Review

Plant diterpene synthases: exploring modularity and metabolic diversity for bioengineering

Philipp Zerbe[1] and Jörg Bohlmann[2]

[1] University of California at Davis, Plant Biology Department, One Shields Avenue, Davis, CA 95616, USA
[2] Michael Smith Laboratories, University of British Columbia, 2185 East Mall, Vancouver, BC, V6T 1Z4, Canada

Plants produce thousands of diterpenoid natural products; some of which are of significant industrial value as biobased pharmaceuticals (taxol), fragrances (sclareol), food additives (steviosides), and commodity chemicals (diterpene resin acids). In nature, diterpene synthase (diTPS) enzymes are essential for generating diverse diterpene hydrocarbon scaffolds. While some diTPSs also form oxygenated compounds, more commonly, oxygenation is achieved by cytochrome P450-dependent mono-oxygenases. Recent genome-, transcriptome-, and metabolome-guided gene discovery and enzyme characterization identified novel diTPS functions that form the core of complex modular pathway systems. Insights into diterpene metabolism may translate into the development of new bioengineered microbial and plant-based production systems.

Multipurpose metabolites: ecological and economic relevance of plant diterpenoids

Plants produce many different, predominantly polycyclic, 20-carbon (C_{20}) isoprenoids (see Glossary), collectively termed diterpenoids. Some diterpenoids occur broadly across the plant kingdom and play key roles in general (i.e., primary) metabolism, growth, and development of plants, such as gibberellin (GA) phytohormones and the phytol side chain of chlorophyll. However, most diterpenoids are specialized (i.e., secondary) metabolites that are of limited taxonomic distribution and often represent signature molecules of certain plant species or families [1,2]. Biosynthesis or accumulation of specialized diterpenoids can further be limited to specific cell types, tissues, or organs, and may be regulated in response to environmental perturbations. In an ecological context, some specialized diterpenoids have been shown to confer resistance against pests or pathogens: for example, diterpene phytoalexins in rice (*Oryza sativa*) and maize (*Zea mays*) [3], diterpene resin acids (DRAs) as major components of the oleoresin defense system of conifer trees (Pinaceae) [4,5], 17-hydroxygeranyllinalool glycosides mediating anti-herbivore defense of wild tobacco [6,7], and the below-ground defense metabolite rhizathalene in *Arabidopsis thaliana* [8] (Figure 1).

Evidence of human appreciation of plant diterpenoids dates back to the Neolithic age, where amber (i.e., fossilized oleoresin) was used as jewelry. Throughout history, diterpenoid-producing plants and their extracts served as ceremonial incense, traditional medicines, fragrances, flavors and biomaterials, leading up to today's wide range of industrial applications worth several billion dollars annually. Major commercial diterpenoids include the anti-cancer drug taxol (paclitaxel) from pacific yew (*Taxus brevifolia*) [9], sclareol from clary sage (*Salvia sclarea*) used as a precursor for ambroxide fragrance and fixatives in perfume manufacture [10,11], the natural sweetener steviol from *Stevia rebaudiana* [12], and conifer DRAs as large-scale feedstock for industrial coatings and inks [13,14] (Figure 1).

Glossary

CPP: copalyl diphosphate; is the product of class II diTPS via conversion of GGPP and serves as key intermediate in diterpenoid biosynthesis.
diTPS: diterpene synthase; form various diterpene scaffolds as the first committed reaction in diterpenoid biosynthesis.
DMAPP: dimethylallyl diphosphate; together with IPP forms the central intermediate in isoprenoid biosynthesis.
DRA: diterpene resin acid; formed as an oleoresin in conifers that function as defense metabolites against insect pests and pathogens.
DXS: 1-deoxy-D-xylulose 5-phosphate synthase; is a key enzyme of the MEP pathway.
GA: gibberellic acid; serves as phytohormone with essential functions in plant growth and development.
GGPP: geranylgeranyl diphosphate; formed through sequential condensation of IPP and DMAPP molecules and represents the common precursor for diterpenoids.
IPP: isopentenyl diphosphate; together with DMAPP forms the central intermediate in isoprenoid biosynthesis.
Isoprenoid: isoprenoids comprise a group of organic compounds characteristically composed of two or more five-carbon isoprene (C_5H_8) units that are derived from the MVA or MEP pathways.
LPP: labda-13-en-8-ol diphosphate (also termed copal-8-ol diphosphate); is an isomer of CPP that contains a hydroxy group at carbon 8.
NNPP: nerylneryl diphosphate; represents the *cisoid* isomer of GGPP.
P450: cytochrome P450-dependent mono-oxygenase; catalyzes functional modifications of diterpene scaffolds, such as oxygenation and carboxylation reactions.
Plasticity: defined as the capability of an enzyme to undergo functional change with only a few amino acid substitutions.
TXS: taxadiene synthase; is the diterpene synthase forming the central intermediate in the biosynthesis of the anti-cancer agent taxol (paclitaxel).

Corresponding author: Bohlmann, J. (bohlmann@mail.ubc.ca).
Keywords: diterpene synthase; plant chemical diversity; synthetic biology; metabolic engineering; natural product biosynthesis.

0167-7799/
 http://dx.doi.org/10.1016/j.tibtech.2015.04.006

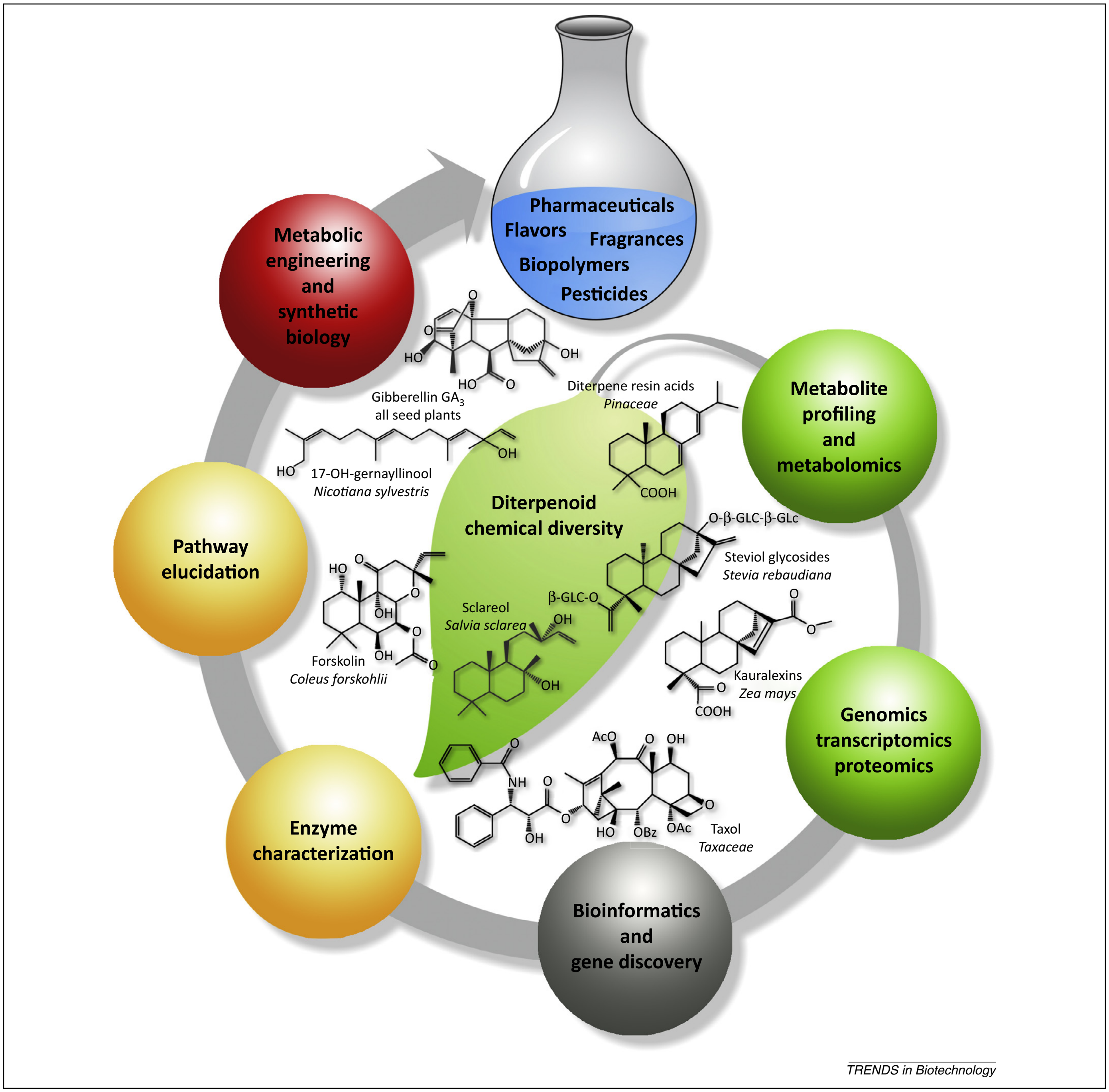

Figure 1. Discovery and application of genes and enzymes of plant diterpenoid metabolism for high-value bioproducts. Plants produce several thousand different diterpenoid metabolites; several of which are used by humans as high-value pharmaceuticals, fragrances, food additives, and precursors for various chemicals and bioproducts. Multidisciplinary systems biology strategies offer new avenues to explore and harness this natural resource. Tissue-specific identification of known and novel compounds through metabolite profiling or metabolomics inform genomics-based discovery and elucidation of diterpenoid-biosynthetic genes, enzymes and pathways. Drawing on the expanding portfolio of modular enzymes and pathways, combinatorial plant and microbial metabolic engineering and synthetic biology approaches can be developed for plant-based bioproducts.

With our increasing need for food, pharmaceuticals, and renewable fuels, plant natural products and biotechnological means for their production, including synthetic biology approaches, have come back to the forefront of a growing attention. A few diterpenoids, such as conifer oleoresin diterpenoids, can be obtained in large quantities directly from plant sources. However, access and availability of some high-value diterpenoids (e.g., taxol) may be limited by low abundance in nature, lack of cultivation or geographic access, and risk of overharvesting of undomesticated plant species. Also, plants typically produce complex diterpenoid mixtures, which can create challenges for isolation of individual target compounds.

Alternative production systems, such as total or semi-synthesis (e.g., (+)-ingenol, the core precursor of the anti-cancer drug ingenol mebutate [15]) and plant cell cultures (e.g., taxol [16]), are being used for commercial diterpenoid production. However, these approaches may not be applicable for many diterpenoid natural products. Beyond plants serving as a rich resource for biologically active