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Metabolic engineering approaches for production of biochemicals in food and medicinal plants

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Historically, plants are a vital source of nutrients and pharmaceuticals. Recent advances in metabolic engineering have made it possible to not only increase the concentration of desired compounds, but also introduce novel biosynthetic pathways to a variety of species, allowing for enhanced nutritional or commercial value. To improve metabolic engineering capabilities, new transformation techniques have been developed to allow for gene specific silencing strategies or stacking of multiple genes within the same region of the chromosome. The 'omics' era has provided a new resource for elucidation of uncharacterized biosynthetic pathways, enabling novel metabolic engineering approaches. These resources are now allowing for advanced metabolic engineering of plant production systems, as well as the synthesis of increasingly complex products in engineered microbial hosts. The status of current metabolic engineering efforts is highlighted for the *in vitro* production of paclitaxel and the *in vivo* production of β-carotene in Golden Rice and other food crops.

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Introduction

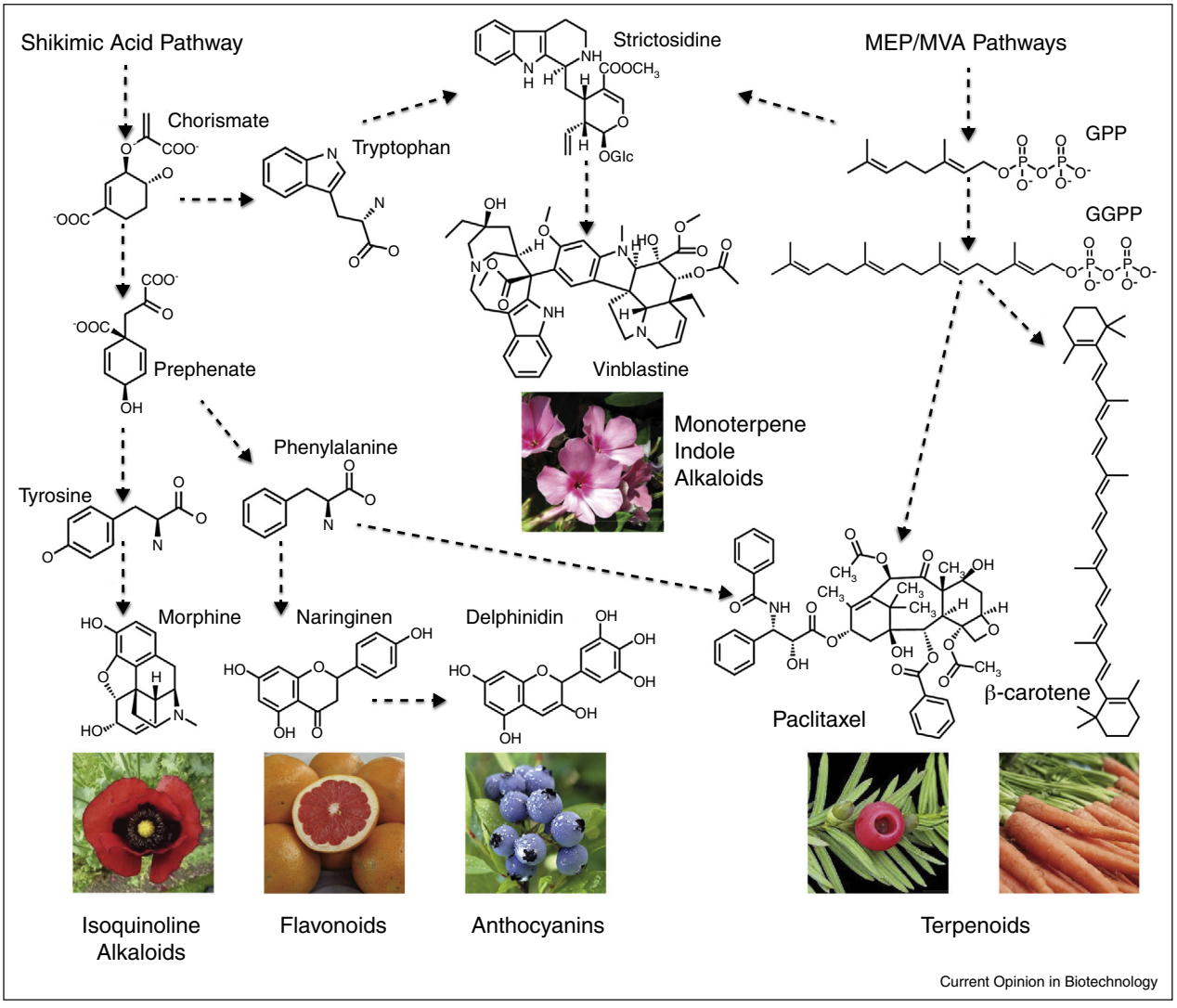
For centuries, plants have been utilized as both a food source and a source of active pharmaceutical agents. As of 2010, 75% of anti-bacterial compounds and 48.6% of anti-cancer compounds were either a natural product or natural product analog [1]. To aid in development and defense against stress, plants synthesize hundreds of thousands of compounds, many of which are produced through species-specific and complex biosynthetic pathways [2]. For instance, the shikimic acid pathway, non-mevalonate (MEP) pathway and mevalonate (MVA) pathway lead to diverse classes of compounds, which include the terpenoids, monoterpene indole alkaloids, isoquinoline alkaloids, flavonoids and anthocyanins (Figure 1). Due to their high degree of structural diversity, these compounds have significant commercial value as pharmaceuticals, nutraceuticals, dyes, fragrances, flavors and pesticides. In addition to their commercial applications, many dietary health benefits can be attributed to specific plant natural products in popular food crops. For instance, the compounds seen in Figure 1 are anti-cancer agents (vinblastine and paclitaxel), analgesics (morphine), antioxidants (naringenin and delphinidin) and provitamins (β-carotene).

Although some valuable plant natural products with simple structures are easily chemically synthesized (e.g., aspirin and ephedrine), many have complex structures with multiple chiral centers, making chemical synthesis both difficult and commercially infeasible [3]. As a result, these compounds are often produced through the exploitation of native biological pathways using natural harvest (e.g., codeine, morphine and dietary food compounds), semi-synthesis (e.g., paclitaxel), heterologous production (e.g., vanilla) or plant cell culture techniques (e.g., paclitaxel, ginseng and anthocyanins) [4]. In these biological systems, metabolic engineering can be used to manipulate flux through both primary and secondary metabolic pathways, allowing for the redirection of carbon flux towards products of interest. This review focuses on the use of metabolic engineering techniques for increasing the production of valuable plant natural products in both medicinal plants and food crops, allowing for decreased production costs and/or increased food nutritional value.

Methods for gene transfer and expression modification

To allow for metabolic engineering of a plant, the system must be amenable to stable transformation. Traditional gene transfer approaches (most commonly *Agrobacterium* transformation and particle bombardment) have advanced greatly over the last decade, allowing for the development of reliable stable transformation methods for many non-model plant species that were previously recalcitrant [5]. Moving forward, much effort has focused on advancing metabolic engineering technologies to allow for transgenics without selective markers and integration of genes in specific regions of the chromosome. Traditionally, plants are transformed with a selective marker (typically an antibiotic or herbicide resistance, such as kanamycin resistance (*npt II*) or hygromycin resistance (*hpt II*)), allowing for selection of successfully transformed

Figure 1



Examples of biosynthetic pathways leading to large classes of pharmaceutical or nutraceutical plant natural compounds. Arrows represent carbon flux through a pathway starting from primary metabolism (shikimic acid and MEP/MVA pathways) and leading to classes of secondary metabolites. Vinblastine and paclitaxel require precursors from both primary metabolic pathways as indicated by the arrows.

cells when efficiencies are low. Due to the risk of transgene migration to weeds or pathogens, selection-free transgenic systems are needed to meet regulatory and biosafety requirements for genetically modified crops. Selectable markers can be removed using techniques such as co-transformation, site-specific recombination, multi-autotransformation vectors, transposition systems and homologous recombination, as recently reviewed [6].

Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have been utilized to allow for site-specific gene mutation, replacement or integration through non-homologous end joining or homologous recombination. These artificial enzymes utilize a DNA cleavage domain fused to a DNA binding domain that can be programmed to target specific regions of the genome. This enables directed mutagenesis of specific genes for silencing, transgene targeting to increase expression or stacking of multiple genes at a single loci to improve genetic transmission over multiple generations. Zinc finger technology was first demonstrated in plants with the model species *Arabidopsis* [7] and has since been used to confer herbicide resistance in both tobacco and *Zea mays* [8,9]. Recently, ZFNs were used to stack genes conferring two herbicide resistances in embryonic maize suspension cultures, allowing for co-segregation of genes to progeny [10•]. TALEN technology has been demonstrated in *Arabidopsis* [11], tobacco [12••], *Brachypodium* [13], rice [13,14], barley [15] and *Brassica* [16]. In rice, TALENs were used to confer