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New developments in engineering plant metabolic pathways

Evangelos C Tatsis and Sarah E O'Connor

Plants contain countless metabolic pathways that are responsible for the biosynthesis of complex metabolites. Armed with new tools in sequencing and bioinformatics, the genes that encode these plant biosynthetic pathways have become easier to discover, putting us in an excellent position to fully harness the wealth of compounds and biocatalysts (enzymes) that plants provide. For overproduction and isolation of high-value plant-derived chemicals, plant pathways can be reconstituted in heterologous hosts. Alternatively, plant pathways can be modified in the native producer to confer new properties to the plant, such as better biofuel production or enhanced nutritional value. This perspective highlights a range of examples that demonstrate how the metabolic pathways of plants can be successfully harnessed with a variety of metabolic engineering approaches.

Address
John Innes Centre, Department of Biological Chemistry, Norwich Research Park, Norwich NR4 7UH, UK

Corresponding author: O'Connor, Sarah E (sarah.o'connor@jic.ac.uk)

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Introduction

Plants provide a seemingly inexhaustible pool of structurally diverse chemicals. *In planta*, the biosynthesis of these compounds is a response to external or environmental cues, and therefore plays a crucial role in shaping the interdependencies and diversity of plant ecosystems. These chemicals impact how effectively plants can be used as food and energy sources. Moreover, many chemicals that are produced by plants promote human health, and numerous plant metabolites are isolated for use in the pharmaceutical industry. Despite the importance of plant metabolites, the biosynthetic processes for only a small fraction of these complicated molecules are known, indicating that the immense diversity of plant metabolism has not been explored. The recent advances in next-generation sequencing technologies, along with the continuous development of new algorithms for bioinformatic analysis of these sequence data, has greatly expedited the process of plant metabolic gene discovery. By extension, these discoveries have allowed advancements in the engineering of plant metabolism.

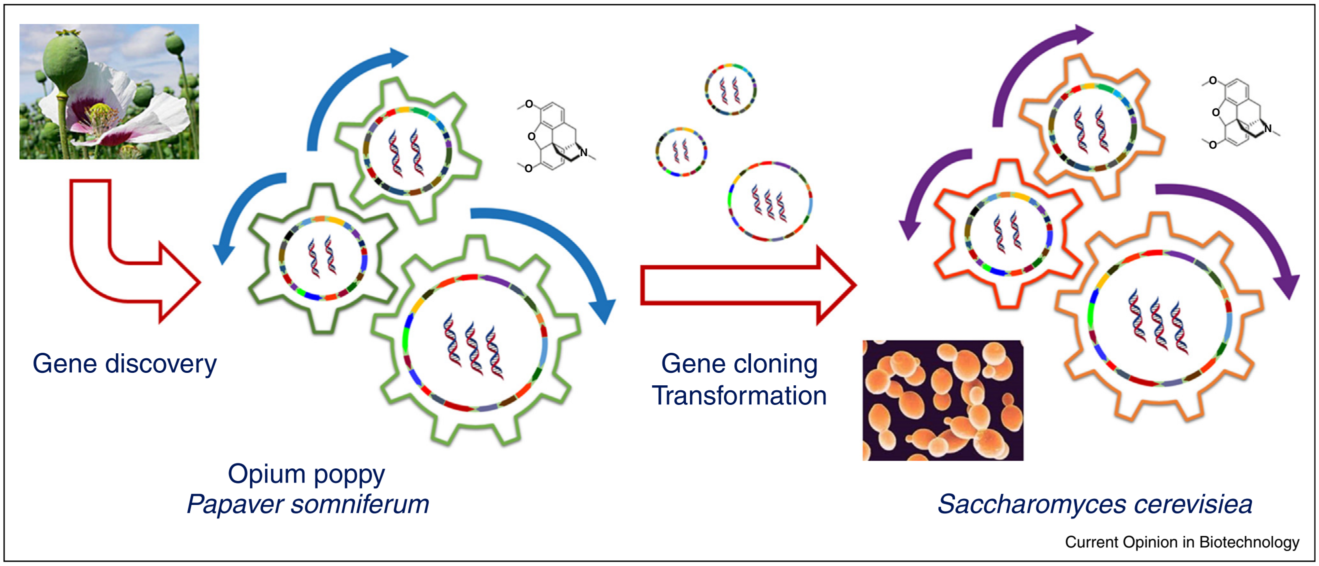
It is of great importance to elucidate and engineer the plant metabolic pathways that construct complex metabolites from simple building blocks. An understanding of these pathways will allow us to fully harness the wealth of compounds and biocatalysts that plants provide. In this perspective, we highlight several important recent examples of metabolic engineering with plant metabolic pathways. These examples demonstrate the wide range of engineering approaches that can be applied to plant pathways, and also illustrate the range of problems that can be addressed by plant metabolic engineering. Collectively, these examples demonstrate the progress that we are making to fully harness the metabolic power of plants.

Heterologous reconstitution of plant metabolic pathways

One approach to harness plant metabolic pathways is to reconstitute the biosynthetic genes into a heterologous organism [1] (Figure 1). Microbial (e.g. *Saccharomyces cerevisiea* and *Escherichia coli*) and plant (e.g. *Nicotiana benthamiana*) hosts can be used, with each system having advantages and disadvantages. For example, plants, which utilize photosynthesis, do not require exogenous carbon feedstocks [2••]. Many plants such as *Nicotiana tabacum* (tobacco) and *N. benthamiana* can generate large amounts of biomass quickly and cheaply [2••,3], making them a robust, sustainable, and scalable platform for large-scale terpene production. On the other hand, microbial hosts can be genetically manipulated in a rapid fashion, are fast growing, and the infrastructure required for microbial production is well established [4]. Below are two representative examples, one utilizing the plant host *N. tabacum* to overproduce high value triterpenoids, and the other using *S. cerevisiea* to produce the plant derived opiate morphine. Other examples using *Nicotiana* [5–7] and *Saccharomyces* [8–12] have also been recently reported in the literature.

Linear, branch-chained triterpenes that are generated by the green alga *Botryococcus braunii* are increasingly recognized as important chemical and biofuel feedstocks [13]. However, the slow-growing *B. braunii* is an impractical production system for large-scale isolation of these compounds [14]. In a recent study, high levels of the *B. braunii*

Figure 1



Heterologous reconstitution of plant pathways in yeast, as exemplified by reconstitution of opiate biosynthetic pathways in yeast. The genes responsible for biosynthesis of opiates were cloned from opium poppy and introduced into the appropriate vectors for expression of enzymes in yeast.

triterpene botryococcene (Figure 2) were produced in *N. tabacum* plants by the overexpression of an avian farnesyl diphosphate synthase along with two versions of botryococcene synthases in the chloroplast [2••]. High yields of methylated botryococcene derivatives could also be obtained when triterpene methyltransferases were expressed in the chloroplast. While approximately 90% of the triterpenes were converted to methylated derivatives when all enzymes were targeted to the chloroplasts, less than 15% of triterpenes were methylated when this metabolic pathway was expressed in the cytoplasm, highlighting the enormous impact that enzyme localization can have on metabolic engineering. Chloroplasts, which have a high flux of carbon passing through the MEP (2-*C*-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate) pathway, appear to be particularly suited for expression of terpenes [2••]. While the plants in this study accumulated 0.2–1.0 mg triterpene per gram of plant fresh weight, the authors of this study pointed out that previously reported engineering efforts with sesquiterpene and monoterpene pathways in plants often resulted in much lower production levels, perhaps because different terpene compounds may have differing effects on physiological homeostasis and growth.

Opioids such as thebaine, codeine and morphine are widely used around the globe to treat pain [15]. Currently, farming of opium poppies and isolation of opiates from the poppy latex is the only commercial source of these compounds. However, in a recent study, yeast (*S. cerevisiea*) was engineered to produce the opiates thebaine and hydrocodone (Figure 2) de novo from an exogenous sugar carbon source [16••]. The resulting strains expressed 21 genes for thebaine production and 23 genes for hydrocodone production. While yields were low (<1 μg/L), this study provides a dramatic proof-of-principle that complex opiates can be produced in yeast. Notably, this work was made possible by the recent discovery of an opiate biosynthetic gene, reticuline epimerase, which researchers had struggled to identify for decades [16••,17••,18••].

Engineering plant pathways to create better biofuels

A major challenge of the modern era is the transition to a bio-based economy. Biofuels are a key part of this landscape, but challenges to efficiently and cost-effectively produce biofuels still remain [19,20]. Bioethanol is currently the major biofuel in use, and it is produced by the easily accessible sugars of sugar cane and corn. However, as food security becomes an increasing concern in an ever-expanding population, other approaches for producing biofuels must be considered [21]. A promising source for next generation biofuels are those produced from lignocellulosic biomass that originates from the residual biomass of crops, such as wheat, corn and sugarcane. Alternatively, the biomass from crops such as poplar and switchgrass that can be grown on marginal land are also possibilities for fuel production [22].

The presence of lignin in plant cell walls undermines the ability to access the polysaccharides of biomass by enzymatic degradation. This biomass must therefore be subjected to hydrolysis under acidic or alkaline conditions to