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Creating plant molecular factories for industrial and nutritional isoprenoid production

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Chemical refining is a highly efficient process that has driven industrialisation and globalisation. However, dwindling fuel reserves and climatic fluctuation are now imposing key societal and economic challenges to health and welfare provision, agriculture, manufacturing outputs and energy. Plants are potentially exploitable 'green' chemical factories, with vast chemical diversity that can be used for the discovery and production of food, feed, medicines and biomaterials. Despite notable advances, plant based production under real-life scenarios remains, in most cases, economically uncompetitive when compared to inherently non-sustainable petrochemical based processes. In the present review the strategies available and those emerging will be described. Furthermore, how can the new evolving molecular tools such as genome editing be utilised to create a new paradigm of plant-based production? To illustrate the present *status quo*, we have chosen the isoprenoids as the class of natural products. These compounds display vast chemical diversity and have been used across multiple industrial sectors as medicines, supplements in food and feedstuffs, colourants and fragrances.

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Introduction

Isoprenoids, also known as terpenoids represent the largest and oldest class of natural products documented, consisting of >40 000 different molecules [1]. Biosynthetically all isoprenoids are related via a common five carbon building block (isopentenyl pyrophosphate; IPP). Commercially isoprenoids are used in cosmetics and fragrances, and as colourants and nutritional supplements in foods and feeds. Additionally, numerous isoprenoids have important medicinal properties, such as the anticancer agents Taxol and the isoprenoid-derived indole alkaloid vincristine and vinblastine [2]. Their industrial relevance also means they are compounds of high value with global markets in the range of $1 billion per annum (www.researchandmarkets.com). A contributing factor to the high cost of these molecules resides in the fact that they are mainly produced in low yields by slow growing plant species that are not readily amenable to agricultural production. It is therefore not surprising that total or semi-synthetic chemical synthesis is presently the method of choice for obtaining many of these isoprenoid molecules. However, their structural complexity makes chemical synthesis expensive, difficult and prone to the formation of non-biological stereoisomers and contamination with reaction intermediates. There is also a significant environmental impact as the precursors used are typically derived from the chemical refining of fossil fuels [3].

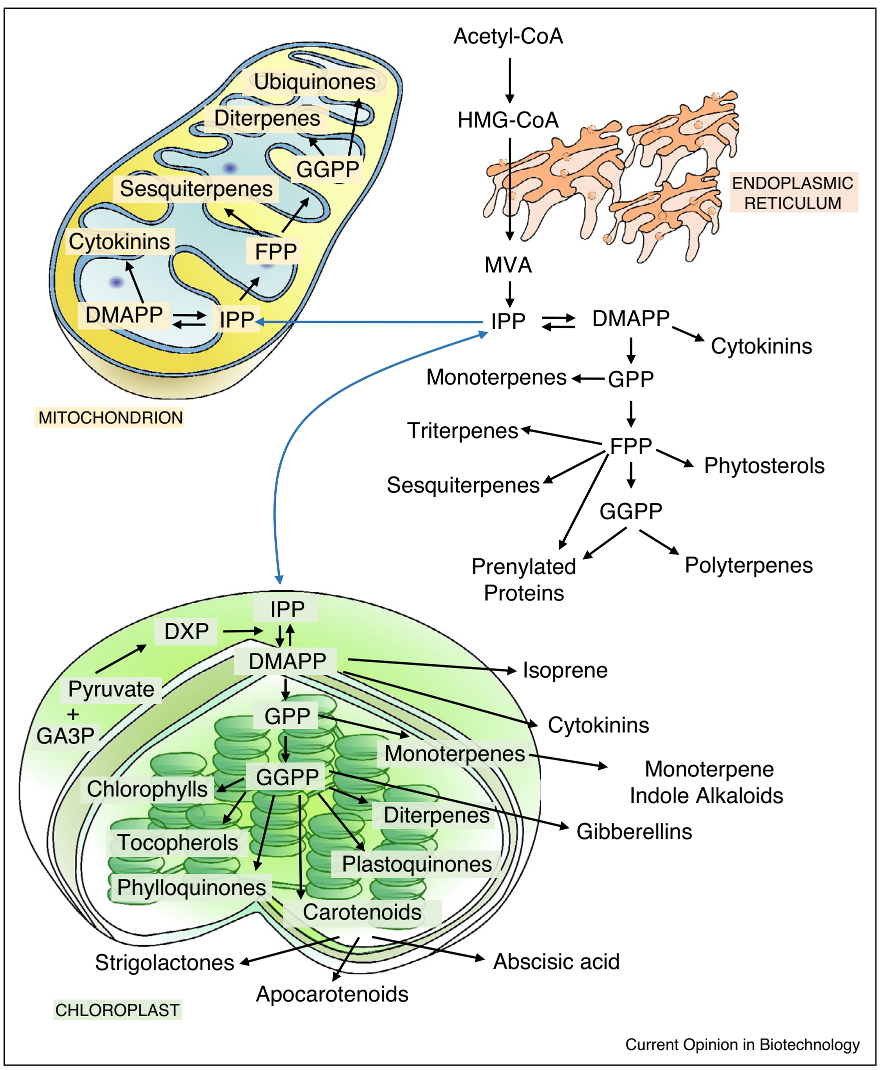
In addition to their value as speciality or bulk chemicals, isoprenoids such as carotenoids (provitamin A) and tocopherols (vitamin E) are essential components of the human diet. Moreover, nutritionally enhanced foods are advocated by most national governments [4]. Therefore, enhancement in crop plants such as rice, maize and tomato to confer improved nutritional and consumer quality traits is an area of intense research and development.

The formation of isoprenoids is a complex process in plants, that is compartmentalised and under developmental regulation. The biosynthetic pathway(s) can be divided into first, the formation of the IPP; second, the formation of prenyl phosphates and third, biosynthesis of isoprenoid subgroups. Since the discovery of the mevalonic acid (MVA) pathway in the 1950s, it was assumed that IPP was synthesised from acetyl-CoA via MVA. However, experimental data consistently indicated that the MVA pathway could not account exclusively for the formation of plastid-derived isoprenoids. In the early 1990s, retro-biosynthetic labelling conclusively established the presence of an alternative MVA-independent pathway for the formation of IPP and dimethylallyl pyrophosphate (DMAPP), termed the methyl D-erythritol phosphate pathway (MEP) [5].

Prenyl phosphates represent the central backbone of the isoprenoid pathway from which all isoprenoid sub-groups are derived. The five carbon substrates used are IPP and DMAPP. These molecules lead to the formation of longer chain prenyl lipids such as geranyl pyrophosphate (GPP, C_{10}), farnesyl pyrophosphate (FPP, C_{15}), geranylgeranyl pyrophosphate (GGPP, C_{20}), octaprenyl pyrophosphate (C_{40}) and solanesyl pyrophosphate (C_{45}). GPP, FPP and GGPP are the main products synthesised in plants. The presence and action of GPP, FPP and GGPP synthases is key to the prenyl lipids produced and thus the formation of independent branches of isoprenoid biosynthesis that utilise these prenyl precursors (Figure 1). A vast array of isoprenoid subgroups exist, these include monoterpenes (C_{10} derived) such as geraniol, sesquiterpenes (C_{15} derived), which includes artemisinin, diterpenes (C_{20} derived) such as taxanes, triterpenes (C_{30} derived), for example, phytosterols and carotenoids (C_{40} derived) such as β-carotene.

Following the elucidation of rudimental knowledge of isoprenoid formation, the last decades have seen an explosion in our ability to implement genetic intervention (metabolic engineering and Marker Assisted Selection, MAS) to alter isoprenoid levels in plants. The following sections will provide notable examples of rational strategies that have been used to achieve successful production of certain isoprenoids or which provide exploitable fundamental knowledge that could lead to future advances. An attempt will be made to judge the levels

Figure 1



Overview of isoprenoid biosynthesis and compartmentalisation in plant cell.
Black arrows indicate either a single or multiple enzymatic steps. Red arrows correspond to cross-membrane transport. HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MVA, mevalonate; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; GA3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose.