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Review

Phytochemical genomics of the Madagascar periwinkle: Unravelling the last twists of the alkaloid engine

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ABSTRACT

The Madagascar periwinkle produces a large palette of Monoterpenoid Indole Alkaloids (MIAs), a class of complex alkaloids including some of the most valuable plant natural products with precious therapeutical values. Evolutionary pressure on one of the hotspots of biodiversity has obviously turned this endemic Malagasy plant into an innovative alkaloid engine. *Catharanthus* is a unique taxon producing vinblastine and vincristine, heterodimeric MIAs with complex stereochemistry, and also manufactures more than 100 different MIAs, some shared with the Apocynaceae, Loganiaceae and Rubiaceae members. For over 60 years, the quest for these powerful anticancer drugs has inspired biologists, chemists, and pharmacists to unravel the chemistry, biochemistry, therapeutic activity, cell and molecular biology of *Catharanthus roseus*. Recently, the "omics" technologies have fuelled rapid progress in deciphering the last secret of strictosidine biosynthesis, the central precursor opening biosynthetic routes to several thousand MIA compounds. Dedicated *C. roseus* transcriptome, proteome and metabolome databases, comprising organ-, tissue- and cell-specific libraries, and other phytogenomic resources, were developed for instance by PhytoMetaSyn, Medicinal Plant Genomic Resources and SmartCell consortium. Tissue specific library screening, orthology comparison in species with or without MIA-biochemical engines, clustering of gene expression profiles together with various functional validation strategies, largely contributed to enrich the toolbox for plant synthetic biology and metabolic engineering of MIA biosynthesis.

Contents

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1. Introduction

The antiarrhythmic drug ajmaline (**1**), the peripheral vasodilators and antihypertensives vincamine (**2**), ajmalicine (**3**) and reserpine (**4**), the pro-erectile yohimbine (**5**) and the highly valuable anticancer drugs vinblastine (**6**) and vincristine (**7**) are prominent examples of numerous pharmacophores present in the Monoterpenoid Indole Alkaloids (MIAs) (see Fig. 1 for examples of specific alkaloids). This large class of alkaloids, with at least 2500 compounds identified, is remarkably restricted in plant kingdom to the Gentianale order families Apocynaceae, Loganiaceae and Rubiaceae. Following the discovery of vinblastine (**6**) and vincristine (**7**) in the late 1950s by the Noble research group in Toronto and the Eli Lilly Pharmaceutical company in Indianapolis; *Catharanthus roseus* (*C. roseus*), a Madagascar endemic plant, has remained the sole source of these drugs (Duffin, 2000; Svoboda and Blake, 1975). However, vinblastine (**6**) and vincristine (**7**) are some of the least abundant (5 and 0.5 ppm, respectively) bioactive natural products extracted from plant material and among the most expensive. Therefore, a tremendous effort has been invested in the search for alternative production platforms (cell and root cultures) and in the understanding of the biochemistry and cell biology of *C. roseus* alkaloid biosynthesis (St-Pierre et al., 2013; Shukla and Khanuja, 2013).

MIAs are complex alkaloids generated from an amino acid-derived amine coupled to an extensively modified monoterpenoid moiety (Fig. 1). The indole moiety of MIAs is derived from the shikimate (**8**) pathway derived amino acid tryptophan (**9**). Allocation of this skeleton from primary to specialized metabolism requires a single reaction catalyzed by tryptophan decarboxylase (TDC) to produce tryptamine (**10**) (De Luca et al., 1989).

The assembly of the monoterpene seco-iridoid moiety, however, requires several reactions to convert the methyl-erythritol phosphate (MEP) pathway-derived monoterpenoid skeleton into secologanin (**11**) (Fig. 1) (Oudin et al., 2007a). Recently, in *C. roseus*, and for the first time in the plant kingdom, reverse genetics strategies have allowed the discovery of the elusive reaction schemes of secologanin (**11**) biosynthesis, which had been only partially described by previous forward genetics approaches. This achievement will have a wide impact since, in addition to being required for MIA biosynthesis, secologanin (**11**) is also an important antiherbivore metabolite produced in a broad range of plants. In the plastid-localized MEP pathway, glyceraldehyde 3-phosphate (GAP, **12**) and pyruvate (**13**) are converted into the universal isoprenoid precursors, isopentenyl diphosphate (IPP, **14**) and dimethylallyl diphosphate (DMAPP, **15**), by the action of seven enzymes, 1-deoxy-D-xylulose 5-phosphate synthase (DXS; Chahed et al., 2000), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR; Veau et al., 2000), 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (CMS; our own unpublished data for the *C. roseus* enzyme), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (CMK; our own unpublished data for the *C. roseus* enzyme), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MECS; Veau et al., 2000), 1-hydroxy-2-methyl-2-butenyl 4-diphosphate synthase (HDS; Oudin et al., 2007b) and 1-hydroxy-2-methyl-2-butenyl 4-diphosphate reductase (HDR; our own unpublished data for the *C. roseus* enzyme). In addition, IPP isomerase (IDI) catalyzes interconversion of the isomers IPP (**14**) and DMAPP (**15**) according to the requirements of different tissues and organs for isoprenoid biosynthesis (Guirimand et al., 2012). Entry of these primary metabolites into iridoid biosynthesis first requires the prenyl-transfer of IPP (**14**)

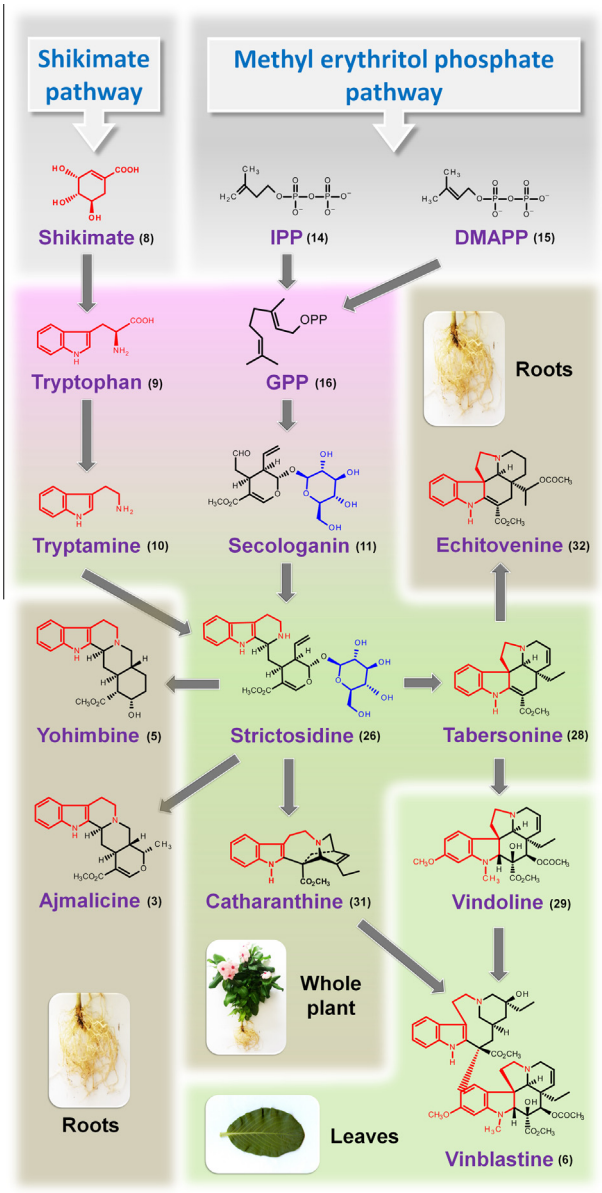

Fig. 1. Overview of the MIA biosynthetic pathway highlighting the preferential distribution of MIAs within *Catharanthus roseus*.

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