to 10 μg g^{-1} fresh weight taxadiene, representing only 10% of the total taxadiene production, suggesting a problem of excretion (Tissier et al., 2012; discussed further in Box 3). However, the amount produced is impressive compared with the 27 μg g^{-1}

BOX 3. The Input of Metabolite Transporters

Terpenoids can be cytotoxic and, because of this, most are usually secreted out of the cells, but only a few studies have reported on how these metabolites get transported. Although they are expected to easily diffuse across membranes, active transport might still be required to keep the internal concentration as low as possible to avoid toxicity (Adebesin et al., 2017; Widhalm et al., 2015). *Nicotiana plumbaginifolia* trichomes secrete large amounts of diterpenes involved in the biotic stress response. Pleiotropic Drug Resistance (PDR) 1, an ATP-binding cassette (ABC) transporter localized to the plasma membrane was shown to be involved in diterpene extrusion (Jasinski et al., 2001; Crouzet el al., 2013). The secretion of diterpenes by tobacco trichomes seems not only to be dependent on PDR1 but also on another class of proteins, the lipid transfer proteins (LTPs). *N. tabacum* plants over-expressing *NtLTP1* have a greater content of cembratiene-diol in the leaf exudate, while the *NtLTP1*-silenced line has a slightly lower content of this diterpene in the leaf exudate (Choi et al., 2012). As another example, Wang et al. (2016a) transiently expressed the metabolic pathway of artemisinin, a sesquiterpene, in *Nicotiana benthamiana*. The expression of both a PDR transporter and an LTP resulted in the increased secretion of artemisinin precursors. Recent studies in yeast and mammalian cells showed that cholesterol is transported through the cytosol, from the endoplasmic reticulum to the plasma membrane, by LTPs (reviewed by Mesmin and Antonny [2016]).

Petunia hybrida produces insecticidal molecules such as petuniasterone and petuniolide (Elliger et al., 1988, 1990). A screening for *P. hybrida PDR* genes expressed in trichomes showed that *PhPDR2* is mainly expressed in the trichomes and leaf margin. A metabolite screening on plants silenced for *PhPDR2* showed a link between the transporter and the accumulation of the steroidal compound as well as herbivore sensitivity (Sasse et al., 2016). Although direct transport of the steroids by PDR2 has not been shown, the transporter has a role during the response to herbivores and the mobilization of the metabolites in the trichomes and leaf margins. Another ABC transporter, PhABCG1, was recently found to actively mediate the passage of volatile compounds across the plasma membrane of petunia petals (Adebesin et al., 2017).

The role of PDR in import or export of metabolites was also investigated in *Artemisia annua*. T-shaped glandular trichomes from the sweet wormwood are involved in the synthesis and accumulation of β-caryophyllene, a sesquiterpene. In this species, AaPDR3, an ABC transporter, was shown to actively transport sesquiterpene (Fu et al., 2017).

The identification of secondary metabolite transporters is only at its beginning, and many transport mechanisms still need to be investigated. But based on recent studies, it can be hypothesized that combining the appropriate LTPs and plasma membrane transporters to trichomes producing squalene and/or triterpenes in the plastids, or overproducing metabolites in the cells, would help prevent cytotoxicity. As a prerequisite, appropriate transporters and LTPs will have to be identified.

dry weight obtained in the best stable transgenic *N. benthamiana* line (Hasan et al., 2014). Hasan et al. (2014) could increase the production in *N. benthamiana* up to 48 μg g^{-1} dry weight by, in addition to constitutively overexpressing the taxadiene synthase, silencing the expression of the gene coding for the phytoene synthase and thereby increasing the geranylgeranyl diphosphate availability. In *N. tabacum*, silencing the genes coding for CBTS did not increase the production of taxadiene (Tissier et al., 2012). Compared with the cultivated tobacco (*N. tabacum*), the wild tobacco (*N. sylvestris*) naturally synthesizes, as diterpenes, cembranoids but no labdanoids. Using the *CBTS* promoter to drive the expression of the 8-hydroxy-copalyl diphosphate synthase and the Z-abienol synthase specifically in *N. sylvestris* trichomes, synthesis of the labdanoid, Z-abienol, reached a yield of 30 μg g^{-1} fresh weight without any major impact on the production of cembranoids or on plant development (Sallaud et al., 2012). Similarly, casbene, another diterpene from

Ricinus communis, also was produced in *N. tabacum* trichomes (Tissier et al., 2012), demonstrating that these structures can really be used as a biofactory for the production of a diverse set of diterpenes. While most of the cembrane and labdane diterpenes are normally found in the cuticle or in the exudate, only a small fraction (10%) of casbene or taxadiene was found in the exudate, suggesting the necessity of associating a transporter when engineering a metabolic pathway (Box 3).

Triterpene Engineering

Could *N. tabacum* trichomes be used as a production platform for terpenoids other than diterpenes? In *N. tabacum* trichomes, the main isoprenoid-derived metabolites are diterpenes from the MEP pathway. Metabolic engineering in trichomes might take advantage of this cross talk to produce, for example, triterpenes instead of the naturally occurring diterpenes by diverting
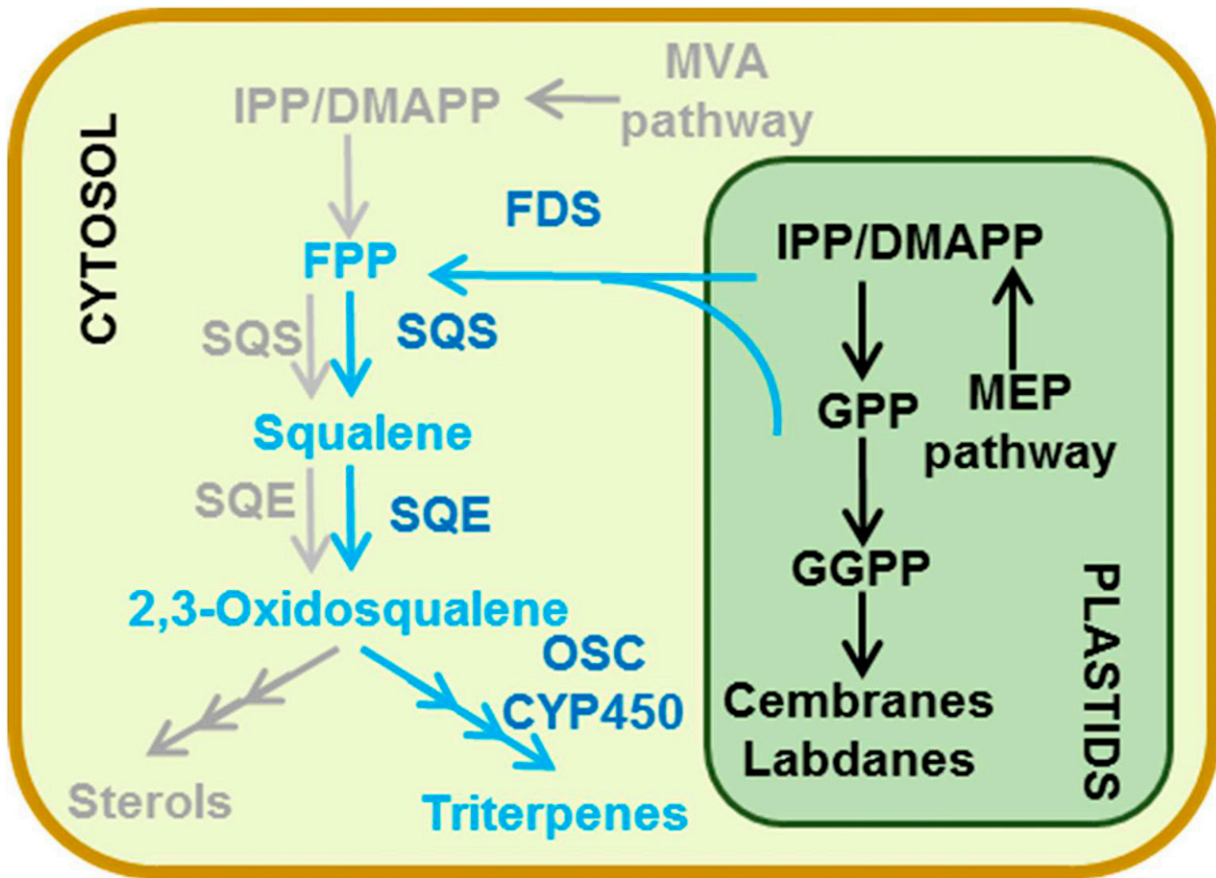

Figure 4. Terpenoid metabolism in engineered tobacco trichomes for triterpene production in the cytosol. The gray pathway represents the normal biosynthetic route for triterpenes and sterols. The enzymes are targeted to the cytosol to enhance the cross talk and sink the plastids from its precursors. Overexpressed enzymes are denoted in dark blue. New products deriving from the engineering metabolism are denoted in light blue. CYP450, Cytochrome P450; FDS, farnesyl diphosphate synthase; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; OSC, 2,3-oxidosqualene cyclase; SQE, squalene epoxidase; SQS, squalene synthase.

the plastidic pool of IPP toward the cytosol to produce the compounds of interest (Fig. 4). Some plants, such as *S. habrochaites*, produce some sesquiterpenes directly in the plastids. The corresponding enzymes, involved in the biosynthesis of those C_{15} terpenes, a sesquiterpene synthase and a farnesyl diphosphate synthase, evolved to be localized to the plastids (Sallaud et al., 2009). Engineering the production of MVA-derived metabolites such as sesquiterpenes (C_{15}) or triterpenes (C_{30}) in the plastids using specifically MEP-derived IPP and DMAPP precursors may require the corresponding enzymes (normally localized to the cytosol or to the endoplasmic reticulum) to be engineered to get targeted to the plastids (Fig. 5).

Triterpenes, the C_{30} family, are of particular interest for their substantial carbon content, making them good targets for biofuel production (Gübitz et al., 1999; Khan et al., 2014) as well as for pharmacological purposes. The biosynthesis of triterpenes requires several enzymatic steps (for review, see Phillips et al., 2006; Thimmappa et al., 2014). The production of squalene is the limiting regulatory step to produce triterpenes. Modifying the production and fate of squalene, a key precursor of phytosterols, is risky if this modification affects the whole plant, as it can lead to dwarfism and loss of fertility (for review, see Clouse, 2002; Schaller, 2003, 2004). Using the CBTS promoter, the production of squalene was achieved in *N. tabacum* trichomes (Wu et al., 2012). The production was greater when the two enzymes for squalene production, squalene synthase

and farnesyl diphosphate synthase, were targeted to the chloroplast. Even though the expression of the transgenes was expected to be restricted to trichomes, those transgenic plants that expressed squalene at the highest level displayed strong phenotypes such as dwarfism and chlorosis, which are similar to the phenotypes observed upon ubiquist expression of transgenes impacting the pool of IPP precursors (Wu et al., 2006; Saxena et al., 2014; Gwak et al., 2017).

Using the same approach, the production of linear triterpenes typical of the green alga *Botryococcus braunii* was achieved in *N. tabacum* trichomes (Jiang et al., 2016). Once again, the best production rate was when the enzymes were targeted to the plastid, which suggests that the best strategy for triterpene production is to exploit the plastidial pool of IPP/DMAPP. However, as for squalene production (Wu et al., 2012), the synthesis of triterpenes led to chlorosis and dwarfism. Plastidial membranes are quite fragile, and the balance of sterols is important for the stability of the plastids (Babiychuk et al., 2008). Thus, the production of squalene or other triterpenes in the plastids might disrupt the chloroplast integrity and lead to chlorosis. Since trichomes are thought to be dispensable, the breakdown of chloroplasts is not expected to have major consequences on the whole plant. A possible explanation for this phenotype is that the promoter was not completely trichome specific, probably due to the presence of 35S enhancers upstream of the promoter (Wu et al., 2012; Jiang et al., 2016) or to some positional
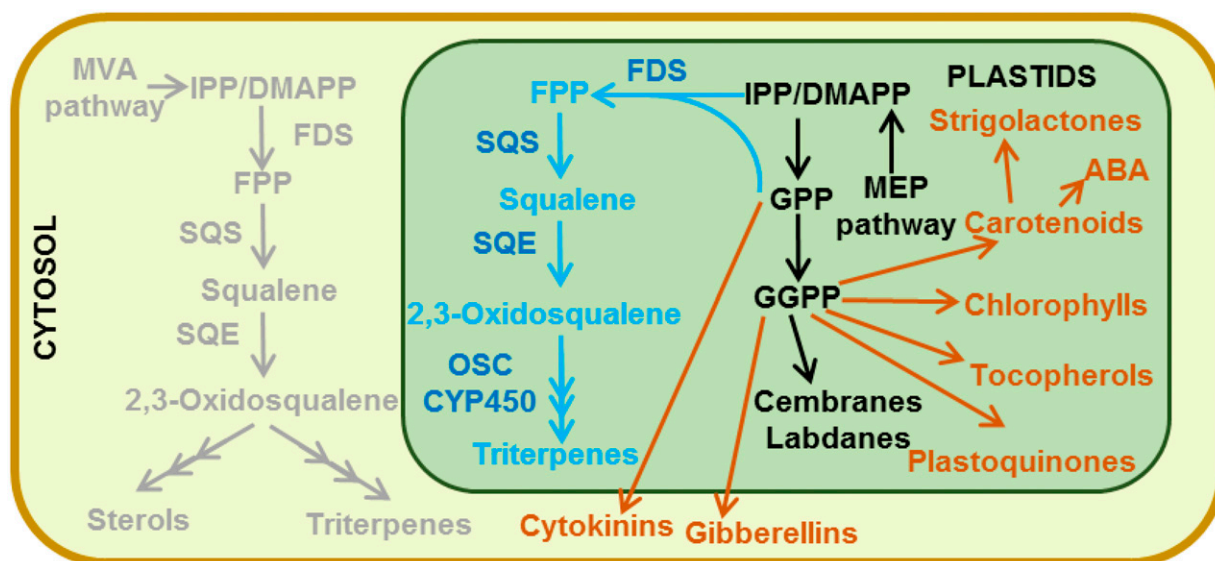

Figure 5. Terpenoid metabolism in engineered tobacco trichomes for triterpene production in plastids. The gray pathway represents the normal biosynthetic route for triterpenes and sterols. The enzymes are targeted to directly produce the triterpenes in the plastids. Overexpressed enzymes are denoted in dark blue. New products deriving from the engineering metabolism are denoted in light blue. The sinking plastidial isoprenoid pool might provoke undesirable consequences. Potentially affected metabolites are denoted in orange. ABA, Abscisic acid; CYP450, cytochrome P450; FDS, farnesyl diphosphate synthase; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; OSC, 2,3-oxidosqualene cyclase; SQE, squalene epoxidase; SQS, squalene synthase.

OUTSTANDING QUESTIONS

- How diverse is the transcriptional control of glandular trichome development in plants? Is there a single transcriptional network at least partially shared between different types of glandular trichomes, or did these structures evolve independently in different (groups of) species (via some evolutionary convergence)?
- Can we engineer trichomes to produce potentially toxic compounds without consequences for the rest of the plant? This also raises the question of finding appropriate gene promoters to drive the different transgenes in plants. Special emphasis should be set on identifying strictly cell-type specific promoters to avoid undesirable effects on plant growth and development.
- To which extent can we exploit the existing crosstalk between the plastidial and cytosolic isoprenoid biosynthesis pathways? Are the cytosolic and plastidial pools of precursors somehow connected via these crosstalks so that depletion of one pool could be compensated by the other one, or are they rather independent?
- How important is it to include transporters in metabolic engineering approaches to help secrete out the compounds of interest (thereby avoiding cytotoxicity) or channel the precursors to the appropriate cell compartment?
- What about the primary metabolism occurring in glandular trichome cells that supports such an intense secondary metabolism? What are the carbon, nitrogen, and energy sources? How do they reach the trichome head cells?

effects of the transgene affecting the specificity of the promoter. The cell type specificity of transgene(s) expression, therefore, should be strictly ascertained in transgenic lines and not based solely on the assessment of the cell type specificity of the promoter via the analysis of transcriptional reporter lines. Another plausible explanation for such a phenotype could be that, although trichomes are not essential, they may transmit a stress signal to other leaf tissues, leading to the observed phenotypes in the transgenic lines (Wu et al., 2012).

Metabolic engineering in tobacco has great potential. The amount of terpenoids that the plant can naturally produce is impressive. Using the plastidial pool for the production of terpenoids seems much more efficient than using the cytosolic one (Wu et al., 2006). However, ubiquist expression alters plant development and directly impairs the yield. To be economically viable, the yield of terpenoids should be increased with no or only a slight impact on the plant phenotype. Lack of excretion of the potentially cytotoxic metabolites is another issue (discussed in Box 3). In addition, comprehension of the exchange of prenyl diphosphates between the plastids and the cytosol (Box 2) also should be investigated so as to reroute the plastidic flux toward the cytosol and avoid disrupting the stability of the chloroplast with a change in sterol profile (Babiychuk et al., 2008).

CONCLUSION

At present, plant biologists are trying to go beyond the Arabidopsis model and to move to nonmodel plant species. In this respect, the study of glandular trichome biology is benefiting greatly from such a change of model system. Recent advances in DNA sequencing, omics technology, and reverse genetics, including plant genome editing, now offer new technical resources to investigate such biological aspects in a wide variety of species.

Our current understanding of both the development of glandular trichomes and of the biosynthetic pathways going on in these structures is improving, but at this point it is still quite fragmentary. However, an increasing number of research groups are now focusing on various aspects of glandular trichome biology, including developmental aspects and bioengineering.

Understanding the way glandular trichomes develop to finally turn into highly efficient biochemical factories in the epidermis of nonmodel plant species is of key importance and could lead to more applied outcomes. This calls for basic research to address these fascinating aspects. It is now time to consider the real biotechnological potential of glandular trichomes as biochemical factories and use up-to-date technology to fully exploit the cellular machinery. Increased knowledge of these fundamental aspects will, in the mid term, allow researchers to tap the up-to-now largely unexploited biotechnological potential of glandular trichomes to engineer plants that would exhibit increased resistance to pests or that would produce compounds of immense industrial/pharmaceutical interest (molecular pharming).

LITERATURE CITED

Adam KP, Thiel R, Zapp J (1999) Incorporation of 1-[1-^{13}C]deoxy-D-xylulose in chamomile sesquiterpenes. Arch Biochem Biophys **369:** 127–132

Adebesin F, Widhalm JR, Boachon B, Lefèvre F, Pierman B, Lynch JH, Alam I, Junqueira B, Benke R, Ray S, et al (2017) Emission of volatile organic compounds from petunia flowers is facilitated by an ABC transporter. Science **356:** 1386–1388

Adrian J, Chang J, Ballenger CE, Bargmann BO, Alassimone J, Davies KA, Lau OS, Matos JL, Hachez C, Lanctot A, et al (2015) Transcriptome dynamics of the stomatal lineage: birth, amplification, and termination of a self-renewing population. Dev Cell **33:** 107–118

Aharoni A, Jongsma MA, Bouwmeester HJ (2005) Volatile science? Metabolic engineering of terpenoids in plants. Trends Plant Sci **10:** 594–602

Akhtar MQ, Qamar N, Yadav P, Kulkarni P, Kumar A, Shasany AK (2017) Comparative glandular trichome transcriptome-based gene characterization reveals reasons for differential (−)-menthol biosynthesis in Mentha species. Physiol Plant 160: 128–141

An L, Zhou Z, Yan A, Gan Y (2011) Progress on trichome development regulated by phytohormone signaling. Plant Signal Behav 6: 1959–1962

Arigoni D, Sagner S, Latzel C, Eisenreich W, Bacher A, Zenk MH (1997) Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. Proc Natl Acad Sci USA 94: 10600–10605

Aschenbrenner AK, Amrehn E, Bechtel L, Spring O (2015) Trichome differentiation on leaf primordia of Helianthus annuus (Asteraceae): morphology, gene expression and metabolite profile. Planta 241: 837–846

Babiychuk E, Bouvier-Navé P, Compagnon V, Suzuki M, Muranaka T, Van Montagu M, Kushnir S, Schaller H (2008) Allelic mutant series reveal distinct functions for Arabidopsis cycloartenol synthase 1 in cell viability and plastid biogenesis. Proc Natl Acad Sci USA 105: 3163–3168

Back K, Chappell J (1996) Identifying functional domains within terpene cyclases using a domain-swapping strategy. Proc Natl Acad Sci USA 93: 6841–6845

Balcke GU, Bennewitz S, Bergau N, Athmer B, Henning A, Majovsky P, Jiménez-Gómez JM, Hoehenwarter W, Tissier A (2017) Multi-omics of tomato glandular trichomes reveals distinct features of central carbon metabolism supporting high productivity of specialized metabolites. Plant Cell 29: 960–983

Balkunde R, Pesch M, Hülskamp M (2010) Trichome patterning in Arabidopsis thaliana from genetic to molecular models. Curr Top Dev Biol 91: 299–321

Bartram S, Jux A, Gleixner G, Boland W (2006) Dynamic pathway allocation in early terpenoid biosynthesis of stress-induced lima bean leaves. Phytochemistry 67: 1661–1672

Bergau N, Bennewitz S, Syrowatka F, Hause G, Tissier A (2015) The development of type VI glandular trichomes in the cultivated tomato Solanum lycopersicum and a related wild species S. habrochaites. BMC Plant Biol 15: 289

Bergau N, Navarette Santos A, Henning A, Balcke GU, Tissier A (2016) Autofluorescence as a signal to sort developing glandular trichomes by flow cytometry. Front Plant Sci 7: 949

Besumbes O, Sauret-Güeto S, Phillips MA, Imperial S, Rodríguez-Concepción M, Boronat A (2004) Metabolic engineering of isoprenoid biosynthesis in Arabidopsis for the production of taxadiene, the first committed precursor of taxol. Biotechnol Bioeng 88: 168–175

Bick JA, Lange BM (2003) Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membrane. Arch Biochem Biophys 415: 146–154

Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A, Kant MR, Prins M, de Vos M, Haring MA, Schuurink RC (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proc Natl Acad Sci USA 109: 20124–20129

Bolger A, Scossa F, Bolger ME, Lanz C, Maumus F, Tohge T, Quesneville H, Alseekh S, Sørensen I, Lichtenstein G, et al (2014) The genome of the stress-tolerant wild tomato species Solanum pennellii. Nat Genet 46: 1034–1038

Bombo AB, Appezzato-da-Glória B, Aschenbrenner AK, Spring O (2016) Capitate glandular trichomes in Aldama discolor (Heliantheae-Asteraceae): morphology, metabolite profile and sesquiterpene biosynthesis. Plant Biol (Stuttg) 18: 455–462

Bosch M, Wright LP, Gershenzon J, Wasternack C, Hause B, Schaller A, Stintzi A (2014) Jasmonic acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. Plant Physiol 166: 396–410

Bose SK, Yadav RK, Mishra S, Sangwan RS, Singh AK, Mishra B, Srivastava AK, Sangwan NS (2013) Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in Mentha arvensis L. Plant Physiol Biochem 66: 150–158

Bouvier F, Rahier A, Camara B (2005) Biogenesis, molecular regulation and function of plant isoprenoids. Prog Lipid Res 44: 357–429

Cha M, Shim SH, Kim SH, Kim OT, Lee SW, Kwon SY, Baek KH (2012) Production of taxadiene from cultured ginseng roots transformed with taxadiene synthase gene. BMB Rep 45: 589–594

Champagne A, Boutry M (2013) Proteomics of nonmodel plant species. Proteomics 13: 663–673

Chang SY, Grunwald C (1976) Duvatrienediols in cuticular wax of Burley tobacco leaves. J Lipid Res 17: 7–11

Chen C, Liu M, Jiang L, Liu X, Zhao J, Yan S, Yang S, Ren H, Liu R, Zhang X (2014) Transcriptome profiling reveals roles of meristem regulators and polarity genes during fruit trichome development in cucumber (Cucumis sativus L.). J Exp Bot 65: 4943–4958

Choi YE, Lim S, Kim HJ, Han JY, Lee MH, Yang Y, Kim JA, Kim YS (2012) Tobacco NtLTP1, a glandular-specific lipid transfer protein, is required for lipid secretion from glandular trichomes. Plant J 70: 480–491

Clouse SD (2002) Arabidopsis mutants reveal multiple roles for sterols in plant development. Plant Cell 14: 1995–2000

Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). Biochem Mol Biol Plants 24: 1250–1319

Cui JY, Miao H, Ding LH, Wehner TC, Liu PN, Wang Y, Zhang SP, Gu XF (2016) A new glabrous gene (csgl3) identified in trichome development in cucumber (Cucumis sativus L.). PLoS ONE 11: e0148422

Dai X, Wang G, Yang DS, Tang Y, Broun P, Marks MD, Sumner LW, Dixon RA, Zhao PX (2010) TrichOME: a comparative omics database for plant trichomes. Plant Physiol 152: 44–54

Deschamps C, Gang D, Dudareva N, Simon JE (2006) Developmental regulation of phenylpropanoid biosynthesis in leaves and glandular trichomes of basil (Ocimum basilicum L.). Int J Plant Sci 167: 447–454

Dixon RA (2005) Engineering of plant natural product pathways. Curr Opin Plant Biol 8: 329–336

Dokládal L, Obořil M, Stejskal K, Zdráhal Z, Ptáčková N, Chaloupková R, Damborský J, Kašparovský T, Jeandroz S, Zd'árská M, et al (2012) Physiological and proteomic approaches to evaluate the role of sterol binding in elicitin-induced resistance. J Exp Bot 63: 2203–2215

Dudareva N, Andersson S, Orlova I, Gatto N, Reichelt M, Rhodes D, Boland W, Gershenzon J (2005) The nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. Proc Natl Acad Sci USA 102: 933–938

Edwards KD, Fernandez-Pozo N, Drake-Stowe K, Humphry M, Evans AD, Bombarely A, Allen F, Hurst R, White B, Kernodle SP, et al (2017) A reference genome for Nicotiana tabacum enables map-based cloning of homeologous loci implicated in nitrogen utilization efficiency. BMC Genomics 18: 448

Elliger CA, Benson ME, Haddon WF, Lundin RE, Waiss AC, Wong RY (1988) Petuniasterones, novel ergostane-type steroids of Petunia hybridia Vilm. (Solanaceae) having insect-inhibitory activity: x-ray molecular structure of the 22,24,25-[(methoxycarbonyl)orthoacetate] of 7[α],22,24,25-tetrahydroxy ergosta-1,4-dien-3-one and of 1[α]-acetoxy-24,25-epoxy-7[α]-hydroxy-22-(methylthiocarbonyl)acetoxyergost-4-en-3-one. J Chem Soc Perkin Trans 1 711–717

Elliger CA, Wong RY, Waiss AC, Benson M (1990) Petuniolides: unusual ergostanoid lactones from Petunia species that inhibit insect development. J Chem Soc Perkin Trans 1 525–531

Ennajdaoui H, Vachon G, Giacalone C, Besse I, Sallaud C, Herzog M, Tissier A (2010) Trichome specific expression of the tobacco (Nicotiana sylvestris) cembratrien-ol synthase genes is controlled by both activating and repressing cis-regions. Plant Mol Biol 73: 673–685

Fahn A (2000) Structure and function of secretory cells. In DL Hallahan, JC Gray, JA Callow, eds, Plant Trichomes. Advances in Botanical Research Incorporating Advances in Plant Pathology, Vol 31. Academic Press, London

Farhi M, Marhevka E, Ben-Ari J, Algamas-Dimantov A, Liang Z, Zeevi V, Edelbaum O, Spitzer-Rimon B, Abeliovich H, Schwartz B, et al (2011) Generation of the potent anti-malarial drug artemisinin in tobacco. Nat Biotechnol 29: 1072–1074

Flügge UI, Gao W (2005) Transport of isoprenoid intermediates across chloroplast envelope membranes. Plant Biol (Stuttg) 7: 91–97

Fridman E, Wang J, Iijima Y, Froehlich JE, Gang DR, Ohlrogge J, Pichersky E (2005) Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species Lycopersicon hirsutum identify a key enzyme in the biosynthesis of methylketones. Plant Cell 17: 1252–1267

Fu X, Shi P, He Q, Shen Q, Tang Y, Pan Q, Ma Y, Yan T, Chen M, Hao X, et al (2017) AaPDR3, a PDR transporter 3, is involved in sesquiterpene β-caryophyllene transport in Artemisia annua. Front Plant Sci 8: 723

Gang DR, Wang J, Dudareva N, Nam KH, Simon JE, Lewinsohn E, Pichersky E (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiol 125: 539–555

Gerber E, Hemmerlin A, Hartmann M, Heintz D, Hartmann MA, Mutterer J, Rodríguez-Concepción M, Boronat A, Van Dorsselaer A, Rohmer M,

et al (2009) The plastidial 2-C-methyl-D-erythritol 4-phosphate pathway provides the isoprenyl moiety for protein geranylgeranylation in tobacco BY-2 cells. Plant Cell 21: 285–300

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3: 408–414

Gershenzon J, McCaskill D, Rajaonarivony JI, Mihaliak C, Karp F, Croteau R (1992) Isolation of secretory cells from plant glandular trichomes and their use in biosynthetic studies of monoterpenes and other gland products. Anal Biochem 200: 130–138

Glas JJ, Schimmel BC, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13: 17077–17103

Glover BJ, Perez-Rodriguez M, Martin C (1998) Development of several epidermal cell types can be specified by the same MYB-related plant transcription factor. Development 125: 3497–3508

Gübitz GM, Mittelbach M, Trabi M (1999) Exploitation of the tropical oil seed plant Jatropha curcas L. Bioresour Technol 67: 73–82

Guo Z, Wagner GJ (1995) Biosynthesis of labdenediol and sclareol in cell-free extracts from trichomes of Nicotiana glutinosa. Planta 197: 627–632

Gutensohn M, Nguyen TT, McMahon RD III, Kaplan I, Pichersky E, Dudareva N (2014) Metabolic engineering of monoterpene biosynthesis in tomato fruits via introduction of the non-canonical substrate neryl diphosphate. Metab Eng 24: 107–116

Gutiérrez-Alcalá G, Calo L, Gros F, Caissard JC, Gotor C, Romero LC (2005) A versatile promoter for the expression of proteins in glandular and non-glandular trichomes from a variety of plants. J Exp Bot 56: 2487–2494

Gwak YS, Han JY, Adhikari PB, Ahn CH, Choi YE (2017) Heterologous production of a ginsenoside saponin (compound K) and its precursors in transgenic tobacco impairs the vegetative and reproductive growth. Planta (in press)

Hampel D, Swatski A, Mosandl A, Wüst M (2007) Biosynthesis of monoterpenes and norisoprenoids in raspberry fruits (Rubus idaeus L.): the role of cytosolic mevalonate and plastidial methylerythritol phosphate pathway. J Agric Food Chem 55: 9296–9304

Hasan MM, Kim HS, Jeon JH, Kim SH, Moon B, Song JY, Shim SH, Baek KH (2014) Metabolic engineering of Nicotiana benthamiana for the increased production of taxadiene. Plant Cell Rep 33: 895–904

Hemmerlin A, Harwood JL, Bach TJ (2012) A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? Prog Lipid Res 51: 95–148

Hemmerlin A, Hoeffler JF, Meyer O, Tritsch D, Kagan IA, Grosdemange-Billiard C, Rohmer M, Bach TJ (2003) Cross-talk between the cytosolic mevalonate and the plastidial methylerythritol phosphate pathways in tobacco Bright Yellow-2 cells. J Biol Chem 278: 26666–26676

Hezari M, Lewis NG, Croteau R (1995) Purification and characterization of taxa-4(5),11(12)-diene synthase from Pacific yew (Taxus brevifolia) that catalyzes the first committed step of taxol biosynthesis. Arch Biochem Biophys 322: 437–444

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P, et al (2009) The genome of the cucumber, Cucumis sativus L. Nat Genet 41: 1275–1281

Huchelmann A, Brahim MS, Gerber E, Tritsch D, Bach TJ, Hemmerlin A (2016) Farnesol-mediated shift in the metabolic origin of prenyl groups used for protein prenylation in plants. Biochimie 127: 95–102

Huchelmann A, Gastaldo C, Veinante M, Zeng Y, Heintz D, Tritsch D, Schaller H, Rohmer M, Bach TJ, Hemmerlin A (2014) S-Carvone suppresses cellulase-induced capsidiol production in Nicotiana tabacum by interfering with protein isoprenylation. Plant Physiol 164: 935–950

Itoh D, Kawano K, Nabeta K (2003) Biosynthesis of chloroplastidic and extrachloroplastidic terpenoids in liverwort cultured cells: 13C serine as a probe of terpene biosynthesis via mevalonate and non-mevalonate pathways. J Nat Prod 66: 332–336

Jaffé FW, Tattersall A, Glover BJ (2007) A truncated MYB transcription factor from Antirrhinum majus regulates epidermal cell outgrowth. J Exp Bot 58: 1515–1524

Jiang Z, Kempinski C, Bush CJ, Nybo SE, Chappell J (2016) Engineering triterpene and methylated triterpene production in plants provides biochemical and physiological insights into terpene metabolism. Plant Physiol 170: 702–716

Jin J, Panicker D, Wang Q, Kim MJ, Liu J, Yin JL, Wong L, Jang IC, Chua NH, Sarojam R (2014) Next generation sequencing unravels the biosynthetic ability of spearmint (Mentha spicata) peltate glandular trichomes through comparative transcriptomics. BMC Plant Biol 14: 292

Jozwiak A, Lipko A, Kania M, Danikiewicz W, Surmacz L, Witek A, Wojcik J, Zdanowski K, Pączkowski C, Chojnacki T, et al (2017) Modeling of dolichol mass spectra isotopic envelopes as a tool to monitor isoprenoid biosynthesis. Plant Physiol 174: 857–874

Kandra L, Wagner GJ (1988) Studies of the site and mode of biosynthesis of tobacco trichome exudate components. Arch Biochem Biophys 265: 425–432

Kang JH, Campos ML, Zemelis-Durfee S, Al-Haddad JM, Jones AD, Telewski FW, Brandizzi F, Howe GA (2016) Molecular cloning of the tomato Hairless gene implicates actin dynamics in trichome-mediated defense and mechanical properties of stem tissue. J Exp Bot 67: 5313–5324

Kang JH, Liu G, Shi F, Jones AD, Beaudry RM, Howe GA (2010) The tomato odorless-2 mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. Plant Physiol 154: 262–272

Kang JH, McRoberts J, Shi F, Moreno JE, Jones AD, Howe GA (2014) The flavonoid biosynthetic enzyme chalcone isomerase modulates terpenoid production in glandular trichomes of tomato. Plant Physiol 164: 1161–1174

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA (2008) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc Natl Acad Sci USA 105: 7100–7105

Keasling JD (2010) Manufacturing molecules through metabolic engineering. Science 330: 1355–1358

Keene CK, Wagner GJ (1985) Direct demonstration of duvatrienediol biosynthesis in glandular heads of tobacco trichomes. Plant Physiol 79: 1026–1032

Khan NE, Myers JA, Tuerk AL, Curtis WR (2014) A process economic assessment of hydrocarbon biofuels production using chemoautotrophic organisms. Bioresour Technol 172: 201–211

Kim S, Park M, Yeom SI, Kim YM, Lee JM, Lee HA, Seo E, Choi J, Cheong K, Kim KT, et al (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet 46: 270–278

Kim SH, Chang YJ, Kim SU (2008) Tissue specificity and developmental pattern of amorpha-4,11-diene synthase (ADS) proved by ADS promoter-driven GUS expression in the heterologous plant, Arabidopsis thaliana. Planta Med 74: 188–193

Kirby J, Keasling JD (2009) Biosynthesis of plant isoprenoids: perspectives for microbial engineering. Annu Rev Plant Biol 60: 335–355

Koepp AE, Hezari M, Zajicek J, Vogel BS, LaFever RE, Lewis NG, Croteau R (1995) Cyclization of geranylgeranyl diphosphate to taxa-4(5),11(12)-diene is the committed step of taxol biosynthesis in Pacific yew. J Biol Chem 270: 8686–8690

Koo AJK, Howe GA (2009) The wound hormone jasmonate. Phytochemistry 70: 1571–1580

Kortbeek RW, Xu J, Ramirez A, Spyropoulou E, Diergaarde P, Otten-Bruggeman I, de Both M, Nagel R, Schmidt A, Schuurink RC, et al (2016) Engineering of tomato glandular trichomes for the production of specialized metabolites. Methods Enzymol 576: 305–331

Kovacs K, Zhang L, Linforth RS, Whittaker B, Hayes CJ, Fray RG (2007) Redirection of carotenoid metabolism for the efficient production of taxadiene [taxa-4(5),11(12)-diene] in transgenic tomato fruit. Transgenic Res 16: 121–126

Kroumova AB, Wagner GJ (2003) Different elongation pathways in the biosynthesis of acyl groups of trichome exudate sugar esters from various solanaceous plants. Planta 216: 1013–1021

Lange BM, Ahkami A (2013) Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenes: current status and future opportunities. Plant Biotechnol J 11: 169–196

Lange BM, Mahmoud SS, Wildung MR, Turner GW, Davis EM, Lange I, Baker RC, Boydston RA, Croteau RB (2011) Improving peppermint essential oil yield and composition by metabolic engineering. Proc Natl Acad Sci USA 108: 16944–16949

Lange BM, Turner GW (2013) Terpenoid biosynthesis in trichomes: current status and future opportunities. Plant Biotechnol J 11: 2–22

Laterre R, Pottier M, Remacle C, Boutry M (2017) Photosynthetic trichomes contain a specific Rubisco with a modified pH-dependent activity. Plant Physiol 173: 2110–2120

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell 16: 126–143

Li Q, Cao C, Zhang C, Zheng S, Wang Z, Wang L, Ren Z (2015) The identification of Cucumis sativus Glabrous 1 (CsGL1) required for the formation of trichomes uncovers a novel function for the homeodomain-leucine zipper I gene. J Exp Bot 66: 2515–2526

Liakopoulos G, Stavrianakou S, Karabourniotis G (2006) Trichome layers versus dehaired lamina of Olea europaea leaves: differences in flavonoid distribution, UV-absorbing capacity, and wax yield. Environ Exp Bot 55: 294–304

Lipko A, Swiezewska E (2016) Isoprenoid generating systems in plants—A handy toolbox how to assess contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthetic process. Progress in lipid research 63: 70–92

Liu J, Xia KF, Zhu JC, Deng YG, Huang XL, Hu BL, Xu X, Xu ZF (2006) The nightshade proteinase inhibitor IIb gene is constitutively expressed in glandular trichomes. Plant Cell Physiol 47: 1274–1284

Liu X, Bartholomew E, Cai Y, Ren H (2016) Trichome-related mutants provide a new perspective on multicellular trichome initiation and development in cucumber (Cucumis sativus L). Front Plant Sci 7: 1187

Liu Y, Liu D, Hu R, Hua C, Ali I, Zhang A, Liu B, Wu M, Huang L, Gan Y (2017) AtGIS, a C2H2 zinc-finger transcription factor from Arabidopsis regulates glandular trichome development through GA signaling in tobacco. Biochem Biophys Res Commun 483: 209–215

Lücker J, Schwab W, Franssen MCR, Van Der Plas LHW, Bouwmeester HJ, Verhoeven HA (2004) Metabolic engineering of monoterpene biosynthesis: two-step production of (+)-trans-isopiperitenol by tobacco. Plant J 39: 135–145

Luu VT, Weinhold A, Ullah C, Dressel S, Schoettner M, Gase K, Gaquerel E, Xu S, Baldwin IT (2017) O-Acyl sugars protect a wild tobacco from both native fungal pathogens and a specialist herbivore. Plant Physiol 174: 370–386

Ma D, Hu Y, Yang C, Liu B, Fang L, Wan Q, Liang W, Mei G, Wang L, Wang H, et al (2016) Genetic basis for glandular trichome formation in cotton. Nat Commun 7: 10456

Maluf WR, Barbosa LV, Costa Santa-Cecília LV (1997) 2-Tridecanone-mediated mechanisms of resistance to the South American tomato pinworm Scrobipalpuloides absoluta (Meyrick, 1917) (Lepidoptera-Gelechiidae) in Lycopersicon spp. Euphytica 93: 189–194

Marienhagen J, Bott M (2013) Metabolic engineering of microorganisms for the synthesis of plant natural products. J Biotechnol 163: 166–178

Matías-Hernández L, Aguilar-Jaramillo AE, Cigliano RA, Sanseverino W, Pelaz S (2016) Flowering and trichome development share hormonal and transcription factor regulation. J Exp Bot 67: 1209–1219

Matías-Hernández L, Jiang W, Yang K, Tang K, Brodelius PE, Pelaz S (2017) AaMYB1 and its orthologue AtMYB61 affect terpene metabolism and trichome development in Artemisia annua and Arabidopsis thaliana. Plant J 90: 520–534

Mesmin B, Antonny B (2016) The counterflow transport of sterols and PI4P. Biochim Biophys Acta 1861: 940–951

Moses T, Pollier J, Thevelein JM, Goossens A (2013) Bioengineering of plant (tri)terpenoids: from metabolic engineering of plants to synthetic biology in vivo and in vitro. New Phytol 200: 27–43

Muravnik LE, Kostina OV, Shavarda AL (2016) Glandular trichomes of Tussilago farfara (Senecioneae, Asteraceae). Planta 244: 737–752

Nakashima T, Wada H, Morita S, Erra-Balsells R, Hiraoka K, Nonami H (2016) Single-cell metabolite profiling of stalk and glandular cells of intact trichomes with internal electrode capillary pressure probe electrospray ionization mass spectrometry. Anal Chem 88: 3049–3057

Natsume S, Takagi H, Shiraishi A, Murata J, Toyonaga H, Patzak J, Takagi M, Yaegashi H, Uemura A, Mitsuoka C, et al (2015) The draft genome of hop (Humulus lupulus), an essence for brewing. Plant Cell Physiol 56: 428–441

Okada Y, Ito K (2001) Cloning and analysis of valerophenone synthase gene expressed specifically in lupulin gland of hop (Humulus lupulus L.). Biosci Biotechnol Biochem 65: 150–155

Paetzold H, Garms S, Bartram S, Wieczorek J, Urós-Gracia EM, Rodríguez-Concepción M, Boland W, Strack D, Hause B, Walter MH (2010) The isogene 1-deoxy-D-xylulose 5-phosphate synthase 2 controls isoprenoid profiles, precursor pathway allocation, and density of tomato trichomes. Mol Plant 3: 904–916

Pan Y, Bo K, Cheng Z, Weng Y (2015) The loss-of-function GLABROUS 3 mutation in cucumber is due to LTR-retrotransposon insertion in a class IV HD-ZIP transcription factor gene CsGL3 that is epistatic over CsGL1. BMC Plant Biol 15: 302

Pattanaik S, Patra B, Singh SK, Yuan L (2014) An overview of the gene regulatory network controlling trichome development in the model plant, Arabidopsis. Front Plant Sci 5: 259

Payne T, Clement J, Arnold D, Lloyd A (1999) Heterologous myb genes distinct from GL1 enhance trichome production when overexpressed in Nicotiana tabacum. Development 126: 671–682

Phillips DR, Rasbery JM, Bartel B, Matsuda SP (2006) Biosynthetic diversity in plant triterpene cyclization. Curr Opin Plant Biol 9: 305–314

Puterka GJ, Farone W, Palmer T, Barrington A (2003) Structure-function relationships affecting the insecticidal and miticidal activity of sugar esters. J Econ Entomol 96: 636–644

Qi T, Huang H, Wu D, Yan J, Qi Y, Song S, Xie D (2014) *Arabidopsis* DELLA and JAZ proteins bind the WD-repeat/bHLH/MYB complex to modulate gibberellin and jasmonate signaling synergy. Plant Cell 26: 1118–1133

Ralston L, Kwon ST, Schoenbeck M, Ralston J, Schenk DJ, Coates RM, Chappell J (2001) Cloning, heterologous expression, and functional characterization of 5-epi-aristolochene-1,3-dihydroxylase from tobacco (Nicotiana tabacum). Arch Biochem Biophys 393: 222–235

Rohmer M (2003) Mevalonate-independent methylerythritol phosphate pathway for isoprenoid biosynthesis: elucidation and distribution. Pure Appl Chem 75: 375–388

Rontein D, Onillon S, Herbette G, Lesot A, Werck-Reichhart D, Sallaud C, Tissier A (2008) CYP725A4 from yew catalyzes complex structural rearrangement of taxa-4(5),11(12)-diene into the cyclic ether 5(12)-oxa-3(11)-cyclotaxane. J Biol Chem 283: 6067–6075

Sallaud C, Giacalone C, Töpfer R, Goepfert S, Bakaher N, Rösti S, Tissier A (2012) Characterization of two genes for the biosynthesis of the labdane diterpene Z-abienol in tobacco (Nicotiana tabacum) glandular trichomes. Plant J 72: 1–17

Sallaud C, Rontein D, Onillon S, Jabès F, Duffé P, Giacalone C, Thoraval S, Escoffier C, Herbette G, Leonhardt N, et al (2009) A novel pathway for sesquiterpene biosynthesis from Z,Z-farnesyl pyrophosphate in the wild tomato *Solanum habrochaites*. Plant Cell 21: 301–317

Sasse J, Schlegel M, Borghi L, Ullrich F, Lee M, Liu GW, Giner JL, Kayser O, Bigler L, Martinoia E, et al (2016) Petunia hybrida PDR2 is involved in herbivore defense by controlling steroidal contents in trichomes. Plant Cell Environ 39: 2725–2739

Saxena B, Subramaniyan M, Malhotra K, Bhavesh NS, Potlakayala SD, Kumar S (2014) Metabolic engineering of chloroplasts for artemisinic acid biosynthesis and impact on plant growth. J Biosci 39: 33–41

Schaller H (2003) The role of sterols in plant growth and development. Prog Lipid Res 42: 163–175

Schaller H (2004) New aspects of sterol biosynthesis in growth and development of higher plants. Plant Physiol Biochem 42: 465–476

Schilmiller A, Shi F, Kim J, Charbonneau AL, Holmes D, Jones AD, Last RL (2010) Mass spectrometry screening reveals widespread diversity in trichome specialized metabolites of tomato chromosomal substitution lines. Plant J 62: 391–403

Schwarz MK (1994) Terpen-Biosynthese in Ginkgo biloba: eine berraschende Geschichte, 2nd Ed. Thesis No. 10951, ETH Zurich.

Serna L, Martin C (2006) Trichomes: different regulatory networks lead to convergent structures. Trends Plant Sci 11: 274–280

Severson RF, Arrendale RF, Chortyk OT, Johnson AW, Jackson DM, Gwynn GR, Chaplin JF, Stephenson MG (1984) Quantitation of the major cuticular components from green leaf of different tobacco types. J Agric Food Chem 32: 566–570

Shangguan XX, Xu B, Yu ZX, Wang LJ, Chen XY (2008) Promoter of a cotton fibre MYB gene functional in trichomes of Arabidopsis and glandular trichomes of tobacco. J Exp Bot 59: 3533–3542

Sierro N, Battey JN, Ouadi S, Bovet L, Goepfert S, Bakaher N, Peitsch MC, Ivanov NV (2013) Reference genomes and transcriptomes of Nicotiana sylvestris and Nicotiana tomentosiformis. Genome Biol 14: R60

Sierro N, Battey JND, Ouadi S, Bakaher N, Bovet L, Willig A, Goepfert S, Peitsch MC, Ivanov NV (2014) The tobacco genome sequence and its comparison with those of tomato and potato. Nat Commun 5: 3833

Simmons AT, Gurr GM, McGrath D, Martin PM, Nicol HI (2004) Entrapment of Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) on glandular trichomes of Lycopersicon species. Aust J Entomol 43: 196–200

Soetaert SS, Van Neste CM, Vandewoestyne ML, Head SR, Goossens A, Van Nieuwerburgh FC, Deforce DL (2013) Differential transcriptome analysis of glandular and filamentous trichomes in Artemisia annua. BMC Plant Biol 13: 220

Soliman S, Tang Y (2015) Natural and engineered production of taxadiene with taxadiene synthase. Biotechnol Bioeng 112: 229–235

Spring O, Pfannstiel J, Klaiber I, Conrad J, Beifuß U, Apel L, Aschenbrenner AK, Zipper R (2015) The nonvolatile metabolome of sunflower linear glandular trichomes. Phytochemistry 119: 83–89

Spyropoulou EA, Haring MA, Schuurink RC (2014) Expression of Terpenoids 1, a glandular trichome-specific transcription factor from tomato that activates the terpene synthase 5 promoter. Plant Mol Biol 84: 345–357

Starks CM, Back K, Chappell J, Noel JP (1997) Structural basis for cyclic terpene biosynthesis by tobacco 5-epi-aristolochene synthase. Science 277: 1815–1820

Stoessl A, Stothers JB, Ward EWB (1976) Sesquiterpenoid stress compounds of the Solanaceae. Phytochemistry 15: 855–872

Tattini M, Gravano E, Pinelli P, Mulinacci N, Romani A (2000) Flavonoids accumulate in leaves and glandular trichomes of Phillyrea latifolia exposed to excess solar radiation. New Phytol 148: 69–77

Thimmappa R, Geisler K, Louveau T, O'Maille P, Osbourn A (2014) Triterpene biosynthesis in plants. Annu Rev Plant Biol 65: 225–257

Tian D, Peiffer M, De Moraes CM, Felton GW (2014) Roles of ethylene and jasmonic acid in systemic induced defense in tomato (Solanum lycopersicum) against Helicoverpa zea. Planta 239: 577–589

Tissier A (2012a) Glandular trichomes: what comes after expressed sequence tags? Plant J 70: 51–68

Tissier A (2012b) Trichome specific expression: promoters and their applications. In YO Çiftçi, ed, Transgenic Plants: Advances and Limitations. InTech, pp 353–378

Tissier A, Sallaud C, Rontein D (2012) Tobacco trichomes as a platform for terpenoid biosynthesis engineering. In Bach TJ, Rohmer M, eds, Isoprenoid Synthesis in Plants and Microorganisms. Springer, New York, New York, pp 271–283

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485: 635–641

Tominaga-Wada R, Ishida T, Wada T (2011) New insights into the mechanism of development of Arabidopsis root hairs and trichomes. Int Rev Cell Mol Biol 286: 67–106

Trikka FA, Nikolaidis A, Ignea C, Tsaballa A, Tziveleka LA, Ioannou E, Roussis V, Stea EA, Božić D, Argiriou A, et al (2015) Combined metabolome and transcriptome profiling provides new insights into diterpene biosynthesis in S. pomifera glandular trichomes. BMC Genomics 16: 935

Valkama E, Salminen JP, Koricheva J, Pihlaja K (2003) Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish birch species. Ann Bot (Lond) 91: 643–655

van Bakel H, Stout JM, Cote AG, Tallon CM, Sharpe AG, Hughes TR, Page JE (2011) The draft genome and transcriptome of Cannabis sativa. Genome Biol 12: R102

Venditti A, Bianco A, Frezza C, Serafini M, Giacomello G, Giuliani C, Bramucci M, Quassinti L, Lupidi G, Lucarini D, et al (2016) Secondary metabolites, glandular trichomes and biological activity of Sideritis montana L. subsp. montana from central Italy. Chem Biodivers 13: 1380–1390

Verpoorte R, Memelink J (2002) Engineering secondary metabolite production in plants. Curr Opin Biotechnol 13: 181–187

Vining KJ, Johnson SR, Ahkami A, Lange I, Parrish AN, Trapp SC, Croteau RB, Straub SCK, Pandelova I, Lange BM (2017) Draft genome sequence of Mentha longifolia and development of resources for mint cultivar improvement. Mol Plant 10: 323–339

Voirin B, Bayet C, Colson M (1993) Demonstration that flavone aglycones accumulate in the peltate glands of Mentha × piperita leaves. Phytochemistry 34: 85–87

Wagner GJ, Wang E, Shepherd RW (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. Ann Bot (Lond) 93: 3–11

Wang E, Gan S, Wagner GJ (2002) Isolation and characterization of the CYP71D16 trichome-specific promoter from Nicotiana tabacum L. J Exp Bot 53: 1891–1897

Wang E, Wagner GJ (2003) Elucidation of the functions of genes central to diterpene metabolism in tobacco trichomes using posttranscriptional gene silencing. Planta 216: 686–691

Wang H, Han J, Kanagarajan S, Lundgren A, Brodelius PE (2013) Trichome-specific expression of the amorpha-4,11-diene 12-hydroxylase (cyp71av1) gene, encoding a key enzyme of artemisinin biosynthesis in Artemisia annua, as reported by a promoter-GUS fusion. Plant Mol Biol 81: 119–138

Wang H, Olofsson L, Lundgren A, Brodelius PE (2011) Trichome-specific expression of amorpha-4,11-diene synthase, a key enzyme of artemisinin biosynthesis in Artemisia annua L., as reported by a promoter-GUS fusion. Am J Plant Sci 2: 619–628

Wang Q, Reddy VA, Panicker D, Mao HZ, Kumar N, Rajan C, Venkatesh PN, Chua NH, Sarojam R (2016a) Metabolic engineering of terpene biosynthesis in plants using a trichome-specific transcription factor MsYABBY5 from spearmint (Mentha spicata). Plant Biotechnol J 14: 1619–1632

Wang Y, Luo SH, Hua J, Liu Y, Jing SX, Li XN, Li SH (2015) Capitate glandular trichomes of Paragutzlaffia henryi harbor new phytotoxic labdane diterpenoids. J Agric Food Chem 63: 10004–10012

Wang YL, Nie JT, Chen HM, Guo CL, Pan J, He HL, Pan JS, Cai R (2016b) Identification and mapping of Tril, a homeodomain-leucine zipper gene involved in multicellular trichome initiation in Cucumis sativus. Theor Appl Genet 129: 305–316

Wei S, Marton I, Dekel M, Shalitin D, Lewinsohn E, Bravdo BA, Shoseyov O (2004) Manipulating volatile emission in tobacco leaves by expressing Aspergillus niger beta-glucosidase in different subcellular compartments. Plant Biotechnol J 2: 341–350

Weinhold A, Baldwin IT (2011) Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation. Proc Natl Acad Sci USA 108: 7855–7859

Widhalm JR, Jaini R, Morgan JA, Dudareva N (2015) Rethinking how volatiles are released from plant cells. Trends Plant Sci 20: 545–550

Williams WG, Kennedy GG, Yamamoto RT, Thacker JD, Bordner J (1980) 2-Tridecanone: a naturally occurring insecticide from the wild tomato Lycopersicon hirsutum f. glabratum. Science 207: 888–889

Wu S, Chappell J (2008) Metabolic engineering of natural products in plants: tools of the trade and challenges for the future. Curr Opin Biotechnol 19: 145–152

Wu S, Jiang Z, Kempinski C, Nybo SE, Husodo S, Williams R, Chappell J (2012) Engineering triterpene metabolism in tobacco. Planta 236: 867–877

Wu S, Schalk M, Clark A, Miles RB, Coates R, Chappell J (2006) Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. Nat Biotechnol 24: 1441–1447

Wungsintaweekul J, De-Eknamkul W (2005) Biosynthesis of plaunotol in Croton stellatopilosus proceeds via the deoxyxylulose phosphate pathway. Tetrahedron Lett 46: 2125–2128

Xie Z, Kapteyn J, Gang DR (2008) A systems biology investigation of the MEP/terpenoid and shikimate/phenylpropanoid pathways points to multiple levels of metabolic control in sweet basil glandular trichomes. Plant J 54: 349–361

Xu X, Pan S, Cheng S, Zhang B, Mu D, Ni P, Zhang G, Yang S, Li R, Wang J, et al (2011) Genome sequence and analysis of the tuber crop potato. Nature 475: 189–195

Yan T, Chen M, Shen Q, Li L, Fu X, Pan Q, Tang Y, Shi P, Lv Z, Jiang W, et al (2017) HOMEODOMAIN PROTEIN 1 is required for jasmonate-mediated glandular trichome initiation in Artemisia annua. New Phytol 213: 1145–1155

Yang C, Gao Y, Gao S, Yu G, Xiong C, Chang J, Li H, Ye Z (2015) Transcriptome profile analysis of cell proliferation molecular processes during multicellular trichome formation induced by tomato Wov gene in tobacco. BMC Genomics 16: 868

Yang C, Li H, Zhang J, Luo Z, Gong P, Zhang C, Li J, Wang T, Zhang Y, Lu Y, et al (2011a) A regulatory gene induces trichome formation and embryo lethality in tomato. Proc Natl Acad Sci USA 108: 11836–11841

Yang C, Li H, Zhang J, Wang T, Ye Z (2011b) Fine-mapping of the woolly gene controlling multicellular trichome formation and embryonic development in tomato. Theor Appl Genet 123: 625–633

Yu G, Nguyen TTH, Guo Y, Schauvinhold I, Auldridge ME, Bhuiyan N, Ben-Israel I, Iijima Y, Fridman E, Noel JP, et al (2010) Enzymatic functions of wild tomato methylketone synthases 1 and 2. Plant Physiol 154: 67–77

Yu G, Pichersky E (2014) Heterologous expression of methylketone synthase1 and methylketone synthase2 leads to production of methylketones and myristic acid in transgenic plants. Plant Physiol 164: 612–622

Zhao JL, Pan JS, Guan Y, Nie JT, Yang JJ, Qu ML, He HL, Cai R (2015) Transcriptome analysis in Cucumis sativus identifies genes involved in multicellular trichome development. Genomics 105: 296–303