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The effect of an enhanced isopentenyl monophosphate pool on terpenoid biosynthesis *in vivo*

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ABSTRACT

Found in all living organisms, terpenoids make up the largest group of natural products and are essential compounds for many major processes, including photosynthesis, respiration, hormone production, and electron transport. Additionally, they have commercial and medical value in products including fragrances, cosmetics, and medicines. Terpenoids originate from the five-carbon building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are synthesized by the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways. An alternative MVA pathway was discovered in Archaea with the final two enzymes being phosphomevalonate decarboxylase (MPD) and isopentenyl phosphate kinase (IPK). Even though this alternative pathway is not present in plants, presence of IPK was retained. The overexpression of IPK in plants indicates that IPK plays a significant role in the MVA pathway by synthesizing IPP/DMAPP from an IP/DMAP pool for terpenoid biosynthesis. It has been suggested that this monophosphate pool regulates downstream carbon flux by inhibiting farnesyl diphosphate synthase (FPPS). By utilizing MPD from the archaeobacterium *Roseiflexus castenholzii*, we can see how an increased isopentenyl (IP) pool affects downstream terpenoid biosynthesis. To do this, RcMPD was overexpressed in the background of *Arabidopsis thaliana* T-DNA insertion lines of a knockdown of IPK. These lines were tested for expression of MPD/IPK using qRT-PCR and terpenoids were analyzed via sterol extraction and scent collection. Levels of monoterpenes (MEP pathway products) and sesquiterpenes (MVA pathway products) were significantly reduced, suggesting that a larger monophosphate pool reduces downstream synthesis of farnesyl diphosphate, a precursor for sterol and sesquiterpene biosynthesis.

KEYWORDS

Plant terpenoids, prenyltransferases, isopentenyl phosphate, metabolic engineering, mevalonate phosphate decarboxylase