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**The Madagascar periwinkle anticancer drugs: a happy end for a 60 year’s story**

*Abstract: The Madagascar periwinkle is the only source of the anticancer compounds Vinblastine and Vincristine. While their production is tributary of the plant resource, elucidation of the biosynthetic pathway has been engaged several years ago to initiate heterologous production. This lengthy quest has been complete with our work describing the last enzymes of the synthesis of these valuables compounds.*

*Suggested Picture:*

From the analgesic morphine produced by Opium poppy to the antiasthma ephedrine extracted from Ephedra spp, the bioactive compounds produced by plants are countless. The capacity of plants to architect and decorate molecules goes beyond our own imagination. One of those remarkable plants is the Madagascar periwinkle, source of vinblastine and vincristine, two alkaloids that constitute the first natural drugs used in chemotherapy, with special emphases on the most severe types of cancer such as Hodgkin’s lymphoma, lung and brain cancer. Up to now, the leaves of Madagascar periwinkle are the exclusive font of these compounds while the low abundance, estimated 1 g of vinblastine extracted from 5 tons of leaves, make us totally dependent on the natural sourcing.

The recent last developments in synthetic biology allow us to consider other “biological factories” for the production of these valuables molecules, such as genetically modified yeast or bacteria. For instance, the anti-malaria artemisinin, previously extracted from *Artemisia annua* can now be obtained through the synthesis of its precursor artemisinic acid by both types of engineered microbes. Conferring to other organisms the capacity to produce compounds of interest on demand relies on the transfer of the *ad hoc* producing machineries. Such creation of cellular factories thus requires deciphering how plants produce the valuable molecules and unravelling the corresponding biosynthetic pathways. This is where the periwinkle story begins, 60 years ago, with the discovery of vinblastine and vincristine followed by identification of the first genes involved in their synthesis, up to our work (Caputi et al., 2018 Science 360:1235-1239), revealing the missing enzymes catalysing the ultimate bioconversion steps of the last known biosynthetic intermediate named stemmadenine acetate into the valuable alkaloids. Elucidating the entire synthetic route of tabersonine and catharanthine, the precursors of the two aforementioned anticancer alkaloids revealed to be a staggering task, since it encompasses no less of 31 enzymatic steps whose identification gathered the efforts of several scientific teams over decades. The complex distribution of the pathway *in planta*, passing through different organelles and distinct cell types probably explained the difficulty to discover all these biosynthetic steps. However, a major breakthrough in enzyme identification was accomplished with OMIC’s approaches that provide access to the sequences of all the genes simultaneously expressed in the different cells of plants. Considering that genes displaying a similar expression profile are potentially involved in the same physiological processes, we used previously characterized genes involved in alkaloid production, as bait to select candidate genes encoding the missing biosynthetic enzymes. Using this strategy combined to biochemical assays, we identified the genes encoding two enzymes able to successively convert the biosynthetic intermediate stemmadenine acetate into dihydroprecondylocarpine acetate (PAS and DPAS) and the genes encoding the sister enzymes catalysing the final step of catharanthine and tabersonine synthesis, namely catharanthine synthase (CS) and tabersonine synthase (TS).

To establish the function of the newly identified enzymes, we took advantage of the capacity to diminish their abundance in periwinkle by a technique called virus-induced gene silencing (VIGS). This approach uses virus-derived tools to specifically block the expression of a dedicated gene, encoding CS or TS for example, during several weeks. In the CS and TS-silenced plants, we thus monitored a huge decrease of catharanthine and tabersonine amounts respectively, confirming that these enzymes were required to alkaloid synthesis. Definitive evidences of their functions were obtained by recreating this part of the biosynthetic pathway in *Nicotiana benthamiana* conferring to this plant the capacity to convert stemmadenine acetate, into tabersonine or catharanthine, and thus paving the way for future complete pathway reconstitution.

By identifying the missing enzymes of the vinblastine and vincristine precursor synthesis, this work - conducted in close collaboration between researchers from the John Innes Centre (UK) and University of Tours (France) - not only signs off the periwinkle alkaloid story but also opens new opportunities to produce anticancer alkaloids independently from the plants. By gathering all biosynthetic enzymes in yeast, the synthesis of catharanthine and vindoline (directly derived from tabersonine) will be possible soon and, following chemical condensation, will ensure the supply of vinblastine and vincristine independently from the periwinkle. Such wedding between plants and yeasts can result in a “green sourcing” of alkaloids, an approach of importance in the general context of biological resource depletion.