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Plant secretory structures: more than just reaction bags

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Plants have a remarkable capacity for the production of a wide range of metabolites. Much has been reported and reviewed on the diversity of these metabolites and how it is achieved, for example through the evolution of enzyme families. In comparison, relatively little is known on the extraordinary metabolic productivity of dedicated organs where many of these metabolites are synthesized and accumulate. Plant glandular trichomes are such specialized metabolite factories, for which recent omics analyses have shed new light on the adaptive metabolic strategies that support high metabolic fluxes. In photosynthetic trichomes such as those of the Solanaceae, these include CO_2 refixation and possibly C4-like metabolism which contribute to the high productivity of these sink organs.

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Introduction

Plants are known to be masters at generating chemical diversity. One invoked reason for this extraordinary diversity is their sessile nature. Being unable to escape adverse conditions, plants have evolved various processes to compensate for this immobility, one of them being the production of compounds that will protect them against aggressors or an unfavourable environment. An argument often used in articles about the biosynthesis of plant secondary metabolites to justify the work is that by elucidating the biosynthesis pathway it will become possible to make them more readily available by engineering easily cultivable microorganisms such as *Escherichia coli* or the yeast *Saccharomyces cerevisiae*. While this argument may hold true for substances produced by rare, endangered or difficult to cultivate species, or for substances that are in particularly low amounts such as Taxol, in many cases the substances of interest are actually present in fairly large quantities in the plant. In Table 1, some examples are given that illustrate this point.

This capacity to produce specialized metabolites in large quantities means that developing processes based on metabolic engineering and fermentation of microorganisms is a tough challenge. A revealing story in this regard is that of the engineering of artemisinic acid, the precursor of the antimalarial artemisinin in yeast. In a series of remarkable metabolic engineering efforts, production of artemisinic acid in yeast reached over 20 g/L [2–4]. The industrial development of this process was taken over by a non-profit organization and ultimately by the pharmaceutical giant Sanofi-Aventis [5]. Major investments were made in a production facility to supply this compound for the manufacture of anti-malarial medication. However, the production costs were still above those of plant-produced artemisinin and a better organization of artemisinin production by small farmers led to a stabilization of the prices and of the supply. This rendered the fermented route no longer competitive. The production plant was sold, and is now faced with an uncertain future [6•]. The lesson of this is that plants can be hard to beat and that it may be worth understanding how they manage to achieve this high productivity. There have been efforts to try and exploit this inherent capacity for metabolic engineering purposes to develop the production of industrial compounds in plants. Current efforts and perspectives for the production of isoprenoids in plant cell factories are reviewed in this issue [7]. The combination of large scale sequencing data, particularly from non-model species, and of recent developments in synthetic biology such as modular assembly schemes based on Golden Gate cloning [8,9], create exciting opportunities for the production of secondary metabolites in plants. In this regard, one nice and recent example is the gram-scale production of triterpenoids in *Nicotiana benthamiana* [10].

Many, if not all, plant species possess dedicated organs or tissues where these metabolites are produced and stored, typically in large quantities. These specialized structures can be classified in two major classes based on their localization in the plant: at the surface or internal. At the surface, probably the most broadly represented type of secretory structures are glandular trichomes. They can adopt a myriad of shapes and sizes but have in common the capacity to synthesize large quantities of metabolites and to secrete them at the surface or store them in dedicated compartments depending on the nature of the compounds [11].

Table 1

Some examples of the productivity of specialized metabolites by plants

Species	Substance(s) produced	Yield	Use	Ref.
Peppermint (*Mentha x piperita*)	Menthol	≈100 kg/ha	Aroma and fragrance industry	[1]
Clary sage (*Salvia sclarea*)	Sclareol	30–50 kg/ha	Fragrance industry	http://www.crieppam.fr/publications/itemlist/category/7-sauge-sclaree
Artemisia annua	Artemisinin	>50 kg/ha	Antimalarial	https://www.york.ac.uk/org/cnap/artemisiaproject/artemisiaF1seed/hybrids.html
Rubber tree (*Hevea brasiliensis*)	Rubber	World average: 1200 kg/ha Max: 3300 kg/ha	Industry	FAO statistics (http://www.fao.org/faostat/en/#data/QC)

Inside the plant, a number of secretory structures or specialized cells dedicated to the biosynthesis and storage of metabolites have been described. These include laticifers, resin ducts, secretory cavities and oil glands. These structures have evolved to accumulate and store large quantities of metabolites or polymers. The purpose of this review is to survey the latest knowledge in our understanding of how these specialized structures work, specifically what are the metabolic features that allow these structures to be so productive. Because most of the work in this area is on glandular trichomes, the content of this review will be strongly biased on these organs.

Transcriptional activation of committed pathways and occurrence of specific isoforms

Probably the clearest feature of secretory structures accumulating a particular class of compounds, is the high transcriptional activation of genes encoding enzymes of the committed pathways for these compounds. In most cases, the compounds which accumulate in these organs are also synthesized there, explaining these high expression levels. For example, in glandular trichomes, this has been a tremendous help to identify and characterize a number of pathways, including those of menthol in peppermint (*Mentha* x *piperita*), artemisinin in *Artemisia annua*, phenylpropenes in basil (*Ocimum basilicum*), acylsugars and terpenoids in cultivated and wild tomato (*Solanum* sp.) or diterpenoids in tobacco [12–37]. Similarly, in opium poppy the final steps in the biosynthesis of the benzylisoquinoline alkaloids are localized in laticifers, although earlier steps take place in neighbouring sieve elements and companion cells [38]. Also, biosynthesis of the abundant conifer resin acids occurs exclusively in resin ducts [39,40].

The high fluxes through the downstream part of the pathways also imply a supply of the corresponding precursors in adequate amounts. These are provided by intermediate pathways which connect central and energy metabolism with secondary metabolism. Again in glandular trichomes, transcriptome analysis revealed high level expression of precursor pathways. For example in tomato (*Solanum lycopersicum* LA4024 and *Solanum habrochaites* LA1777), type VI trichomes which produce terpenoids from both from the cytosolic mevalonate (MEV) and the plastidic methyl-erythritol phosphate (MEP) pathways show significantly increased expression of genes of these pathways [41••]. However the increase in expression is not uniform across the pathways. In the MEP pathway, the first two steps, deoxy-xylulose phosphate synthase (DXS) and deoxy-xylulose phosphate reductase (DXR), as well as the last two steps, hydroxymethylbutenyl 4-diphosphate synthase (HDS) and hydroxymethylbutenyl 4-diphosphate reductase (HDR), are strongly overexpressed in trichomes whereas the three genes of the middle of the pathway (2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (CMK) and 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS)), are barely differentially regulated [41••]. A somewhat similar picture emerges in glandular trichomes of basil, although this is more the case at the protein than at the transcript levels [42]. This suggests that these enzymes of the MEP pathway could constitute bottlenecks whose high level expression in the glandular trichomes is required for high flux through this pathway. However, in a recent study using Illumina RNA-sequencing, this expression profile across the MEP pathway does not seem to be present in spearmint (*Mentha spicata*), suggesting there may be species to species variations [43]. Nonetheless, at least DXS has been shown repeatedly to constitute a bottleneck in the flux through the MEP pathway, whether in bacterial or in plant systems [44–46]. Interestingly in plants, DXS is consistently the only enzyme of the pathway encoded by two isogenes, *DXS1* and *DXS2*, with *Arabidopsis thaliana* being a notable exception. Phylogenetic analysis shows that these two isoforms are conserved throughout plant evolution and constitute two distinct clades [47–49]. There is good evidence that the *DXS1* clade has house-keeping functions, mainly associated with the supply of isoprenoid precursors for photosynthesis, whereas the *DXS2* is associated to the production of abundant