the plant journal

SEB Society for Experimental Biology

The Plant Journal (2012) **70**, 51–68 doi: 10.1111/j.1365-313X.2012.04913.x

HIGH-RESOLUTION MEASUREMENTS IN PLANT BIOLOGY

Glandular trichomes: what comes after expressed sequence tags?

Alain Tissier*

Department of Metabolic and Cell Biology, Leibniz-Institute of Plant Biochemistry, Weinberg 3, 06120 Halle (Saale), Germany

Received 21 November 2011; revised 16 January 2012; accepted 17 January 2012.
*For correspondence (e-mail alain.tissier@ipb-halle.de).

SUMMARY

Glandular trichomes cover the surface of many plant species. They exhibit tremendous diversity, be it in their shape or the compounds they secrete. This diversity is expressed between species but also within species or even individual plants. The industrial uses of some trichome secretions and their potential as a defense barrier, for example against arthropod pests, has spurred research into the biosynthesis pathways that lead to these specialized metabolites. Because complete biosynthesis pathways take place in the secretory cells, the establishment of trichome-specific expressed sequence tag libraries has greatly accelerated their elucidation. Glandular trichomes also have an important metabolic capacity and may be considered as true cell factories. To fully exploit the potential of glandular trichomes as breeding or engineering objects, several research areas will have to be further investigated, such as development, patterning, metabolic fluxes and transcription regulation. The purpose of this review is to provide an update on the methods and technologies which have been used to investigate glandular trichomes and to propose new avenues of research to deepen our understanding of these specialized structures.

Keywords: glandular trichomes, transcriptomics, proteomics, development, modeling.

INTRODUCTION

When one looks at plant surfaces with a scanning electron microscope, a picture of bewildering diversity of shapes and structures comes to view. The first level of complexity lies at the very surface of the cells where the outermost layer of the cuticle, i.e. the epicuticle, produces various elements such as wax crystals (Samuels *et al.*, 2008). This is in the sub-micrometer range and is not within the scope of this review. When one zooms out, a surprising micro-landscape of tree- and bush-like structures is encountered in many species. These protuberances, which range in size from a few microns to several centimeters, are called trichomes. Their diversity is almost as great as the number of species on which they are encountered, as they can be single-celled or multicellular, curved or straight, non-secretory or glandular, and with many more descriptive criteria possible (Werker, 2000). A glossary of terms depicting trichomes with over 700 entries was even compiled, giving us an idea of the range of shapes that may be encountered (Payne, 1978). There is, however, one criterion which is commonly used to classify trichomes, namely whether they are glandular or not. Glandular trichomes also encompass a great variety of shapes and structures, but have in common the presence of metabolically active cells with, for many of them, the capacity to secrete or store large quantities of specialized metabolites. The distribution of glandular trichomes in the plant kingdom suggests a fairly ancient evolution. Peltate trichomes can be observed in fossilized leaf material from seed ferns such as *Barthelopteris germarii* of the late Paleozoic (Krings and Kerp, 1998) and multicellular glandular trichomes from the seed fern *Blanzyopteris praedentata* of the late Carboniferous (Krings *et al.*, 2003). The latter are compared with the explosive glandular trichomes of extant angiosperms such as *Sicana odorifera* (Cucurbitaceae), suggesting that these fern glandular trichomes may have been part of a defensive strategy against arthropod pests (Krings *et al.*, 2002, 2003). Studies on living ferns, in particular the Pteridaceae, also indicate the existence of glandular trichomes which are involved in the production of a farinose wax composed mostly of flavonoid aglycones (Wollenweber *et al.*, 1998; reviewed in Wollenweber and Schneider, 2000).

No record of glandular trichomes from bryophytes could be found, suggesting that glandular trichomes are an invention of vascular plants. Although hairs are found on twigs of gymnosperms, such as *Picea* sp. where they are used as taxonomic markers (Fernald, 1950; cited in Johnson, 1975), glandular trichomes *per se* are rare and were reported only in juvenile leaves of *Pinus cembra* and *Pinus lambertiana* (Napp-Zinn, 1966). In contrast, gymnosperms are endowed with another type of secretory cell, namely those of the resin ducts, which are located inside the plant body (Fahn, 1988). Glandular trichomes occur very frequently in angiosperms where they can be found in many plant families (Fahn, 1988; Werker, 2000; Wagner *et al.*, 2004). However, a comprehensive survey of the occurrence and types of glandular trichomes across the angiosperm clade is missing and would certainly provide more insight into the evolutionary patterns of these structures. Species of some angiosperm dicotyledonous families such as the Lamiaceae, Solanaceae, Asteraceae and Cannabaceae are particularly rich in glandular trichomes. Monocotyledons are less known for their glandular trichomes, but there are well-studied cases of the oil-producing trichomes which occur in flowers of species from the Liliaceae and the Orchidaceae and play critical roles in pollination by specialized bees (Buchmann, 1987). Fahn (1988, 2002) has summarized and reviewed the current hypotheses on the evolution of plant secretory structures and of glandular trichomes in particular. It is suggested that in the course of evolution, the secretory cells were originally located in the mesophyll and subsequently migrated either to internal vascular tissues or to the outside to generate glandular trichomes. According to this theory, glandular trichomes would represent the most recent and advanced type of secretory tissue (Fahn, 1988). There is, however, no molecular support for this hypothesis, and the sheer diversity of structures and shapes of glandular trichomes would indicate that they may not be the result of a single evolutionary event. On the contrary, a study on basal angiosperms suggests that trichomes may have evolved from stomata several times independently (Carpenter, 2006). Even within a single genus, *Sisyrinchium* (Iridaceae), phylogenetic analysis indicates that glandular trichomes have evolved three times independently (Chauveau *et al.*, 2011). In addition, in some cases, morphological analogies between multicellular non-glandular and glandular trichomes have suggested that glandular trichomes have evolved from non-glandular trichomes through the differentiation of apical cells into secretory cells (Uphof, 1962; Fahn and Shimony, 1977). One such example is provided by leaf trichomes of tomato species (*Solanum lycopersicum* and *Solanum habrochaites*) where the capitate trichomes of Type 1 only differ from the long non-glandular trichomes of Type 3 by the presence of a single secretory cell at the tip (Luckwill, 1943) (see Figure 1A).

Glandular trichomes can also be classified according to the type of compounds they produce: hydrophilic, lipophilic, proteins, poly- or monosaccharides. Further, they may be classified according to the volatility of the compounds they produce. Volatile compounds such as mono- and sesquiterpenes, phenylpropenes or methylketones, if not retained one way or another would simply diffuse into the headspace. Some glandular trichomes, like the peltate trichomes of the Lamiaceae (mint family) are particularly well adapted to retain and store volatile compounds [in mint (*Mentha* sp.) or sage (*Salvia officinalis*) (see Figure 1G–I) for example]; a subcuticular storage cavity which results from the separation of the cuticle from the cell wall lies above the plate of eight glandular cells. Upon rupture of the cuticle, which may occur when an insect treads upon the trichome, or when the leaves are crushed, the volatile compounds of this storage space are released and can then diffuse into the headspace. Similar storage spaces are also found in glandular trichomes of Asteraceae, for example *Tanacetum parthenium* (see Figure 1E,F). On the contrary, capitate trichomes, such as those of tobacco, synthesize compounds, which are not volatile or are poorly volatile and are directly exuded onto the surface of the trichome (Figure 1D). The droplets of exudate can be seen coming out of the glandular cells at the tip of the trichome and dripping down the trichome stalk. In some cases, as in tobacco or in the wild tomato *Solanum pennellii*, the exudate is highly sticky and sets a trap for insects or small arthropods, which can become glued to the leaf and die (Fobes *et al.*, 1985). In most species however, several types of trichomes, non-glandular and glandular, capitate and peltate, coexist. The complexity of the trichome landscape is illustrated by a scanning electron microscopy image of a young leaf of a wild species of tomato (*Solanum habrochaites* ssp. *glabratum*) (Figure 1A). The diversity of trichomes is mirrored by the metabolic diversity of compounds they produce. As an example of this diversity, some compounds produced by tomato glandular trichomes, which will be referred to later in this review, are presented in Figure 2.

The secretions of glandular trichomes have been exploited by humans for a number of uses and are only illustrated by a couple of examples here. For a more detailed presentation of this topic, readers are referred to a recent review (Schilmiller *et al.*, 2008). Probably one of the most ancient uses stems from the highly fragrant and aromatic properties of some of those secretions. Collected by distillation they are referred to as essential oils. The plant family which contributes the most essential oils is the Lamiaceae, with popular species such as mint, basil, lavender, oregano or thyme; clary sage (*Salvia sclarea*), is grown for its essential oil, but particularly for its concrete, which contains a high content (up to 50%) of sclareol. Sclareol, a labdanoid diterpene, is produced in capitate trichomes of the inflorescences and is used as a synthesis precursor of fixative agents such as Ambrox or Ambroxane, which are highly prized ingredients in the fragrance and detergent industry

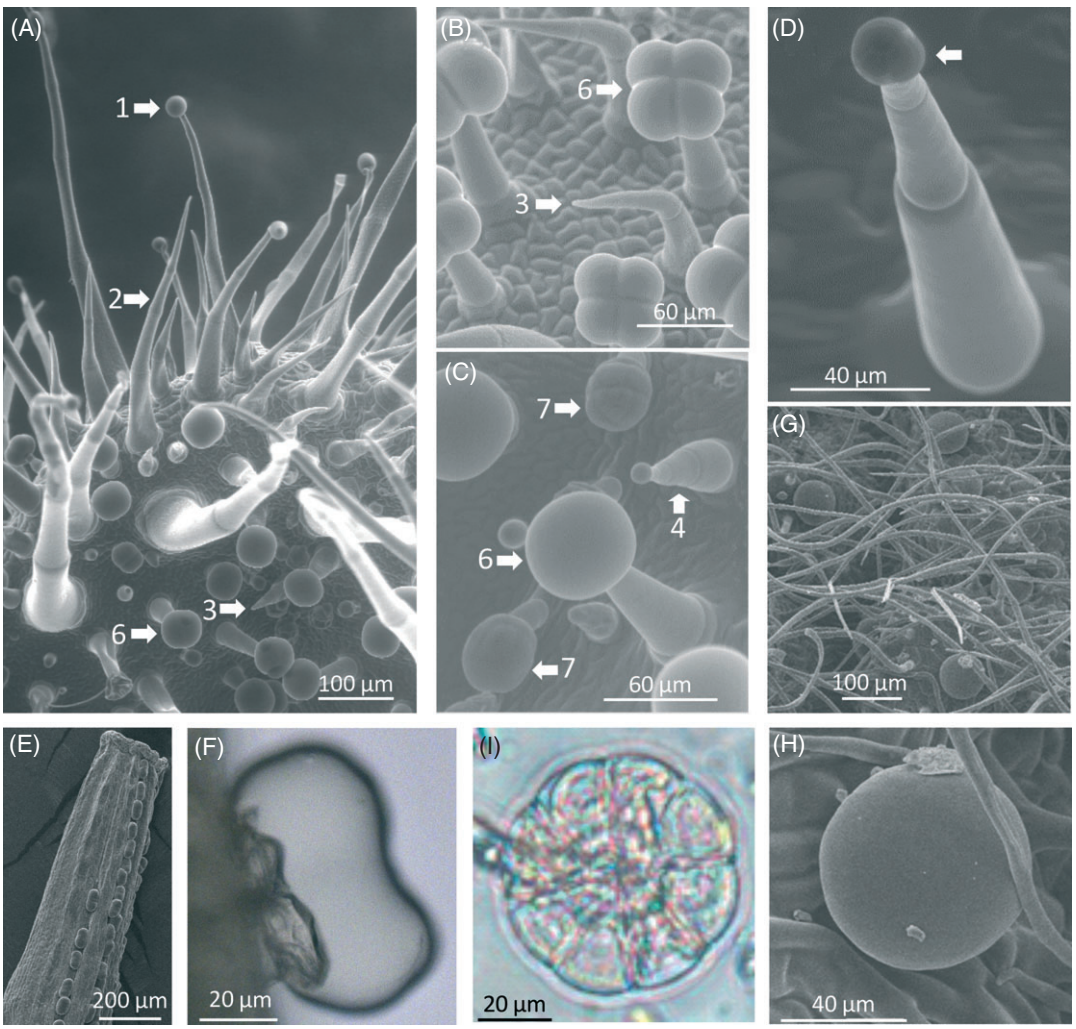

Figure 1. A sample of plant glandular trichome diversity.
(A) Scanning electron microscopy (SEM) image of a young wild tomato (*Solanum habrochaites*, accession LA2409) leaf. This picture illustrates the complexity of the leaf trichome landscape. At least five trichome types, numbered according to Luckwill (1943) can be observed: Type 1, tall glandular trichomes with a single secretory cell; Type 2, tall non-glandular trichomes; Type 3, short hooked non-glandular trichome; Type 6, glandular trichomes with four head cells; Type 7, short glandular trichomes.
(B) Detail of a tomato leaf (*Solanum lycopersicum* LA 4024) showing Type 6 and Type 3 trichomes.
(C) Detail of a wild tomato leaf (*S. habrochaites* LA 1777) showing Type 4, Type 6 and Type 7 trichomes. In *S. habrochaites*, the four glandular cells of Type 6 trichomes are enveloped in a peri-cellular cuticle, whereas in the cultivated tomato the four glandular cells can be distinctly seen (see B).
(D) Capitate trichome of *Nicotiana sylvestris*, similar to Type 4 trichomes of tomato. A droplet of diterpenoid-rich exudate can be seen on the side of the glandular head (white arrow).
(E) Scanning electron microscopy (SEM) image of a floret from *Tanacetum parthenium* showing files of glandular trichomes.
(F) A close-up view of the same type of glandular trichome in light microscopy showing the large subcuticular cavity where hydrophobic volatile compounds are stored, similar to the peltate trichomes of the Lamiaceae.
(G) Scanning electron microscopy (SEM) image of a *Salvia officinalis* leaf, showing peltate trichomes in a network of hairs.
(H, I) Detailed view of a peltate trichome of *S. officinalis*, showing the envelope delimiting the subcuticular storage space (H), and the plate of eight glandular cells (I).

(Moulines *et al.*, 2001, 2004; Barrero *et al.*, 2004; Frija *et al.*, 2011). Secretions of glandular trichomes have been used for their medicinal properties, and in some cases active ingredients have been marketed as drugs. Artemisinin is a sesquiterpene lactone produced in glandular trichomes of *Artemisia annua* and used for the treatment of malaria as an alternative to quinine drugs, which face increasing resistance from emerging strains of the malarial parasite (Covello, 2008; Weathers *et al.*, 2011). Pharmaceutical companies have developed derivatives of artemisinin (artemeter, artesunate) which are now widely marketed (Shanks, 2006).

In this review, I will provide an update on the methods and techniques which have been used to study glandular trichomes, and how this has allowed the elucidation of novel pathways for specialized metabolites. However, the interest in glandular trichomes also lies beyond the

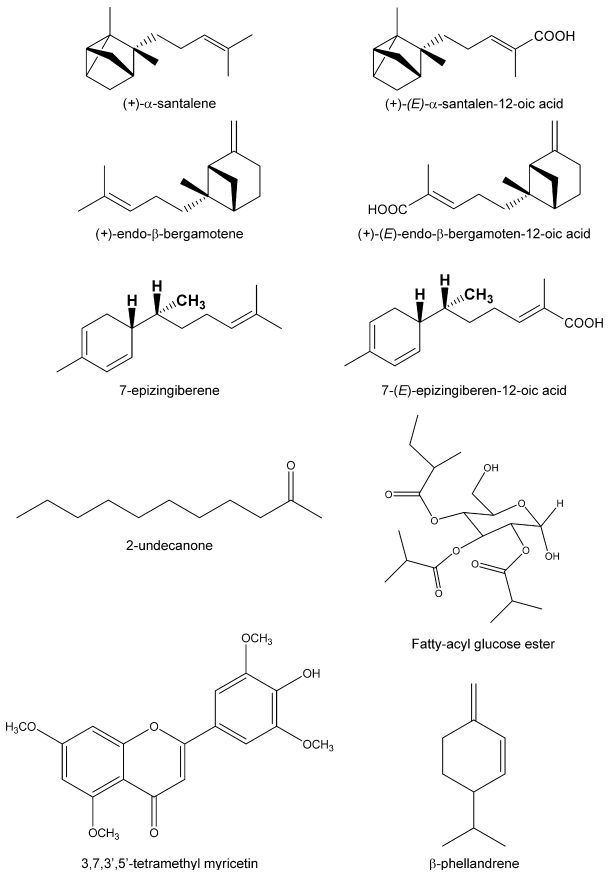

Figure 2. Examples of metabolites found in glandular trichomes of various tomato species.
(+)-α-santalene, (+)-endo-β-bergamotene, 7-epizingiberene and their respective carboxylic acids are found in Type 6 trichomes of *Solanum habrochaites*. 2-Undecanone is found in Type 6 trichomes of *S. habrochaites* ssp *glabratum*. Glucose esters of short chain fatty acids are produced by Type 4 trichomes of *Solanum pennellii*. The tetra-methylated myricetin is found preferentially in Type 4 trichomes *of S. habrochaites*. β-Phellandrene is produced in Type 6 trichomes of *S. lycopersicum*.

characterization of biochemical pathways, namely in the understanding of how these cell factories develop and function in order to be in a position to fully exploit their potential. Research avenues on how to achieve this goal are proposed, with, among others, the adoption of model species, comprehensive metabolic profiling integrating primary and specialized metabolism, detailed developmental studies and the functional analysis of genes by forward or reverse screens.

TECHNIQUES FOR COLLECTING AND PURIFYING TRICHOMES

Different methods have been tested and developed to specifically harvest plant trichomes, and in particular glandular trichomes. The peculiarities of different types of glandular trichomes have necessitated the development of adapted techniques which, while preserving the integrity of the glandular cells, allowed a sufficiently pure preparation. The most basic technique relies on abrasion of the leaf surface with a brush or a microscope glass slide. This was applied to harvest mint (Croteau and Winters, 1982) and tobacco trichomes (Keene and Wagner, 1985) and provided material that demonstrated that enzymes for the biosynthesis of compounds which are secreted by trichomes are also present in trichomes (see below). Later, the techniques were somewhat refined with the use of glass beads to detach glands followed by either a density gradient, e.g. in Percoll, or a succession of meshes of appropriate sizes to purify trichomes (Gershenzon *et al.*, 1987, 1992). This glass bead–mesh technique has turned out to be the most successful and it has been applied to numerous other species, including basil (Gang *et al.*, 2001), tomato (van Der Hoeven *et al.*, 2000; Fridman *et al.*, 2005; Slocombe *et al.*, 2008; Schilmiller *et al.*, 2010b), hops (Nagel *et al.*, 2008; Wang *et al.*, 2008), *Artemisia annua* (Teoh *et al.*, 2006), and sage (*Salvia fruticosa*) (Chatzopoulou *et al.*, 2010). Abrasion with powdered dry ice followed by mesh filtration was also used successfully to prepare heads of glandular trichomes from geranium, potato and tomato among others (Yerger *et al.*, 1992). This method presents the advantage that the glandular cells are frozen as they are harvested, thus providing high-quality material for RNA and protein isolation. Glandular trichomes from a number of species, including *Cistus creticus*, *Cistus laurifolius*, *Cannabis sativa* and *Nepeta racemosa*, were successfully prepared with this method, demonstrating its robustness and reliability (Clark *et al.*, 1997; Hallahan *et al.*, 1998; Sirikantaramas *et al.*, 2005; Falara *et al.*, 2008; Pateraki and Kanellis, 2010). Variations of these techniques were developed, for example in tobacco or *Medicago sativa*, by using leaves frozen in liquid nitrogen but abrading them with glass beads followed by mesh filtering (Ranger *et al.*, 2004; Amme *et al.*, 2005). Other methods rely on the trapping of glandular trichomes on adhesive tape and release of the trichomes from the tape by water (Yamaura *et al.*, 1992) or the use of double-sided tape stuck to a glass slide (Gopfert *et al.*, 2006). More recently, RNA from single glandular cells of *A. annua* trichomes could be recovered after laser microdissection. The *A. annua* glandular trichomes contain several layers of secretory cells, and the expression of artemisinin pathway genes could be shown to be preferentially located in the apical cells (Olsson *et al.*, 2009). However, the same authors recently published an improved method and demonstrated that genes of the artemisinin pathway are expressed in all cells (apical and sub-apical) (Olofsson *et al.*, 2012). Furthermore, specific expression in all secretory cells of the *Artemisia* glandular trichomes could be shown in transgenic plants carrying *AaAS-promoter:*GUS and *CYP71AV1-promoter:*GUS fusions (Wang *et al.*, 2011).

GLANDULAR TRICHOMES: SECRETING AND PRODUCING GLANDS

Early studies on the biochemistry and physiology of glandular trichomes pointed to the fact that intense metabolic activity takes place in the glandular cells and that they could be the site of biosynthesis of the secreted compounds. This was first observed by ultrastructural and cytochemical studies, for example in peppermint (Amelunxen, 1965; Amelunxen *et al.*, 1969; Lange *et al.*, 2000). The first biochemical evidence came with the feeding of ^{14}C-acetate to tobacco leaf epidermal peels containing trichomes, or whole trichomes collected by 'shaving' the stems. ^{14}C was incorporated into cembratrien-diols (CBT-diols, formerly called duvatrien-diols), the major diterpenes secreted by glandular trichomes (Michie and Reid, 1968). An apparently contradictory view was supported by the observation that CBT-diols were found on the whole surface of the epidermis, suggesting that they may not be synthesized exclusively in the trichomes but also in the epidermal cells (Chang and Grunwald, 1980). However, this can be easily attributed to the fact that the secretion from glandular heads, although viscous, is fluid and slowly spreads over the surface of the leaf (Figure 1D). A subsequent labeling study, performed with purified trichome heads and not just a whole trichome fraction, convincingly demonstrated incorporation into CBT-diols (Keene and Wagner, 1985). In retrospect, ^{14}C-acetate was certainly not the best precursor for such feeding studies. Its use was based on the assumption that diterpenes would be synthesized through the mevalonate pathway, which was the only known isoprenoid precursor pathway at the time. It is now well established that the alternative 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway provides isoprenoid precursors in the plastids of higher plants, and that diterpenes, which are synthesized in plastids, are produced with those MEP-derived precursors (Phillips *et al.*, 2008). This pathway starts with pyruvate and glyceraldehyde-3-phosphate, which would be better suited to perform labeling studies. Interestingly, the low level of incorporation of acetate into tobacco diterpenoids indicates either a recycling of acetate into pyruvate through the citric acid cycle or alternatively import of mevalonate-derived precursors into the chloroplast. Further biochemical evidence that tobacco glandular trichomes are the site of biosynthesis of secreted compounds was provided by feeding ^{14}C-sucrose and ^{14}C-glucose. Both sucrose esters and diterpenoids could thus be labeled in detached trichome glands (Kandra and Wagner, 1988).

Another line of evidence for the *in situ* biosynthesis of trichome-secreted compounds came from studies in spearmint, where limonene hydroxylase and carveol dehydrogenase activities from extracts of glandular trichomes could be demonstrated (Gershenzon *et al.*, 1989). Further, immunolocalization studies in spearmint and peppermint provided an even more refined picture. The limonene synthase and both subunits of the geranyl diphosphate synthase could be localized in the leucoplasts of the glandular cells, while the cytochrome P450 monooxygenase, 4-*S*-limonene-6-hydroxylase, was detected in the smooth endoplasmic reticulum of spearmint (Turner *et al.*, 1999; Turner and Croteau, 2004). Two other enzymes involved in downstream steps of the pathway, namely (–)-*trans*-isopiperitenol dehydrogenase and peppermint (–)-pulegone reductase, were detected in the mitochondria and the cytosol, respectively (Turner and Croteau, 2004). This shows that in a single cell type several distinct intracellular compartments are involved in the biosynthesis of the final compounds. This raises the question of intracellular transport of pathway intermediates, and it was proposed that a combination of free diffusion and selective active transport could account for this partitioning (Turner and Croteau, 2004).

The cloning of genes involved in the trichome metabolic pathways allowed the characterization of their promoters in transgenic plants. This was done in tobacco, and nicely showed that their expression is restricted to the glandular cells of the trichomes and therefore highly specific (Wang *et al.*, 2002; Ennajdaoui *et al.*, 2010). In addition to these tobacco promoters, however, there are relatively few trichome-specific promoters known from genes of the endogenous pathways. The only ones described in the literature are the promoter of the linalool synthase from lavender (Biswas *et al.*, 2009), the promoter of the *A. annua* amorphadiene synthase (*AaAS*) and that of a WRKY transcription factor which binds to the *AaAS* promoter (Kim *et al.*, 2008; Ma *et al.*, 2009). For more detailed information on trichome-specific promoters, readers are referred to a recent review on the topic (Tissier, 2012).

TRANSCRIPTOMICS AND PROTEOMICS OF GLANDULAR TRICHOMES

The advent of genomics and transcriptomics allowed the large-scale sequencing of expressed sequence tags (ESTs) (Adams *et al.*, 1991). The application of EST sequencing to purified trichomes, generated collections of sequences which have proved extremely useful for the identification and characterization of pathway genes (see below). The EST projects from three species (mint, basil and tomato) will be presented in more detail below, whereas the others are listed in Table 1. In total, glandular trichome EST projects were carried out in 16 different species with more than 2 million sequences. In contrast, only a few proteomic studies are reported, with one in basil, two in tobacco and one in tomato (Amme *et al.*, 2005; Xie *et al.*, 2008; Schilmiller *et al.*, 2010b; Van Cutsem *et al.*, 2011). The first trichome EST sequencing project was carried out in mint with 1316 sequences obtained by the Sanger sequencing method (Lange *et al.*, 2000). Although 1316 is a relatively low number of EST

Table 1 A list of available glandular trichome-specific expressed sequence tag (EST) collections from plants

Species	Accession	Trichome type	Sequencing	No. of ESTs	References
Solanum habrochaites	LA1777	Mixed	Sanger	2 656	1, 2
S. habrochaites	PI126449	Mixed	Sanger	5 494	3
Solanum lycopersicum	NA	Mixed	Sanger	7 254	4
Solanum pennelllii	LA716	Mixed	Sanger	2 917	2
S. lycopersicum	LA3475	Mixed stems	NGS	278 000	5
S. lycopersicum	LA3475	Type VII	Sanger	791	5
S. lycopersicum	LA3475	Type VI	NGS	225 000	5
S. lycopersicum	LA3475	Type I	Sanger	831	5
S. habrochaites	LA1777	Mixed leaves	NGS	108 000	5
S. habrochaites	LA1777	Type I	Sanger	978	5
S. habrochaites	LA1777	Type IV	Sanger	1 425	5
S. habrochaites	LA1777	Type VI	NGS	224 000	5
S. habrochaites	PI126449	Type VI	Sanger	15 000	5
Solanum pimpinellifolium	LA1589	Type VI	NGS	227 000	5
S. pennelllii	LA0716	Type IV	Sanger	1 277	5
S. pennelllii	LA0716	Type VI	Sanger	1 137	5
S. pennelllii	LA0716	Mixed leaves	NGS	275 000	5
S. arcanum	LA1708	Mixed stems	NGS	415 000	5
S. lycopersicum	Moneymaker	Mixed	NGS	195 377	6
S. habrochaites	PI127826	Mixed	NGS	182 386	6
Mentha × *piperita*		Peltate	Sanger	1 316	7
Ocimum basilicum		Peltate	Sanger	1 344	8
O. basilicum		Peltate	Sanger	4 804	9
O. basilicum		Peltate	Sanger	5 422	10
O. basilicum		Peltate	Sanger	7 314	11
Artemisia annua		Glandular trichomes	Sanger	24 947	12
A. annua		Glandular trichomes	NGS	406 044	13
Cannabis sativa		Glandular trichomes from female inflorescences	Sanger	1 540	14
Cistus creticus		Glandular trichomes	Sanger	2 004	15
A. annua		Glandular trichomes subtractive library	Sanger	9 322	16
Humulus lupulus		Glandular trichome	Sanger	10 599	17
H. lupulus		Glandular trichome from female flower	Sanger	12 360	18
Medicago sativa		Stem glandular trichomes	Sanger	9659	19
Medicago truncatula		Glandular trichomes	Sanger	10 377	20
Nicotiana benthamiana		Glandular trichomes	Sanger	6 686	21
Nicotiana tabacum	Burley 21	Glandular trichomes	Sanger	1 949	22
N. tabacum	K326	Glandular trichomes	Sanger	1 161	22
N. tabacum	Samsun	Glandular trichomes	Sanger	3 179	22
N. tabacum	K326	Glandular trichomes	Sanger	5 139	23
N. tabacum	Xanthi	Glandular trichomes with or without Cd	Sanger	2 100	24
Salvia fruticosa		Glandular trichomes	Sanger	1 459	25

References: 1, van Der Hoeven *et al.* (2000); 2, Fei *et al.* (2004); 3, Fridman *et al.* (2005); 4, Besser *et al.* (2009); 5, McDowell *et al.* (2011); 6, Bleeker *et al.* (2011); 7, Lange *et al.* (2000); 8, Gang *et al.* (2001); 9, Iijima *et al.* (2004a); 10, Iijima *et al.* (2004b); 11, Kapteyn *et al.* (2007); 12, Newman *et al.*, unpublished data; 13, Wang *et al.* (2009); 14, Marks *et al.* (2009); 15, Falara *et al.* (2008); 16, Teoh *et al.* (2006); 17, Nagel *et al.* (2008); 18, Wang *et al.* (2008); 19, Aziz *et al.* (2005); 20, Dai *et al.* (2010); 21, Slocombe *et al.* (2008); 22, Coates *et al.*, unpublished data; 23, Cui *et al.* (2006); 24, Harada *et al.* (2010); 25, Chatzopoulou *et al.* (2010). NGS: Next Generation Sequencing. Cd, Cadmium.

sequences, this was enough to identify genes in the MEP and the menthol biosynthesis pathways. Moreover, 25% of the described sequences in this pool were estimated to be involved in essential oil metabolism. Among the most highly expressed genes in the terpenoid pathway were the (−)-limonene-3-hydroxylase (61.1‰), (−)-limonene synthase (29.6‰), and isopiperitone reductase (22.4‰). Genes of primary metabolism were also highly expressed with glycolysis, pentose phosphate and oxidative phosphorylation pathways accounting for 17, 8.8 and 9.6‰ of the ESTs, respectively. Another important group of highly expressed genes were homologs of lipid transfer proteins (LTPs) with 32‰. Although their function in the trichomes is not yet clearly defined, it is assumed that they play a role in the

transport of the lipophilic terpenoids to the subcuticular storage cavity of the peltate glands. A similar approach was chosen to discover genes involved in phenylpropene and monoterpene metabolism in basil (*Ocimum basilicum*). In this case, EST libraries were generated from several varieties of basil with distinct trichome metabolic profiles, either with high phenylpropene and low terpene content, or the reverse (Gang *et al.*, 2001, 2002b; Iijima *et al.*, 2004a,b). This allowed the successful identification of several key enzymes involved in monoterpene, sesquiterpene and phenylpropene metabolism in these basil varieties (Gang *et al.*, 2002a,b; Iijima *et al.*, 2004a; Koeduka *et al.*, 2006; Vassao *et al.*, 2006; Kapteyn *et al.*, 2007). In total, over 23 000 ESTs were assembled resulting in 7963 unigenes. A complete analysis of these EST databases together with a large-scale proteomics study (881 unique proteins identified) allowed the comparison of the transcriptome and the proteome of basil peltate trichomes (Xie *et al.*, 2008). Several interesting observations could be drawn out of this analysis. In general, there is a good correlation between protein levels and transcript levels, with the most abundant proteins corresponding to the most abundant ESTs. However, 118 of the proteins could not be assigned to any EST, suggesting that the EST dataset of almost 8000 unigenes falls short of covering the proteome that is represented by the most abundant proteins. The availability of four different basil lines with distinct metabolic profiles allowed assessment of the expression of distinct pathways (phenylpropanoid versus terpenoid) and their regulation in a similar or almost identical genetic background. For example, line EMX-1, a poor terpenoid producer, shows drastically reduced transcript and protein levels for the first two enzymes of the MEP pathway, deoxy-xylulose phosphate synthase (DXS) and deoxy-xylulose phosphate reductase (DXR). Interestingly, other steps of the pathway (e.g. the hydroxy-methylbutenyl diphosphate synthase or GCPE) do not seem to be down-regulated, indicating that this differentiation may have occurred fairly recently, only affecting key steps of the pathway. Additionally, the combined transcriptomics–proteomics analysis indicated that the mevalonate pathway is expressed at extremely low levels compared to the plastidic MEP pathway, implying that the cytosolic sesquiterpene biosynthesis relies on IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate) pools originating almost exclusively from the plastidic MEP pathway. This is in agreement with previous observations that the MEP pathway contributes to specialized sesquiterpene biosynthesis in some species, such as snapdragon (*Antirrhinum majus*) and *Arabidopsis thaliana* (Kasahara *et al.*, 2002; Dudareva *et al.*, 2005).

As can be seen from Table 1, certain species have been the focus of important efforts to uncover the transcriptome of their glandular trichomes. Prominent among these are tomato (*S. lycopersicum* and related wild species) and *A. annua*, the plant producing artemisinin. Since artemisinin is produced in leaf glandular trichomes, trichome EST sequencing efforts have allowed the discovery of key biosynthetic enzymes, in particular the oxidizing steps after the committed sesquiterpene synthase step (Covello, 2008).

Over the years a major effort has been devoted to producing large-scale EST sequences from tomato trichomes by different research groups, with an estimated total of over 2 million ESTs, all tomato species included (Table 1) (van Der Hoeven *et al.*, 2000; Fei *et al.*, 2004; Fridman *et al.*, 2005; Slocombe *et al.*, 2008; Besser *et al.*, 2009; Dai *et al.*, 2010; Schilmiller *et al.*, 2010a,b; Bleeker *et al.*, 2011; McDowell *et al.*, 2011). One reason for the interest in tomato glandular trichomes is the role that their secretions play in the resistance to a number of insect pests, including white flies (*Bemisia* sp.) or *Tuta absoluta* (Simmons and Gurr, 2005; Bleeker *et al.*, 2009). Wild species such as *Solanum habrochaites* and *Solanum pennellii*, show much higher resistance levels than the cultivated tomato, and in many cases this could be traced down to glandular trichome secretions (Simmons and Gurr, 2005). Therefore, these species have been the focus of particular attention with regards to their trichome secretions and several transcriptomics studies have been published on the topic in the last decade (van Der Hoeven *et al.*, 2000; Fridman *et al.*, 2005; Slocombe *et al.*, 2008; Ben-Israel *et al.*, 2009; Besser *et al.*, 2009; McDowell *et al.*, 2011). This has created useful resources for the elucidation of the biosynthetic pathways for compounds specific to the glandular trichomes, such as methyl ketones (Fridman *et al.*, 2005; Ben-Israel *et al.*, 2009; Yu *et al.*, 2010), monoterpenes (Schilmiller *et al.*, 2009) and sesquiterpenes (van Der Hoeven *et al.*, 2000; Sallaud *et al.*, 2009). Up to seven different trichome types were described on the aerial parts of tomato plants (Luckwill, 1943), and from those two particular classes of glandular trichomes seem to play major roles in the production of compounds which mediate interactions with insects. Type 4 trichomes, of the capitate type with one to several glandular cells atop a multicellular stalk, produce glucose esters of short chain fatty acids in *S. pennellii* (Fobes *et al.*, 1985; Slocombe *et al.*, 2008). Type 6 trichomes, consisting of four glandular cells on one plane, produce either terpenes (monoterpenes and sesquiterpenes) or methylketones (MKs). Recent publications on the characterization of tomato terpene synthases indicate that some of them (*SlTPS3*, *SlTPS9* and *SlTPS17*) are preferentially expressed in stem trichomes, indicating that trichome metabolic profile may be under organ-specific control (Bleeker *et al.*, 2011; Falara *et al.*, 2011).

METABOLIC PATHWAY DISCOVERY

The specificity and the high-level expression of the trichome pathways combined with the availability of significant EST resources (see above) has greatly enhanced the discovery and characterization of genes involved in those pathways. A pioneering example is that of the menthol pathway, which,

except for one step, was completely elucidated. Since this pathway has been extensively described in excellent reviews, the reader is referred to the most recent of them (Croteau *et al.*, 2005). Identification of the early steps of the artemisinin pathway has also greatly benefited from trichome EST databases, and again this topic is covered in great detail in recent articles, to which the reader is referred (Covello *et al.*, 2007; Covello, 2008; Teoh *et al.*, 2009; Zhang *et al.*, 2009b; Brown, 2010). I will focus here instead on trichome pathways which have been little reviewed but present a particular interest because of their originality. The first concerns the tomato MK pathway which was mentioned earlier. Methyl ketones are fatty-acid-derived molecules and two enzymes could be found to play decisive functions in the last steps from the 3-ketoacyl-acyl carrier proteins (ACPs). *ShMKS2*, encoding a protein with similarity to bacterial thioesterases, was shown to catalyze the hydrolysis of the 3-ketoacyl-ACP thioester bond, while ShMKS1, a protein of the α/β-hydrolase superfamily, could catalyze the decarboxylation of the resulting 3-keto fatty acids (Ben-Israel *et al.*, 2009; Yu *et al.*, 2010). ShMKS1 shares similarity to tomato methyl jasmonate esterase (LeMJE) as well as methyl salicylate esterase from tobacco. These intriguing connections suggest a recent diversification of this enzyme family in the biosynthesis of defense-related compounds (Fridman *et al.*, 2005). The second pathway concerns the class II sesquiterpenes α-santalene and β-bergamotene from the wild tomato *Solanum habrochaites*. The sesquiterpene synthase, ShSBS, is a member of the family of plant kaurene synthases, which were, up until then, known to be involved in the biosynthesis of gibberellins and related polycyclic labdanoid diterpenes (Sallaud *et al.*, 2009). More intriguingly, the substrate of ShSBS is not the usual *trans*, *trans*-farnesyl diphosphate (*E,E*-FPP), but rather its *cis* isomer *Z,Z*-FPP, itself produced by a short-chain *cis*-isoprenyl diphosphate synthase called zFPS. Both proteins are targeted to the chloroplast, strongly suggesting that the MEP pathway supplies the isoprenyl precursors for these sesquiterpenes (Sallaud *et al.*, 2009). Similarly, it was found that in cultivated tomato (*S. lycopersicum*) the major monoterpene, β-phellandrene, is synthesized by related enzymes. The monoterpene synthase, SlPHS1, is a kaurene synthase-like terpene synthase, which shares high levels of sequence identity with ShSBS (Schilmiller *et al.*, 2009). Its substrate, neryl diphosphate, the *cis* isomer of GPP, is synthesized by a short-chain *cis*-isoprenyl diphosphate synthase highly similar to zFPS. More recently, this sub-family of kaurene synthase-like enzymes was expanded by the identification of the tobacco *cis*-abienol synthase, which is also expressed specifically in glandular trichomes and involved in the biosynthesis of the labdanoid *cis*-abienol (Sallaud, C. and Tissier, A. *et al.*, unpublished data). This high sequence similarity between this tobacco diterpene synthase, using 8-hydroxy-copalyl diphosphate as a substrate, and the tomato ShSBS and SlPHS1, which use acyclic *cis*-isoprenyl diphosphates, provides interesting material to study terpene synthase substrate specificity, an area that is still poorly investigated. The elucidation of both pathways (MKs and class II sesquiterpenes) was largely facilitated by their trichome specificity and the high-level expression of the corresponding genes and provide good examples of the value of glandular trichomes for gaining insight into novel enzymatic activities.

METABOLIC PROFILING OF GLANDULAR TRICHOMES

The location of trichomes on the surface offers the possibility of recovering trichome-borne secretions by simple solvent washes without extracting internal leaf metabolites. This is a highly efficient and simple method, which restricts the complexity of the extract to compounds located on the surface of the plants provided extraction times remain short, i.e. one to a few minutes. Since many compounds synthesized by trichomes are hydrophobic, organic solvents such as hexane, heptane, methylene chloride, methanol, ethanol or acetonitrile are often used. Depending on the chemical characteristics of the compounds, different analytical methods may be used. A robust method, but with relatively poor resolution, is thin layer chromatography (TLC). Identification of the compounds can only be done by comparison with purified authentic standards, and even so assignments have to be done with care. The advantage of TLC is that it does not require extensive sample preparation and crude extracts can be loaded without fear of damaging the equipment. High-performance TLC (HP-TLC) instruments allow a much higher reproducibility and sensitivity and can even be coupled to a mass spectrometer; alternatively plates may be analyzed by matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) for the identification of compounds (Fuchs *et al.*, 2008). The large choice of solid phases combined with appropriate mobile phases offers a virtually endless continuum of separation conditions. In early reports of tobacco trichome exudates, TLC was often used in combination with gas chromatography (GC) to rapidly evaluate sample composition (Keene and Wagner, 1985; Kandra and Wagner, 1988; Kandra *et al.*, 1990).

However, since in many cases glandular trichomes produce compounds that are volatile or semi-volatile in ambient conditions, GC is often the method of choice for analyzing them. It should be noted that GC also offers flexibility in terms of sample preparation, injection, solid phase and detection. Detection is typically done either with flame ionization detection (FID) or electron impact (EI) ionization followed by mass spectrometry, in general with a simple quadrupole instrument, although GC-TOF and GC-triple quadrupole MS coupling are also available. With FID, compound identification will be essentially done by comparison of the retention time with an authentic standard, or

by comparison to the Kovats retention index. This is fairly accurate but not error-proof, particularly in the case of enantiomers. These may, however, be resolved by the use of chiral columns. Gas chromatography-MS offers an extra level of confidence for compound identification by comparison of EI mass spectra with those from a database (e.g. National Institute of Standards and Technology, NIST), but relying solely on MS comparison is certainly not advised to ascertain compound assignment.

The wide range of suitable injection techniques in GC may have a considerable impact on compound detection and identification. The most widely used injection technique is the *splitless*, whereby samples are first vaporized in the injector, then recondense at the top of the column. Although convenient and fairly trouble-free, this method is not adapted for compounds which are temperature-sensitive and will undergo rearrangements due to the increased temperature during the injection. A typical example of these are the germacrene sesquiterpenes, which can be thermally converted to elemenes (van Der Hoeven *et al.*, 2000; Schilmiller *et al.*, 2010b). To avoid this phenomenon, cool on-column injection (OCI) should be used. In this injection technique, the sample is directly injected onto the column and the temperature is increased progressively to proceed with the separation. Although thermal degradation can still occur during the separation, this method results in much less degradation and produces chromatograms that better reflect the composition of the extract.

To gain a more comprehensive overview of trichome metabolism, in particular primary metabolism, with GC-MS, sample derivatization would be required. However, to the best of my knowledge, this has not been done on any of the major plants bearing glandular trichomes. One issue is the difficulty in collecting a sufficient amount of trichome-specific material for an exhaustive analysis. An alternative to GC is LC-MS metabolic profiling, which offers the advantage of increased sensitivity. This was used as a non-targeted analytical method (LC-TOF) in an investigation of tomato glandular trichomes (McDowell *et al.*, 2011). In this study, exudates from specific trichome types (1, 4, 6 and 7) and from different tomato species (*S. lycopersicum*, *S. habrochaites*, *S. arcanum*, *S. pennelllii*, *S. pimpinellifolium*) were analyzed and the results processed by a clustering analysis. Intriguingly, there were fewer differences between trichome types within a species than there were amongst the same types between species. However, this result should be taken with caution since the authors themselves admit that 'pure' trichome preparations could not be guaranteed and that cross-contamination between exudates from different trichome types could not be excluded. Liquid chromatography MS was also used as a targeted analytical method for the measurement of flavonoids in trichomes of *S. habrochaites* (accession LA1777). Methylated myricetins were detected preferentially in the type 1 and 4 trichomes, rather than in Type 6 trichomes. This is consistent with the observation that Type 1 and Type 4 trichomes look much alike, the difference between them being their size (Schmidt *et al.*, 2011). However, it should be noted that Type 6 trichomes also produced significant amounts of those myricetin derivatives. This confirms the data of McDowell *et al.* (2011) and suggests that, although the different trichome types have distinct profiles, there is also a significant metabolite overlap between them, indicating the presence of common regulatory mechanisms. In both reports (McDowell *et al.*, 2011; Schmidt *et al.*, 2011), exudates were collected from individual trichomes with glass capillaries. The non-targeted analysis described in McDowell *et al.* (2011) did not show any primary metabolites. Since primary metabolites should be present in lower quantities than the specialized compounds secreted by the trichomes, this may be because insufficient amounts of metabolites were analyzed or because the MS method did not allow for a comprehensive coverage of such metabolites. This is a relevant issue if one wishes to understand how these cell factories achieve such high metabolic fluxes towards restricted classes of compounds, in particular how these specialized metabolic pathways are connected to primary metabolism.

GLANDULAR TRICHOME MODELING

Modeling can be applied to simulate developmental processes or metabolic fluxes. Discrepancies between model predictions and real-life data may be used to iteratively optimize the models and improve their predictive ability. Trichome patterning has been explored in depth in *A. thaliana*, and a model based on interacting activator and inhibitor complexes has been proposed (Digiuni *et al.*, 2008; Pesch and Hulskamp, 2009; Balkunde *et al.*, 2010). To the best of my knowledge, no patterning models for glandular trichomes have been reported so far. This is probably due on one hand to the lack of genetic and molecular data concerning glandular trichome development and on the other hand to the complexity of patterns, with often several types of glandular trichome (see Figure 1). What has been observed is the apparent competition between stomata and trichomes, or between trichome types, suggesting that common regulatory mechanisms may underlie the patterning of these epidermal differentiations (reviewed in Martin and Glover, 2007). There is a single reported attempt at metabolic modeling of glandular trichomes of mint (Rios-Estepa *et al.*, 2008, 2010). Peppermint glandular trichomes make an interesting model in this respect, because the pathway to the major monoterpenes is well known, enzyme kinetic properties for the corresponding steps are available and in addition the pathway has branches, which bring an additional layer of complexity (Croteau *et al.*, 2005). Moreover, the monoterpene profile changes during the course of plant development, implicating a dynamic regulation of the

pathway (McConkey *et al.*, 2000). The first model included a number of parameters such as trichome number, volume of the trichome cells and their subcuticular storage space, amount of monoterpenes present in each trichome, enzyme activity levels and kinetic values (Rios-Estepa *et al.*, 2008). Using those parameters, a set of differential equations was established to simulate monoterpene production in peppermint peltate trichomes. The first simulations were then iteratively optimized by comparing the simulated values with experimental results, leading to a basic model for unstressed conditions. Then, to validate the robustness of the model, stress conditions which alter the secondary compound profile of the essential oil were simulated and tested experimentally. Reduced light led to a 50% reduction of total oil yield and to a modification of the dynamic profile with a transient increase in pulegone and a hyperbolic accumulation of menthofuran, which is a side product of the pathway branching out from pulegone. Previous studies using transgenic plants overexpressing menthofuran synthase (MFS) had pointed to a possible role of menthofuran in the inhibition of pulegone reductase (PR) expression, the committed step to menthol from pulegone (Mahmoud and Croteau, 2001). When simply modulating the expression levels of MFS and PR in the model, no satisfactory simulation could be obtained. However, when inhibitory effects of menthofuran on PR enzyme activity were included, a much better fit could be reached with a competitive inhibition mechanism. This was then further tested experimentally by demonstrating that menthofuran is indeed a competitive inhibitor of PR (Rios-Estepa *et al.*, 2008). Further, it was shown that menthofuran accumulated in the glandular cells and was poorly transported to the storage space, lending additional support to its function as an intracellular inhibitor of PR (Rios-Estepa *et al.*, 2008). In a subsequent study, these authors updated their model by taking into account further experimental data obtained from wild-type plants grown under various environmental stresses (reduced fertilizer, reduced light and reduced light with higher night temperature) and from transgenic plants with reduced levels of MFS (Mahmoud and Croteau, 2001) grown in the same conditions. The model was expanded by taking into account enzymes of the isoprenoid precursor pathway, dynamics of glandular trichome size and density, estimating enzyme concentrations from real-time PCR data, and feedback control of two enzymes (GPS and PR) by products of the pathway (Rios-Estepa *et al.*, 2010). This expanded model was then used to predict essential oil composition and yield from two transgenic lines, one with reduced MFS expression and the other with reduced limonene-3-hydroxylase expression. Two main conclusions could be drawn from this study. First, variations in gene expression levels have an impact on essential oil composition, but relatively little on total oil yield. Second, a determining factor in oil yield is trichome density and size. The fits of the model were in good agreement with the experimental data with non-significant differences as validated with chi-square tests. However, it is difficult to estimate the true predictive ability of the model. A better test would be to predict outcomes in conditions or transgenic plants, which were not tested, and compare those *a posteriori*. For now, one of the limitations of the model may lie in the relatively limited set of enzymes and pathway steps which it includes. Indeed, only the isoprenoid precursor MEP pathway and the menthol pathway were taken into account. It is likely, however, that primary metabolism plays a crucial role, since the precursors of the MEP pathway, glyceraldehyde-3-phosphate and pyruvate, are directly connected to glycolysis. A more comprehensive model should probably include the primary metabolism network and influx of photosynthetates into the glandular trichome. This in turn could be correlated to growing conditions (light, temperature, nutrient availability). However, the required data sets are missing and it would necessitate a combination of transcriptomics and metabolomics, including fluxomics, to allow the elaboration of a more comprehensive model.

FUTURE DIRECTIONS

So far, glandular trichomes have mostly been studied to elucidate the biochemical pathways of the major compounds they produce and secrete. While this has allowed significant contributions to be made in the field of specialized plant metabolism, particularly in the terpenoid, lipid and phenylpropanoid areas, a deeper understanding of the development, regulation and wiring of the metabolic networks in these specialized cell factories is required to fully exploit their potential. A number of research directions are proposed below to fill these gaps and to make glandular trichomes the fully accomplished research topic that they deserve to be.

A model species for glandular trichome studies?

Arabidopsis has become the reference for molecular genetic studies of non-glandular trichome biology. Similarly a model species for glandular trichome research would also contribute to significantly increase our knowledge in this area by bringing together efforts from laboratories over the world. Although Lamiaceae species like mint or basil appear to be potential candidates because of the early work on glandular trichomes, they suffer from a number of difficulties, especially in the case of mint. These include poor genetics due to the polyploid nature of many cultivated mint species and lack of genomic sequences. The reduced costs and increased throughput of the latest sequencing technologies could, however, overcome this barrier. Recently, *Mentha longifolia*, a diploid mint ancestor of *Mentha spicata* has been proposed as a model for mint genetic research (Vining *et al.*, 2005). Similar problems (lack of genetic resources, difficult transformation) concern other plants such as the Cannabaceae (cannabis, hops). *Artemisia annua*

also has the potential to become a model for glandular trichome biology. A genetic map and a mutant collection were developed in the frame of the Center for Novel Agricultural Products (UK) *Artemisia* project (http://www.york.ac.uk/org/cnap/artemisiaproject/; Graham *et al.*, 2010). In addition, genetic transformation, although not routine, is feasible as attested by several recent publications (Han *et al.*, 2006; Wang *et al.*, 2007; Yang *et al.*, 2008; Feng *et al.*, 2009; Zhang *et al.*, 2009a; Banyai *et al.*, 2010; Alam and Abdin, 2011; Chen *et al.*, 2011; Liu *et al.*, 2011; Nafis *et al.*, 2011). Further, *A. annua* is related to sunflower (*Helianthus annuus*), an important crop plant, for which important genetic resources are available. However, the genetic resources of *Artemisia* itself are still relatively limited and the *Artemisia* project mentioned above is an effort from a single institute which, although impressive, would benefit from the contribution of the international community, for example by undertaking the sequencing of the *Artemisia* genome.

In contrast to the plants mentioned above, tomato, as a horticultural crop of worldwide importance, has benefited from many years of research effort in breeding, physiology, development and molecular biology. Therefore, tomato currently looks like a strong candidate to serve as a reference for the field of glandular trichome biology. Its genome sequence is now available, genetic resources are almost second to none with mutant collections, introgression lines derived from crosses with related wild species, and already rich EST databases of trichome-specific sequences are available (see above). In addition, tomato is easily transformed, further expanding the list of tools for probing and validating gene functions *in vivo*. The species suffers from a lack of exhaustive insertion mutant collections (with T-DNA or transposons), although it should be pointed out that a significant, albeit partial, insertion collection based on Ac/Ds insertions is already developed (Meissner *et al.*, 2000). Yet, a large-scale insertion mutant collection is an effort which would benefit tomato scientists as a whole. TILLING-based or equivalent resources are also available for tomato and already provide adequate reverse genetics populations (Gady *et al.*, 2009; Piron *et al.*, 2010; Sreelakshmi *et al.*, 2010). Moreover, virus-induced gene silencing (VIGS) works well in various tomato species, including for genes which are specifically expressed in trichomes, and may thus be used to rapidly probe gene function (Besser *et al.*, 2009; Becker and Lange, 2010). Additionally, the availability of a 'miniature' tomato line, the Micro-tom variety, also makes it possible to perform genetic screens with limited greenhouse space (Meissner *et al.*, 1997; Dan *et al.*, 2006) and several mutant resources have already been generated in this line (Meissner *et al.*, 2000; Okabe *et al.*, 2011; Saito *et al.*, 2011). Thus, exhaustive genetic screens for glandular trichome-related phenotypes are definitely within reach. Studies in tomato could also benefit from work in other Solanaceae, in particular tobacco (*N. tabacum*). Many pioneering glandular trichome studies were done in this species, for which extensive EST resources and a partial genomic sequence are available. Moreover, it should be pointed out that the genome of 100 Solanaceae species will be sequenced (http://solgenomics.net/organism/sol100/view) including *S. pennellii*, *Nicotiana sylvestris*, *N. tabacum*, *N. benthamiana* and many others. Through this unique resource, cross-species comparisons will be made easier, allowing the identification of genes which determine distinct glandular trichome types or secretions. Thus, rather than tomato alone, the Solanaceae as a whole – thanks to its diversity, growing genetic and sequence resources – may emerge as the reference plant family to uncover basic processes of glandular trichome biology. However, given the diversity of glandular trichomes and their secretions, it can be argued that no single species will serve as a single model. Rather, certain species or groups of species will emerge as references for certain types of trichomes and corresponding secretions, such as Lamiaceae for capitate trichomes and their capacity to store volatiles, or Solanaceae for capitate trichomes. A summary of the main contending species with their main pros and cons is provided in Table 2.

Development

Investigations of non-glandular trichomes in the model species Arabidopsis have been very fruitful and have provided a molecular genetic framework for the development of specialized organs (Balkunde *et al.*, 2010). Equivalent approaches for glandular trichomes are missing, in part because of the absence of model plant species (see above). There is some indication that the development of glandular and non-glandular trichomes may be under the control of distinct transcription factor networks (Serna and Martin, 2006). This suggests that simply looking for homologs of Arabidopsis genes involved in trichome development in species with glandular trichomes may not prove a successful strategy, and therefore dedicated studies in the species of interest are required to tackle this issue. Understanding how glandular trichomes develop and how their distribution on the surface of the aerial parts of the plants is controlled will open up new possibilities for engineering and breeding glandular trichome productivity and density. Specific types of glandular trichomes seem to have a rigidly defined developmental plan with a constant number of cells and cell types. For example, the tomato Type 6 trichomes always have four glandular cells, one intermediate cell, a stalk cell and a basal cell (Figure 3). A similar organization is seen in Lamiaceae peltate trichomes, although the intermediate cell is absent and the stalk cell is much shorter (Turner *et al.*, 2000). If glandular trichomes are to be considered as plausible biofactories for the production of renewable chemicals (Schilmiller *et al.*, 2008), significant efforts will have to be devoted to increase their productivity, for example by increasing trichome

Table 2 A summary of characteristics, advantages and difficulties of the major plant species which could serve as models for the study of glandular trichomes

Common name	Family	Latin names of main and related species	Type of trichomes	Main applied interest	Advantages	Difficulties
Mint	Lamiaceae	*Mentha* × *piperita*; *M. spicata*	Peltate trichomes which accumulate volatiles	Essential oil industry. Concerns other related species (Sage, Lavender, Rosemary, Thyme, Oregano)	Good background knowledge	Dearth of genetic resources. Polyploid species. Propagation and stability through seeds difficult. No genome sequences.
Basil	Lamiaceae	*Ocimum basilicum*	Peltate trichomes which accumulate volatiles			Dearth of genetic resources. No genome sequences
Tomato	Solanaceae	*Solanum lycopersicum*; *S. pennellii*; *S. habrochaites*	Capitate and Type 6 (similar to peltate trichomes of Lamiaceae)	Pest resistance	Large EST resources; sequenced genome; extensive genetic (forward and reverse) resources; genetic transformation; large scientific community	
Tobacco	Solanaceae	*Nicotiana tabacum*; *N. sylvestris*; *N. glutinosa*	Capitate trichomes	Pest resistance; precursors of fragrance compounds (Labdane diterpenoids)	Large EST resources; partially sequenced genome; genetic transformation	Relative lack of easily accessible forward and reverse genetic resources
Sweet wormwood	Asteraceae	*Artemisia annua*	Biseriate	Pharmaceutical ingredients	Large EST resources; genetic map; mutant collection; related to sunflower	Small community (but growing); limited genetic resources; no genome sequences
Marijuana	Cannabaceae	*Cannabis sativa*	Multicellular glands with sub-cuticular storage space	Pharmaceutical ingredients	EST resource	Dioecious species; no genome sequence; genetic transformation not routine; 'Drug' status
Hops	Cannabaceae	*Humulus lupulus*	Multicellular glands with sub-cuticular storage space	Aroma ingredients	EST resource	Dioecious species; no genome sequence; genetic transformation not routine
Alfalfa	Fabaceae	*Medicago sativa*	Capitate trichomes	Pest resistance	EST resource	Tetraploid species; genetic transformation difficult
Barrel Medic	Fabaceae	*Medicago truncatula*	Capitate trichomes (few)	ND	EST resource; genetics; genome sequence	Few glandular trichomes; genetic transformation difficult

EST, expressed sequence tag.

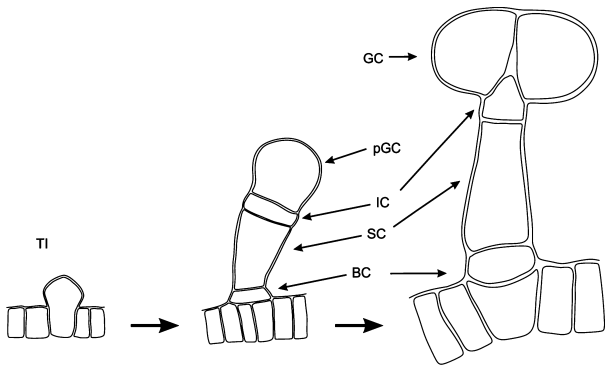

Figure 3. Initiation and developmental stages of tomato Type 6 glandular trichomes from *Solanum lycopersicum*.
Drawing freely adapted from sections visualized by light microscopy. Left: appearance of a trichome initial (TI) in the epidermal layer. Middle: Intermediate developmental stage with the established overall plan of the trichome. BC, basal cell; SC, stalk cell; IC, intermediate cell; pGC, pre-glandular cell. Right: mature trichome. The pre-glandular cell has divided twice to give four glandular cells of equal size.

density or size. Glandular trichome density appears to be under the control of numerous environmental and hormonal factors. In particular, jasmonates seem to play an important role, as confirmed by several independent reports (Thaler *et al.*, 2002; Ament *et al.*, 2004; Li *et al.*, 2004; Boughton *et al.*, 2005; Maluf *et al.*, 2007). However, the signaling pathways are still largely unknown. In addition to genetic screens, which can be performed, for example, on tomato as mentioned above, novel EST resources should be devoted to specific stages of development. Because most of the interest has focused on elucidating biosynthetic pathways, glandular trichome EST libraries have been generated up to now from mature and actively secreting glands. Gathering EST sequences from early and distinct phases of development of glandular trichomes would be one step towards this goal. Since early phases of trichome development are intimately linked to leaf development, the current techniques for trichome isolation will most likely prove irrelevant. This will require establishing novel methods to isolate homogeneous populations of developing trichomes at specific phases of development. This could be done for example by laser microdissection of developing glandular cells or, once a promoter for early phases of trichome development is available, by flow cytometry of leaf protoplasts from a plant expressing a fluorescent protein under the control of this promoter. Such methods have proved successful in characterizing transcriptomes of specific plant cell types (Chen *et al.*, 2010a; Matas *et al.*, 2010; Sozzani *et al.*, 2010; Schiebold *et al.*, 2011). Access to transcriptomes of specific development phases of glandular trichomes will allow us to probe the function of putative transcription or other regulatory factors by reverse genetics strategies.

Understanding the glandular trichome cell factories

Another key and unique aspect of glandular trichome biology is their ability to synthesize and secrete large amounts of a limited number of metabolites relative to their size (Schilmiller *et al.*, 2008). As such, they qualify as true cell factories, making them relevant targets for metabolic engineering. This suggests that dedicated metabolic networks have been put in place in these cells. To date, however, there is almost no information on how this high level of specialized metabolite productivity is achieved, in particular how primary metabolism contributes to this high metabolic flux. Although existing EST data can be mined to look for cDNAs encoding enzymes of primary metabolism, comprehensive metabolic profiling of trichome content should be performed to address this issue. For example, it could help unravel novel metabolic shunts that are used in those organs to escape the tight regulation that usually controls specialized metabolism in other non-secretory tissues. Better yet would be fluxomics studies whereby the dynamics of metabolite fluxes through primary metabolite pathways could be analyzed. This would require labeling of plants with stable isotopes, for example $^{13}CO_2$- or ^{13}C-labeled precursors. Sensitive LC-MS measurements, preferably with high mass precision (i.e. TOF instruments) should allow the relative quantification of $^{12}C/^{13}C$ ratios of known metabolites and contribute to the identification of unknown metabolites. Such methods are already available and have been implemented in plants (Feldberg *et al.*, 2009; Giavalisco *et al.*, 2011). In the dynamic phase, $^{13}CO_2$ labeling experiments will permit the identification of the photosynthetates which are incorporated into trichome secretions. Such labeling studies have been performed recently on the cyanobacterium *Synechocystis* sp. allowing the identification of metabolic pathway fluxes in a photosynthetic organism (Young *et al.*, 2011). Subsequently, feeding with specific precursors which have been identified as important flux nodes could be used to further validate the findings of the $^{13}CO_2$ labeling. The role of the different intracellular compartments will be more difficult to address because of current technical limitations and challenges in combining metabolite quenching and pure organelle isolation. This was recently applied to analyze the vacuolar metabolome of *Chara australis*, which, however, benefits from an extraordinarily large cell size (Oikawa *et al.*, 2011). The establishment of a flux map of glandular trichomes combined with the analysis of deep RNA sequencing and proteomics data should allow the identification of potential metabolic bottlenecks and of genes which contribute to their high metabolic productivity. Further, the subcellular targeting of pathway enzymes can be determined by expressing fluorescent protein (FP) fusions in transgenic plants, to identify or confirm the compartmentalization of distinct pathway steps. Such FP fusions may also be used to demonstrate protein–protein

interactions *in planta* via fluorescence lifetime imaging microscopy (FLIM)–fluorescence resonance energy transfer (FRET) measurements (Chen *et al.*, 2010b). More generally, high-throughput two-hybrid screens could allow the production of an interactome map, potentially revealing substrate channeling or regulatory networks.

This can be used to model the functioning of the trichome cell factories and may then be transposed to other plant tissues or other organisms. It will most likely require the co-expression of a relatively large number of genes. Here, the recent spectacular advances in synthetic biology for the cloning of large fragments of DNA (see for example Gibson *et al.*, 2010) make it possible to contemplate the introduction of multiple genes in plants or in microorganisms to 'reconstitute' a trichome cell factory. In plants, multigene constructs, either with artificial mini-chromosomes or with large T-DNA, are now possible. Up to 150–190 kb of DNA may thus be transferred to plant chromosomes (for review see Birchler *et al.*, 2010; Naqvi *et al.*, 2010).

Transport and storage of metabolites in glandular cells also require careful inspection. Concerning transport, LTPs in particular have been suspected to be involved due to their relatively high representation in trichome EST databases. Up until now, their contribution to the secretion of metabolites had not been demonstrated, but a recent article indicates that in *N. tabacum*, NtLTP1, a trichome-specific LTP, seems to play a critical role in the secretion of tobacco terpenoids (Choi *et al.*, 2012). ABC transporters are an important class of proteins, which have been shown to be involved in the transport of a variety of metabolites across membranes. Of relevance to glandular trichomes is the identification and characterization of NpPDR1, an ABC transporter from *Nicotiana plumbaginifolia*, which was shown to support transport of sclareol and is expressed in trichomes, although not specifically (Stukkens *et al.*, 2005). Furthermore, a proteomics study on tobacco trichomes led to the identification of several transporters, including a number from the ABC class (Van Cutsem *et al.*, 2011). However, validation of their role in transport of trichome metabolites awaits experimental evidence. Storage of volatile compounds in subcuticular spaces, as in the peltate trichomes of the Lamiaceae for example, requires not only transport but also a highly restricted and polarized digestion of the apical cell wall of the secretory cells to dissociate the cuticle from the cell wall. Currently nothing is known about this process and the actual composition of cell wall lining this storage cavity, although they could have interesting practical implications beyond the field of plant biology. This process is intimately linked to trichome development and its elucidation will require transcriptomics and functional studies of pre-secretory stages.

In conclusion, plant glandular trichomes constitute a highly active area of research, fueled by interests in the elucidation of the biosynthetic pathways of industrially relevant compounds such as essential oils, pharmaceutical ingredients or substances, which may be used in plant defense. As cellular factories, glandular trichomes are also attractive targets for metabolic engineering and breeding, particularly regarding the possibility of use plants as biofactories for the renewable production of organic compounds. To reach this long-term goal, a multidisciplinary approach including genetic, developmental, transgenic, metabolomic and fluxomic analyses will have to be implemented.

ACKNOWLEDGEMENTS

I wish to thank Frank Syrowatka from the Multidisciplinary Centre of Materials Science, Halle (Saale), Germany, for the environmental SEM pictures of tomato leaves and Ana Simonovic and Sladjana Todorović for providing SEM pictures of *Tanacetum parthenium* florets and *Salvia officinalis* leaves and Angelos Kanellis for providing pictures of Salvia trichomes. Work in my laboratory is funded by the EU under the project TERPMED (grant number KBBE-227448) and by the Leibniz Institute of Plant Biochemistry. I would also like to thank Bettina Hause, Thomas Vogt and Michael Walter for critical reading, and both reviewers for suggestions which significantly improved the manuscript. I would like to apologize to authors whose work could not be cited due to space limitations. The author declares no competing financial interests.

REFERENCES

Adams, M.D., Kelley, J.M., Gocayne, J.D. *et al.* (1991) Complementary-DNA sequencing – expressed sequence tags and human genome project. *Science*, **252**, 1651–1656.

Alam, P. and Abdin, M.Z. (2011) Over-expression of HMG-CoA reductase and amorpha-4,11-diene synthase genes in *Artemisia annua* L. and its influence on artemisinin content. *Plant Cell Rep.* **30**, 1919–1928.

Amelunxen, F. (1965) Elektronenmikroskopische untersuchungen an den Srüsenschuppen von *Mentha piperita* L.1. *Planta Med.* **13**, 457–473.

Amelunxen, F., Wahlig, T. and Arbeiter, H. (1969) On presence of essential oil I isolated glandular hairs and trichomes of *Mentha piperita* L. *Z. Pflanzenphysiol.* **61**, 68–72.

Ament, K., Kant, M.R., Sabelis, M.W., Haring, M.A. and Schuurink, R.C. (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiol.* **135**, 2025–2037.

Amme, S., Rutten, T., Melzer, M., Sonsmann, G., Vissers, J.P., Schlesier, B. and Mock, H.P. (2005) A proteome approach defines protective functions of tobacco leaf trichomes. *Proteomics*, **5**, 2508–2518.

Aziz, N., Paiva, N.L., May, G.D. and Dixon, R.A. (2005) Transcriptome analysis of alfalfa glandular trichomes. *Planta*, **221**, 28–38.

Balkunde, R., Pesch, M. and Hulskamp, M. (2010) Trichome patterning in *Arabidopsis thaliana*: from genetic to molecular models. In *Plant Development*, Vol. 91 (Timmermans, M.C.P., ed.). San Diego: Elsevier Academic Press, pp. 299–321.

Banyai, W., Kirdmanee, C., Mii, M. and Supaibulwatana, K. (2010) Over-expression of farnesyl pyrophosphate synthase (FPS) gene affected artemisinin content and growth of *Artemisia annua* L. *Plant Cell Tissue Organ Cult.* **103**, 255–265.

Barrero, A.F., Alvarez-Manzaneda, E.J., Chahboun, R. and Arteaga, A.F. (2004) Degradation of the side chain of (–)-sclareol: a very short synthesis of nor-Ambreinolide and ambrox. *Synth. Commun.* **34**, 3631–3643.

Becker, A. and Lange, M. (2010) VIGS – genomics goes functional. *Trends Plant Sci.* **15**, 1–4.

Ben-Israel, I., Yu, G., Austin, M.B., Bhuiyan, N., Auldridge, M., Nguyen, T., Schauvinhold, I., Noel, J.P., Pichersky, E. and Fridman, E. (2009) Multiple biochemical and morphological factors underlie the production of methylketones in tomato trichomes. *Plant Physiol.* **151**, 1952–1964.

Besser, K., Harper, A., Welsby, N., Schauvinhold, I., Slocombe, S., Li, Y., Dixon, R.A. and Broun, P. (2009) Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. *Plant Physiol.* **149**, 499–514.

Birchler, J.A., Krishnaswamy, L., Gaeta, R.T., Masonbrink, R.E. and Zhao, C.Z. (2010) Engineered minichromosomes in plants. *Crit. Rev. Plant Sci.* **29**, 135–147.

Biswas, K.K., Foster, A.J., Aung, T. and Mahmoud, S.S. (2009) Essential oil production: relationship with abundance of glandular trichomes in aerial surface of plants. *Acta Physiol. Plant.* **31**, 13–19.

Bleeker, P.M., Diergaarde, P.J., Ament, K., Guerra, J., Weidner, M., Schutz, S., de Both, M.T.J., Haring, M.A. and Schuurink, R.C. (2009) The role of specific tomato volatiles in tomato-whitefly interaction. *Plant Physiol.* **151**, 925–935.

Bleeker, P., Spyropoulou, E., Diergaarde, P. *et al.* (2011) RNA-seq discovery, functional characterization, and comparison of sesquiterpene synthases from *Solanum lycopersicum* and *Solanum habrochaites* trichomes. *Plant Mol. Biol.* **77**, 323–336.

Boughton, A.J., Hoover, K. and Felton, G.W. (2005) Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. *J. Chem. Ecol.* **31**, 2211–2216.

Brown, G.D. (2010) The biosynthesis of Artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules*, **15**, 7603–7698.

Buchmann, S.L. (1987) The ecology of oil flowers and their bees. *Ann. Rev. Ecol. Syst.* **18**, 343–369.

Carpenter, K.J. (2006) Specialized structures in the leaf epidermis of basal angiosperms: morphology, distribution, and homology. *Am. J. Bot.* **93**, 665–681.

Chang, S.Y. and Grunwald, C. (1980) Structural organization of tobacco leaf polar cuticular lipids. *Bot. Gaz.* **141**, 360–365.

Chatzopoulou, F.M., Makris, A.M., Argiriou, A., Degenhardt, J. and Kanellis, A.K. (2010) EST analysis and annotation of transcripts derived from a trichome-specific cDNA library from *Salvia fruticosa*. *Plant Cell Rep.* **29**, 523–534.

Chauveau, O., Eggers, L., Raquin, C. *et al.* (2011) Evolution of oil-producing trichomes in Sisyrinchium (Iridaceae): insights from the first comprehensive phylogenetic analysis of the genus. *Ann. Bot.* **107**, 1287–1312.

Chen, C.B., Farmer, A.D., Langley, R.J., Mudge, J., Crow, J.A., May, G.D., Huntley, J., Smith, A.G. and Retzel, E.F. (2010a) Meiosis-specific gene discovery in plants: RNA-Seq applied to isolated Arabidopsis male meiocytes. *BMC Plant Biol.* **10**, 280.

Chen, Y., Johnson, J., Macdonald, P., Wu, B. and Mueller, J.D. (2010b) Observing protein interactions and their stoichiometry in living cells by brightness analysis of fluorescence fluctuation experiments. In *Methods in Enzymology, Vol. 472: Single Molecule Tools, Pt A: Fluorescence Based Approaches*, Vol. 472 (Walter, N.G., ed.). San Diego, CA: Elsevier Academic Press Inc, pp. 345–363.

Chen, J.L., Fang, H.M., Ji, Y.P. *et al.* (2011) Artemisinin biosynthesis enhancement in transgenic *Artemisia annua* plants by downregulation of the beta-caryophyllene synthase gene. *Planta Med.* **77**, 1759–1765.

Choi, Y.E., Lim, S., Kim, H.-J., Han, J.Y., Lee, M.-H., Yang, Y., Kim, J.-A. and Kim, Y.-S. (2012) Tobacco NtLTP1, a glandular-specific lipid transfer protein, is required for lipid secretion from glandular trichomes. *Plant J.* (Epub ahead of print).

Clark, I.M., Forde, B.G. and Hallahan, D.L. (1997) Spatially distinct expression of two new cytochrome P450s in leaves of *Nepeta racemosa*: identification of a trichome-specific isoform. *Plant Mol. Biol.* **33**, 875–885.

Covello, P.S. (2008) Making artemisinin. *Phytochemistry*, **69**, 2881–2885.

Covello, P.S., Teoh, K.H., Polichuk, D.R., Reed, D.W. and Nowak, G. (2007) Functional genomics and the biosynthesis of artemisinin. *Phytochemistry*, **68**, 1864–1871.

Croteau, R. and Winters, J.N. (1982) Demonstration of the intercellular compartmentation of L-menthone metabolism in peppermint (*Mentha piperita*) leaves. *Plant Physiol.* **69**, 975–977.

Croteau, R.B., Davis, E.M., Ringer, K.L. and Wildung, M.R. (2005) (−)-Menthol biosynthesis and molecular genetics. *Naturwissenschaften*, **92**, 562–577.

Cui, H., Ji, H., Zhang, H. and Chen, L. (2006) Construction of full-length cDNA library from trichomes of *Nicotiana tabacum*. *J. Xiamen Univ.* **45**, 859–862.

Dai, X.B., Wang, G.D., Yang, D.S., Tang, Y.H., Broun, P., Marks, M.D., Sumner, L.W., Dixon, R.A. and Zhao, P.X. (2010) TrichOME: a comparative omics database for plant trichomes. *Plant Physiol.* **152**, 44–54.

Dan, Y., Yan, H., Munyikwa, T., Dong, J., Zhang, Y. and Armstrong, C. (2006) MicroTom – a high-throughput model transformation system for functional genomics. *Plant Cell Rep.* **25**, 432–441.

van Der Hoeven, R.S., Monforte, A.J., Breeden, D., Tanksley, S.D. and Steffens, J.C. (2000) Genetic control and evolution of sesquiterpene biosynthesis in *Lycopersicon esculentum* and *L. hirsutum*. *Plant Cell*, **12**, 2283–2294.

Digiuni, S., Schellmann, S., Geier, F. *et al.* (2008) A competitive complex formation mechanism underlies trichome patterning on Arabidopsis leaves. *Mol. Syst. Biol.* **4**, 217.

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

Ennajdaoui, H., Vachon, G., Giacalone, C., Besse, I., Sallaud, C., Herzog, M. and 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. (1988) Secretory tissues in vascular plants. *New Phytol.* **108**, 229–257.

Fahn, A. (2002) Functions and location of secretory tissues in plants and their possible evolutionary trends. *Isr. J. Plant Sci.* **50**, S59–S64.

Fahn, A. and Shimony, C. (1977) Development of glandular and non-glandular leaf hairs of *Aviceniiia marnia* (Forskal) Vierh. *Bot. J. Linn. Soc.* **74**, 37–46.

Falara, V., Fotopoulos, V., Margaritis, T., Anastasaki, T., Pateraki, I., Bosabalidis, A.M., Kafetzopoulos, D., Demetzos, C., Pichersky, E. and Kanellis, A.K. (2008) Transcriptome analysis approaches for the isolation of trichome-specific genes from the medicinal plant *Cistus creticus* subsp. *creticus*. *Plant Mol. Biol.* **68**, 633–651.

Falara, V., Akhtar, T.A., Nguyen, T.T.H. *et al.* (2011) The tomato terpene synthase gene family. *Plant Physiol.* **157**, 770–789.

Fei, Z., Tang, X., Alba, R.M., White, J.A., Ronning, C.M., Martin, G.B., Tanksley, S.D. and Giovannoni, J.J. (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. *Plant J.* **40**, 47–59.

Feldberg, L., Venger, I., Malitsky, S., Rogachev, I. and Aharoni, A. (2009) Dual labeling of metabolites for metabolome analysis (DLEMMA): a new approach for the identification and relative quantification of metabolites by means of dual isotope labeling and liquid chromatography-mass spectrometry. *Anal. Chem.* **81**, 9257–9266.

Feng, L.L., Yang, R.Y., Yang, X.Q., Zeng, X.M., Lu, W.J. and Zeng, Q.P. (2009) Synergistic re-channeling of mevalonate pathway for enhanced artemisinin production in transgenic *Artemisia annua*. *Plant Sci.* **177**, 57–67.

Fernald, M.L. (1950) *Gray's Manual of Botany*, 8th edn. New York: American Book Co., lxiv: 1632 pp.

Fobes, J.F., Mudd, J.B. and Marsden, M.P.F. (1985) Epicuticular lipid accumulation on the leaves of *Lycopersicon pennellii* (Corr.) D'Arcy and *Lycopersicon esculentum* Mill. *Plant Physiol.* **77**, 567–570.

Fridman, E., Wang, J.H., Iijima, Y., Froehlich, J.E., Gang, D.R., Ohlrogge, J. and 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.

Frija, L.S.M.T., Frade, R.F.M. and Afonso, C.A.M. (2011) Isolation, chemical, and biotransformation routes of Labdane-type diterpenes. *Chem. Rev.* **111**, 4418–4452.

Fuchs, B., Schiller, J., Suess, R., Zscharnack, M., Bader, A., Mueller, P., Schuerenberg, M., Becker, M. and Suckau, D. (2008) Analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS. *Anal. Bioanal. Chem.* **392**, 849–860.

Gady, A.L.F., Hermans, F.W.K., Van de Wal, M., van Loo, E.N., Visser, R.G.F. and Bachem, C.W.B. (2009) Implementation of two high through-put techniques in a novel application: detecting point mutations in large EMS mutated plant populations. *Plant Methods*, **5**, 13.

Gang, D.R., Wang, J., Dudareva, N., Nam, K.H., Simon, J.E., Lewinsohn, E. and Pichersky, E. (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiol.* **125**, 539–555.

Gang, D., Beuerle, T., Ullmann, P., Werck-Reichhart, D. and Pichersky, E. (2002a) Differential production of meta hydroxylated phenylpropanoids in sweet basil peltate glangular trichomes and leaves is controlled by the activities of specific acyltransferase and hydeoxylases. *Plant Physiol.* **130**, 1536–1544.

Gang, D.R., Lavid, N., Zubieta, C., Chen, F., Beuerle, T., Lewinsohn, E., Noel, J.P. and Pichersky, E. (2002b) Characterization of phenylpropene *O*-methyltransferases from sweet basil: facile change of substrate specificity and convergent evolution within a plant *O*-methyltransferase family. *Plant Cell*, **14**, 505–519.

Gershenzon, J., Duffy, M.A., Karp, F. and Croteau, R. (1987) Mechanized techniques for the selective extraction of enzymes from plant epidermal glands. *Anal. Biochem.* **163**, 159–164.

Gershenzon, J., Maffei, M. and Croteau, R. (1989) Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of spearmint (*Mentha spicata*). *Plant Physiol.* **89**, 1351–1357.

Gershenzon, J., McCaskill, D., Rajaonarivony, J.I., Mihaliak, C., Karp, F. and 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.

Giavalisco, P., Li, Y., Matthes, A., Eckhardt, A., Hubberten, H.M., Hesse, H., Segu, S., Hummel, J., Kohl, K. and Willmitzer, L. (2011) Elemental formula annotation of polar and lipophilic metabolites using (13)C, (15)N and (34)S isotope labelling, in combination with high- resolution mass spectrometry. *Plant J.* **68**, 364–376.

Gibson, D.G., Glass, J.I., Lartigue, C. *et al.* (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science*, **329**, 52–56.

Gopfert, J., Conrad, J. and Spring, O. (2006) 5-deoxynevadensin, a novel flavone in sunflower and aspects of biosynthesis during trichome development. *Nat. Prod. Commun.* **1**, 935–940.

Graham, I.A., Besser, K., Blumer, S. *et al.* (2010) The genetic map of *Artemisia annua* L. Identifies loci affecting yield of the antimalarial drug artemisinin. *Science*, **327**, 328–331.

Hallahan, D.L., West, J.M., Smiley, D.W.M. and Pickett, J.A. (1998) Nepetalactol oxidoreductase in trichomes of the catmint *Nepeta racemosa*. *Phytochemistry*, **48**, 421–427.

Han, J.L., Liu, B.Y., Ye, H.C., Wang, H., Li, Z.Q. and Li, G.F. (2006) Effects of overexpression of the endogenous farnesyl diphosphate synthase on the artemisinin content in *Artemisia annua* L. *J. Integr. Plant Biol.* **48**, 482–487.

Harada, E., Kim, J.A., Meyer, A.J., Hell, R., Clemens, S. and Choi, Y.E. (2010) Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. *Plant Cell Physiol.* **51**, 1627–1637.

Iijima, Y., Davidovich-Rikanati, R., Fridman, E., Gang, D.R., Bar, E., Lewinsohn, E. and Pichersky, E. (2004a) The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant Physiol.* **136**, 3724–3736.

Iijima, Y., Gang, D.R., Fridman, E., Lewinsohn, E. and Pichersky, E. (2004b) Characterization of geraniol synthase from the peltate glands of sweet basil. *Plant Physiol.* **134**, 370–379.

Johnson, H.B. (1975) Plant pubescence: an ecological perspective. *Bot. Rev.* **41**, 233–258.

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

Kandra, L., Severson, R. and Wagner, G.J. (1990) Modified branched-chain amino-acid pathways give rise to acyl acids of sucrose esters exuded from tobacco leaf trichomes. *Eur. J. Biochem.* **188**, 385–391.

Kapteyn, J., Qualley, A.V., Xie, Z.Z., Fridman, E., Dudareva, N. and Gang, D.R. (2007) Evolution of cinnamate/p-coumarate carboxyl methyltransferases and their role in the biosynthesis of methylcinnamate. *Plant Cell*, **19**, 3212–3229.

Kasahara, H., Hanada, A., Kuzuyama, T., Takagi, M., Kamiya, Y. and Yamaguchi, S. (2002) Contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthesis of gibberellins in Arabidopsis. *J. Biol. Chem.* **277**, 45188–45194.

Keene, C.K. and Wagner, G.J. (1985) Direct demonstration of duvatrienediol biosynthesis in glandular heads of tobacco trichomes. *Plant Physiol.* **79**, 1026–1032.

Kim, S.H., Chang, Y.J. and Kim, S.U. (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.

Koeduka, T., Fridman, E., Gang, D.R. *et al.* (2006) Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc. Natl. Acad. Sci. USA*, **103**, 10128–10133.

Krings, M. and Kerp, H. (1998) Epidermal anatomy of *Barthelopteris germarii* from the upper carboniferous and lower Permian of France and Germany. *Am. J. Bot.* **85**, 553–562.

Krings, M., Taylor, T.N. and Kellogg, D.W. (2002) Touch-sensitive glandular trichomes: a mode of defence against herbivorous arthropods in the Carboniferous. *Evol. Ecol. Res.* **4**, 779–786.

Krings, M., Kellogg, D.W., Kerp, H. and Taylor, T.N. (2003) Trichomes of the seed fern *Blanzyopteris praedentata*: implications for plant–insect interactions in the late Carboniferous. *Bot. J. Linn. Soc.* **141**, 133–149.

Lange, B.M., Wildung, M.R., Stauber, E.J., Sanchez, C., Pouchnik, D. and Croteau, R. (2000) Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *Proc. Natl. Acad. Sci. USA*, **97**, 2934–2939.

Li, L., Zhao, Y.F., McCaig, B.C., Wingerd, B.A., Wang, J.H., Whalon, M.E., Pichersky, E. and Howe, G.A. (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.

Liu, B.Y., Wang, H., Du, Z.G., Li, G.F. and Ye, H.C. (2011) Metabolic engineering of artemisinin biosynthesis in *Artemisia annua* L. *Plant Cell Rep.* **30**, 689–694.

Luckwill, L.C. (1943) *The Genus Lycopersicon: A Historical, Biological, and Taxonomic Survey of the Wild and Cultivated Tomatoes*. Aberdeen, UK: Aberdeen University Press.

Ma, D.M., Pu, G.B., Lei, C.Y. *et al.* (2009) Isolation and characterization of AaWRKY1, an *Artemisia annua* transcription factor that regulates the Amorpha-4,11-diene synthase gene, a key gene of artemisinin biosynthesis. *Plant Cell Physiol.* **50**, 2146–2161.

Mahmoud, S.S. and Croteau, R.B. (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proc. Natl. Acad. Sci. USA*, **98**, 8915–8920.

Maluf, W.M., Inoue, I.F., Ferreira, R. D. P.D., Gomes, L.A.A., de Castro, E.M. and Cardoso, M. D. G. (2007) Higher glandular trichome density in tomato leaflets and repellence to spider mites. *Pesqu. Agropecu. Bras.* **42**, 1227–1235.

Marks, M.D., Tian, L., Wenger, J.P., Omburo, S.N., Soto-Fuentes, W., He, J., Gang, D.R., Weiblen, G.D. and Dixon, R.A. (2009) Identification of candidate genes affecting delta(9)-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. *J. Exp. Bot.* **60**, 3715–3726.

Martin, C. and Glover, B.J. (2007) Functional aspects of cell patterning in aerial epidermis. *Curr. Opin. Plant Biol.* **10**, 70–82.

Matas, A.J., Agusti, J., Tadeo, F.R., Talon, M. and Rose, J.K.C. (2010) Tissue-specific transcriptome profiling of the citrus fruit epidermis and subepidermis using laser capture microdissection. *J. Exp. Bot.* **61**, 3321–3330.

McConkey, M.E., Gershenzon, J. and Croteau, R.B. (2000) Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. *Plant Physiol.* **122**, 215–224.

McDowell, E.T., Kapteyn, J., Schmidt, A. *et al.* (2011) Comparative functional genomic analysis of Solanum glandular trichome types. *Plant Physiol.* **155**, 524–539.

Meissner, R., Jacobson, Y., Melamed, S., Levyatuv, S., Shalev, G., Ashri, A., Elkind, Y. and Levy, A. (1997) A new model system for tomato genetics. *Plant J.* **12**, 1465–1472.

Meissner, R., Chague, V., Zhu, Q.H., Emmanuel, E., Elkind, Y. and Levy, A.A. (2000) A high throughput system for transposon tagging and promoter trapping in tomato. *Plant J.* **22**, 265–274.

Michie, M.J. and Reid, W.W. (1968) Biosynthesis of complex terpenes in leaf cuticle and trichomes of *Nicotiana tabacum*. *Nature*, **218**, 578.

Moulines, J., Lamidey, A.M. and Desvergnes-Breuil, V. (2001) A practical synthesis of Ambrox (R) from sclareol using no metallic oxidant. *Synth. Commun.* **31**, 749–758.

Moulines, J., Bats, J.P., Lamidey, A.M. and Da Silva, N. (2004) About a practical synthesis of Ambrox (R) from sclareol: a new preparation of a ketone key intermediate and a close look at its Baeyer–Villiger oxidation. *Helv. Chim. Acta*, **87**, 2695–2705.

Nafis, T., Akmal, M., Ram, M., Alam, P., Ahlawat, S., Mohd, A. and Abdin, M.Z. (2011) Enhancement of artemisinin content by constitutive expression of the HMG-CoA reductase gene in high-yielding strain of *Artemisia annua* L. *Plant Biotechnol. Rep.* **5**, 53–60.

Nagel, J., Culley, L.K., Lu, Y.P., Liu, E.W., Matthews, P.D., Stevens, J.F. and Page, J.E. (2008) EST analysis of hop glandular trichomes identifies an *O*-methyltransferase that catalyzes the biosynthesis of xanthohumol. *Plant Cell*, **20**, 186–200.

Napp-Zinn, K. (1966) Anatomie des Blattes. I. Blattanatomie der Gymnospermen. In *Handbuch der Pflanzenanotomie, Bd 8, Teil 1* (Zimmermann, W., Ozenda, P. and Wulff, H.D., eds). Berlin: Bomtraeger, pp. 1–370.

Naqvi, S., Farre, G., Sanahuja, G., Capell, T., Zhu, C.F. and Christou, P. (2010) When more is better: multigene engineering in plants. *Trends Plant Sci.* **15**, 48–56.

Oikawa, A., Matsuda, F., Kikuyama, M., Mimura, T. and Saito, K. (2011) Metabolomics of a single vacuole reveals metabolic dynamism in an Alga Chara australis. *Plant Physiol.* **157**, 544–551.

Okabe, Y., Asamizu, E., Saito, T., Matsukura, C., Ariizumi, T., Brès, C., Rothan, C., Mizoguchi, T. and Ezura, H. (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from micro-tom mutant libraries. *Plant Cell Phys.* **52**, 1994–2005.

Olsson, M.E., Olofsson, L.M., Lindahl, A.L., Lundgren, A., Brodelius, M. and Brodelius, P.E. (2009) Localization of enzymes of artemisinin biosynthesis to the apical cells of glandular secretory trichomes of *Artemisia annua* L. *Phytochemistry*, **70**, 1123–1128.

Olofsson, L., Lundgren, A. and Brodelius, P.E. (2012) Trichome isolation with and without fixation using laser microdissection and pressure catapulting followed by RNA amplification: Expression of genes of terpene metabolism in apical and sub-apical trichome cells of Artemisia annua L. *Plant Sci.* **183**, 9–13.

Pateraki, I. and Kanellis, A.K. (2010) Stress and developmental responses of terpenoid biosynthetic genes in *Cistus creticus* subsp *creticus*. *Plant Cell Rep.* **29**, 629–641.

Payne, W. (1978) A glossary of plant hair terminology. *Brittonia*, **30**, 239–255.

Pesch, M. and Hulskamp, M. (2009) One, two, three... models for trichome patterning in Arabidopsis? *Curr. Opin. Plant Biol.* **12**, 587–592.

Phillips, M.A., Leon, P., Boronat, A. and Rodriguez-Concepcion, M. (2008) The plastidial MEP pathway: unified nomenclature and resources. *Trends Plant Sci.* **13**, 619–623.

Piron, F., Nicolai, M., Minoia, S., Piednoir, E., Moretti, A., Salgues, A., Zamir, D., Caranta, C. and Bendahmane, A. (2010) An induced mutation in tomato eIF4E leads to immunity to two potyviruses. *PLoS ONE*, **5**, 1–10.

Ranger, C.M., Winter, R.E.K., Rottinghaus, G.E., Backus, E.A., Ellersieck, M.R. and Johnson, D.W. (2004) Bioactivity of lipophilic metabolites from glandular trichomes of *Medicago sativa* against the potato leafhopper. *J. Chem. Ecol.* **30**, 1969–1983.

Rios-Estepa, R., Turner, G.W., Lee, J.M., Croteau, R.B. and Lange, B.M. (2008) A systems biology approach identifies the biochemical mechanisms regulating monoterpenoid essential oil composition in peppermint. *Proc. Natl. Acad. Sci. USA*, **105**, 2818–2823.

Rios-Estepa, R., Lange, I., Lee, J.M. and Lange, B.M. (2010) Mathematical modeling-guided evaluation of biochemical, developmental, environmental, and genotypic determinants of essential oil composition and yield in peppermint leaves. *Plant Physiol.* **152**, 2105–2119.

Saito, T., Ariizumi, T., Okabe, Y., Asamizu, E., Hiwasa-Tanase, K., Fukuda, N., Mizoguchi, T., Yamazaki, Y., Aoki, K. and Ezura, H. (2011) TOMATOMA: a novel tomato mutant database distributing micro-tom mutant collections. *Plant Cell Phys.* **52**, 283–296.

Sallaud, C., Rontein, D., Onillon, S. *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.

Samuels, L., Kunst, L. and Jetter, R. (2008) Sealing plant surfaces: cuticular wax formation by epidermal cells. *Annu. Rev. Plant Biol.* **59**, 683–707, Palo Alto: Annual Reviews.

Schiebold, S., Tschiersch, H., Borisjuk, L., Heinzel, N., Radchuk, R. and Rolletschek, H. (2011) A novel procedure for the quantitative analysis of metabolites, storage products and transcripts of laser microdissected seed tissues of *Brassica napus*. *Plant Methods*, **7**, 19.

Schilmiller, A.L., Last, R.L. and Pichersky, E. (2008) Harnessing plant trichome biochemistry for the production of useful compounds. *Plant J.* **54**, 702–711.

Schilmiller, A.L., Schauvinhold, I., Larson, M., Xu, R., Charbonneau, A.L., Schmidt, A., Wilkerson, C., Last, R.L. and Pichersky, E. (2009) Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. *Proc. Natl. Acad. Sci. USA*, **106**, 10865–10870.

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

Schilmiller, A.L., Miner, D.P., Larson, M., McDowell, E., Gang, D.R., Wilkerson, C. and Last, R.L. (2010b) Studies of a biochemical factory: tomato trichome deep expressed sequence tag sequencing and proteomics. *Plant Physiol.* **153**, 1212–1223.

Schmidt, A., Li, C., Shi, F., Jones, A.D. and Pichersky, E. (2011) Polymethylated myricetin in trichomes of the wild tomato species *Solanum habrochaites* and characterization of trichome-specific 3′/5′- and 7/4′-myricetin *O*-methyltransferases. *Plant Physiol.* **155**, 1999–2009.

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

Shanks, G.D. (2006) Treatment of falciparum malaria in the age of drug resistance. *J. Postgrad. Med.* **52**, 277–280.

Simmons, A.T. and Gurr, G.M. (2005) Trichomes of Lycopersicon species and their hybrids: effects on pests and natural enemies. *Agric. For. Entomol.* **7**, 265–276.

Sirikantaramas, S., Taura, F., Tanaka, Y., Ishikawa, Y., Morimoto, S. and Shoyama, Y. (2005) Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Phys.* **46**, 1578–1582.

Slocombe, S.P., Schauvinhold, I., McQuinn, R.P. *et al.* (2008) Transcriptomic and reverse genetic analyses of branched-chain fatty acid and acyl sugar production in *Solanum pennellii* and *Nicotiana benthamiana*. *Plant Physiol.* **148**, 1830–1846.

Sozzani, R., Cui, H., Moreno-Risueno, M.A., Busch, W., Van Norman, J.M., Vernoux, T., Brady, S.M., Dewitte, W., Murray, J.A.H. and Benfey, P.N. (2010) Spatiotemporal regulation of cell-cycle genes by SHORTROOT links patterning and growth. *Nature*, **466**, 128–132.

Sreelakshmi, Y., Gupta, S., Bodanapu, R., Chauhan, V.S., Hanjabam, M., Thomas, S., Mohan, V., Sharma, S., Srinivasan, R. and Sharma, R. (2010) NEATTILL: a simplified procedure for nucleic acid extraction from arrayed tissue for TILLING and other high-throughput reverse genetic applications. *Plant Methods*, **6**, 3.

Stukkens, Y., Bultreys, A., Grec, S., Trombik, T., Vanham, D. and Boutry, M. (2005) NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from Nicotiana plumbaginifolia, plays a major role in plant pathogen defense. *Plant Physiol.* **139**, 341–352.

Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G. and Covello, P.S. (2006) *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. *FEBS Lett.* **580**, 1411–1416.

Teoh, K.H., Polichuk, D.R., Reed, D.W. and Covello, P.S. (2009) Molecular cloning of an aldehyde dehydrogenase implicated in artemisinin biosynthesis in *Artemisia annua*. *Botany*, **87**, 635–642.

Thaler, J.S., Farag, M.A., Pare, P.W. and Dicke, M. (2002) Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. *Ecol. Lett.* **5**, 764–774.

Tissier, A. (2012) Trichome specific expression: promoters and their applications. In *Transgenic Plants - Advances and Limitations* (Çiftçi, Y.O., ed.). Rijeka: InTech, pp. 353–378.

Turner, G.W. and Croteau, R. (2004) Organization of monoterpene biosynthesis in Mentha. Immunocytochemical localizations of geranyl diphosphate synthase, limonene-6-hydroxylase, isopiperitenol dehydrogenase, and pulegone reductase. *Plant Physiol.* **136**, 4215–4227.

Turner, G., Gershenzon, J., Nielson, E.E., Froehlich, J.E. and Croteau, R. (1999) Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiol.* **120**, 879–886.

Turner, G.W., Gershenzon, J. and Croteau, R.B. (2000) Development of peltate glandular trichomes of peppermint. *Plant Physiol.* **124**, 665–680.

Uphof, J.C.T. (1962) Plant hairs. In *Handbuch der Pflanzenanatomie, Bd. 4, Teil 5* (Finsbauer, K., ed.). Berlin: Borntraeger, pp. 1–292.

Van Cutsem, E., Simonart, G., Degand, H., Faber, A.M., Morsomme, P. and Boutry, M. (2011) Gel-based and gel-free proteomic analysis of *Nicotiana tabacum* trichomes identifies proteins involved in secondary metabolism and in the (a)biotic stress response. *Proteomics*, **11**, 440–454.

Vassao, D.G., Gang, D.R., Koeduka, T., Jackson, B., Pichersky, E., Davin, L.B. and Lewis, N.G. (2006) Chavicol formation in sweet basil (*Ocimum basilicum*): cleavage of an esterified C9 hydroxyl group with NAD(P)H-dependent reduction. *Org. Biomol. Chem.* **4**, 2733–2744.

Vining, K.J., Zhang, Q., Tucker, A.O., Smith, C. and Davis, T.M. (2005) *Mentha longifolia* (L.) L.: a model species for mint genetic research. *HortScience*, **40**, 1225–1229.

Wagner, G.J., Wang, E. and Shepherd, R.W. (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann. Bot.* **93**, 3–11.

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

Wang, H., Liu, Y., Chong, K., Liu, B.Y., Ye, H.C., Li, Z.Q., Yan, F. and Li, G.F. (2007) Earlier flowering induced by over-expression of CO gene does not accompany increase of artemisinin biosynthesis in *Artemisia annua*. *Plant Biol.* **9**, 442–446.

Wang, G., Tian, L., Aziz, N., Broun, P., Dai, X., He, J., King, A., Zhao, P.X. and Dixon, R.A. (2008) Terpene biosynthesis in glandular trichomes of hop. *Plant Physiol.* **148**, 1254–1266.

Wang, W., Wang, Y.J., Zhang, Q., Qi, Y. and Guo, D.J. (2009) Global characterization of *Artemisia annua* glandular trichome transcriptome using 454 pyrosequencing. *BMC Genomics*, **10**, 465.

Wang, H., Olofsson, L., Lundgren, A. and Brodelius, P.E. (2011) Studies on the expression of enzymes of artemisinin biosynthesis using promoter-GUS fusions in transgenic *Artemisia annua* plants. In *Terpnet 2011* (Brodelius, P.E., ed.). Kalmar, Sweden: School of Natural Sciences Linnaeus University, pp. 58.

Weathers, P.J., Arsenault, P.R., Covello, P.S., McMickle, A., Teoh, K.H. and Reed, D.W. (2011) Artemisinin production in *Artemisia annua*: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. *Phytochem. Rev.* **10**, 173–183.

Werker, E. (2000) Trichome diversity and development. In *Advances in Botanical Research Incorporating Advances in Plant Pathology, Vol. 31: Plant Trichomes* (Hallahan, D.L. and Gray, J.C., eds). San Diego/Boston/London: Academic Press, pp. 1–35.

Wollenweber, E. and Schneider, H. (2000) Lipophilic exudates of Pteridaceae – chemistry and chemotaxonomy. *Biochem. Syst. Ecol.* **28**, 751–777.

Wollenweber, E., Stevens, J.F., Ivanic, M. and Deinzer, M.L. (1998) Acylphloroglucinols and flavonoid aglycones produced by external glands on the leaves of two Dryopteris ferns and Currania robertiana. *Phytochemistry*, **48**, 931–949.

Xie, Z., Kapteyn, J. and Gang, D.R. (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.

Yamaura, T., Tanaka, S. and Tabata, M. (1992) Localization of the biosynthesis and accumulation of monoterpenoids in glandular trichomes of thyme. *Planta Med.* **58**, 153–158.

Yang, R.Y., Feng, L.L., Yang, X.Q., Yin, L.L., Xu, X.L. and Zeng, Q.P. (2008) Quantitative transcript profiling reveals down-regulation of a sterol pathway relevant gene and overexpression of artemisinin biogenetic genes in transgenic *Artemisia annua* plants. *Planta Med.* **74**, 1510–1516.

Yerger, E.H., Grazzini, R.A., Hesk, D., Coxfoster, D.L., Craig, R. and Mumma, R.O. (1992) A rapid method for isolating glandular trichomes. *Plant Physiol.* **99**, 1–7.

Young, J.D., Shastri, A.A., Stephanopoulos, G. and Morgan, J.A. (2011) Mapping photoautotrophic metabolism with isotopically nonstationary (13)C flux analysis. *Metab. Eng.* **13**, 656–665.

Yu, G., Nguyen, T.T.H., Guo, Y. *et al.* (2010) Enzymatic functions of wild tomato methylketone synthases 1 and 2. *Plant Physiol.* **154**, 67–77.

Zhang, L., Jing, F.Y., Li, F.P., Li, M.Y., Wang, Y.L., Wang, G.F., Sun, X.F. and Tang, K.X. (2009a) Development of transgenic *Artemisia annua* (Chinese wormwood) plants with an enhanced content of artemisinin, an effective anti-malarial drug, by hairpin-RNA-mediated gene silencing. *Biotechnol. Appl. Biochem.* **52**, 199–207.

Zhang, Y.S., Teoh, K.H., Reed, D.W. and Covello, P.S. (2009b) Molecular cloning and characterization of Dbr1, a 2-alkenal reductase from *Artemisia annua*. *Botany*, **87**, 643–649.