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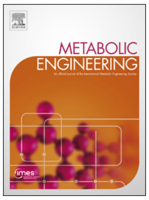

Minireview

Metabolic engineering to enhance the value of plants as green factories

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ABSTRACTS

The promise of plants to serve as the green factories of the future is ever increasing. Plants have been used traditionally for construction, energy, food and feed. Bioactive compounds primarily derived from specialized plant metabolism continue to serve as important scaffold molecules for pharmaceutical drug production. Yet, the past few years have witnessed a growing interest on plants as the ultimate harvesters of carbon and energy from the sun, providing carbohydrate and lipid biofuels that would contribute to balancing atmospheric carbon. How can the metabolic output from plants be increased even further, and what are the bottlenecks? Here, we present what we perceive to be the main opportunities and challenges associated with increasing the efficiency of plants as chemical factories. We offer some perspectives on when it makes sense to use plants as production systems because the amount of biomass needed makes any other system unfeasible. However, there are other instances in which plants serve as great sources of biological catalysts, yet are not necessarily the best-suited systems for production. We also present emerging opportunities for manipulating plant genomes to make plant synthetic biology a reality.

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1. Introduction

Some of the main challenges humanity will face in the next 50–100 years include insufficient (healthy) food, climate change resulting from increased greenhouse gas accumulation, inadequate power for the needs of a growing and increasingly energy-dependent population, and a need for new medicines to treat existing and emerging diseases. Plants are clearly part of the solution to all these challenges. Terrestrial plants capture 121.7×10^9 metric tons of carbon from the atmosphere each year (Beer et al., 2010) using solar light as the sole energy source. Photosynthesized carbon is then chemically converted into a very large number of chemical compounds. Some of these compounds are among the most abundant on earth; others are found in low quantities in a limited number of plant species. Generally (but not always), highly abundant compounds are derived from primary (central) metabolism and include the aromatic polymer lignin, triacylglycerols (vegetable oils) and the polysaccharides cellulose and starch. Providing just one example of the impact that these plant compounds have on humans, the world's annual production of starch exceeds 75 million tons (Data for 2012, http://www.zuckerforschung.at/).

In contrast, most of the less abundant plant compounds derive from specialized metabolism, an aspect of the plant metabolic activity that results in the production of a very large number [estimated to be in excess of 200,000 (Dixon and Strack, 2003)] of compounds which plants use, as sessile organisms, in their interactions with the environment (biotic and abiotic). A large number [20–30% (Raskin et al., 2002)] of pharmaceutical drugs currently in the market were originally obtained from plants, where they often accumulate in very small quantities. For example, 10,000 kg of Pacific yew bark yields less than 1 kg of the potent anti-cancer compound paclitaxel (taxol) (Vidensek et al., 1990).

Thus, while plants have the ability to produce some of the most abundant compounds on Earth (*e.g.* lignin and cellulose), they also produce other chemicals of high utility for humankind in minute quantities. Strategies directed at engineering the metabolism of plant compounds must take this into consideration, as the approaches, tools and business propositions are very different for both (Fig. 1). It is obvious that plants will continue to be the major producers of carbohydrates, vegetable oils and cellulose, and there are significant opportunities to engineer the respective plant pathways to alter the chemical properties or the levels of such compounds. But as we learn more about how plants synthesize specialized metabolites, the reconstitution of these pathways in microorganisms, either *de novo* or from privileged scaffolds using synthetic biology approaches, is becoming increasingly attractive from both economical and

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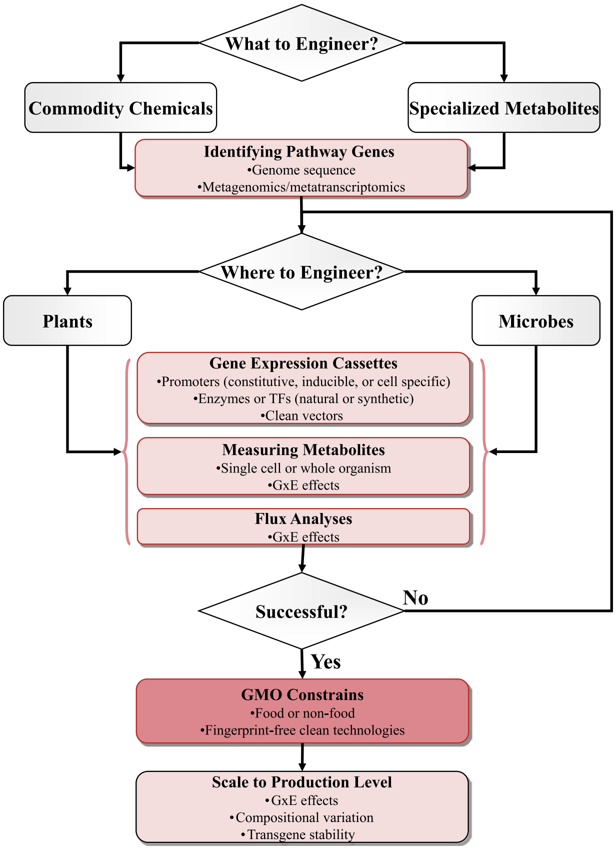

Fig. 1. Outline of the steps and challenges associated with rational metabolic engineering. Bottlenecks are shaded red; the intensity of the color reflects to what extent each aspect is a bottleneck. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

experimental perspectives (Mitchell, 2011; Xu et al., 2012). The promise of synthetic biology for the production of plant-derived specialized metabolites that are expensive and difficult to obtain is exemplified by the production of the antimalarial drug artemisinin in *Saccharomyces cerevisiae* (Paddon and Keasling, 2014; Paddon et al., 2013), and by the high-level production of precursors for the anticancer drug taxol in *Escherichia coli* (Ajikumar et al., 2010). Application of synthetic biology in plants is still at an early stage, but as described in subsequent sections, new breakthroughs in genome engineering offer new opportunities. Plant metabolic engineering can also be used to increase plant performance, for example by increasing resistance to pathogens or tolerance to abiotic stress conditions. However, in this review, we will primarily focus on metabolic engineering as a way to produce plant chemical compounds.

2. Understanding plant metabolism essential for predictive engineering

For many years, plant metabolic engineering consisted of modifying a regulatory or biosynthetic step in a pathway with subsequent evaluation of the outcome, with the consequence that the results were often very different from what was expected (Carrari et al., 2003). Predictive metabolic engineering consists of tinkering with metabolism in ways that result in anticipated and desired outcomes (Chappell and Grotewold, 2008), which requires an exceptional knowledge of plant biosynthetic pathways and plant systems biology (Curran and Alper, 2012). It is not our intention to recapitulate here the wealth of knowledge on plant metabolism, since there are some excellent recent reviews on the topic (Anarat-Cappillino and Sattely, 2014; Farre et al., 2014; Shumskaya and Wurtzel, 2013; Vogt, 2010; Ziegler and Facchini, 2008). But there are a few aspects of plant metabolism that have emerged over the past few years which must be considered in any successful metabolic engineering activity, and are therefore worth highlighting.

2.1. *Enzymes can be promiscuous*

A general assumption has been that enzymes are “specialists”, selected to catalyze just one chemical reaction. While this appears to be true for many catalysts from central metabolism, it is becoming evident that a vast majority of the (specialized) metabolic enzymes in microbes (Nam et al., 2012) and plants (Bar-Even and Tawfik, 2013) are promiscuous (generalists), meaning that they can act on a number of different (often, but not always, related) substrates. This broad substrate specificity of specialized metabolism biosynthetic enzymes provides an outstanding opportunity for evolutionary innovations (Grotewold, 2005; Tawfik, 2010; Weng et al., 2012). The ability of specialized metabolism enzymes to utilize multiple substrates means that many specialized pathways [*e.g.*, phenylpropanoid biosynthesis (Bonawitz and Chapple, 2010)] are structured as complex grids, rather than as straightforward linear pathways (*e.g.*, glycolysis).

2.2. *Enzymes involved in specialized metabolism are inefficient*

Many of the genes encoding enzymes involved in specialized metabolism appear to have arisen from genes of central metabolism, for example by the process of gene duplication and neofunctionalization (Ober, 2005, 2010). Interestingly, however, specialized metabolism enzymes commonly possess significantly lower k_{cat} values compared to their corresponding central metabolism counterparts. This could be a consequence of reduced selective pressures, when compared to enzymes of core metabolism, offering significant opportunities for enzyme optimization (Bar-Even and Tawfik, 2013).

2.3. *Cluster organization of genes encoding metabolic enzymes*

In bacteria, loci belonging to functional groups are often arranged in operons, which are clusters of genes with a related function and under the control of a single regulatory signal that are co-transcribed into a common mRNA. Indeed, it is thought that the organization of genes into co-regulated clusters (or operons) plays a major role in enabling bacteria to quickly adapt to changing environmental conditions, with classical examples such as the *lac* and *trp* operons that have had a major influence on how we perceive genetic regulatory mechanisms in all biological systems (Lawrence, 2002). Operons, as described in bacteria, are not found in plants (Osbourn and Field, 2009), but there is increasing evidence for the presence of gene clusters, particularly for the biosynthesis of specialized metabolites [*e.g.*, oat avenacins (Qi et al., 2004); the maize benzoaxazinoid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) (Frey et al., 1997), the *Papaver somniferum* noscapine alkaloid (Winzer et al., 2012), and the Solanaceae steroidal glycoalkaloids (Itkin et al., in press)]. These clusters are up to several hundred kb in length (Chu et al., 2011), and can harbor from two to more than 10 biosynthesis enzymes (Chu et al., 2011; Winzer et al., 2012).

As of April 2014, approximately 100 plant genomes have been sequenced to a reasonable depth, providing an unparalleled opportunity for identifying enzymes that fit the requirements (*e.g.*, specificity, kinetic parameters) of metabolic engineers. The Plant Metabolic Network (PMN, http://www.plantcyc.org/) attempts to catalog enzymes and catalyzed reactions from a number of plants, but limitations in the amount of biochemical information available makes the resource of modest utility. This is nevertheless a great first step towards developing genome-scale models of metabolism. However, for the

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