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The small subunit of geranyl diphosphate synthase: a tool to improve aroma and flavour by metabolic engineering

Monoterpenes represent a major class of volatile terpenes produced by plants and play important roles in pollination, defense and seed dispersal (Dudareva *et al.* 2006). Also, plant-derived terpenoids, including monoterpenes are used as natural flavour and aroma compounds, and have beneficial impact on humans as health promoting compounds (Wagner and Elmadfa 2003). All monoterpenes are derived from the common precursor, geranyl diphosphate (GPP, C_{10}), which is synthesized by a head-to-tail condensation of one molecule each of isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP) in a reaction catalyzed by GPP synthase (GPPS, EC 2.5.1.1) (figure 1) (Ogura and Koyama 1998).

GPPSs have been isolated and functionally characterized in a limited number of plant species and are known to exist as homodimeric and heterodimeric structures. Heterodimeric GPPSs have been described in peppermint (*Mentha piperita*), snapdragon (*Antirrhinum majus*), Clarkia (*Clarkia breweri*) and hop (*Humulus lupulus*), which produce large amounts of monoterpenes in specific organs (Burke *et al.* 1999; Tholl *et al.* 2004; Wang and Dixon 2009). Structurally, the heterodimeric GPPS constitutes a small

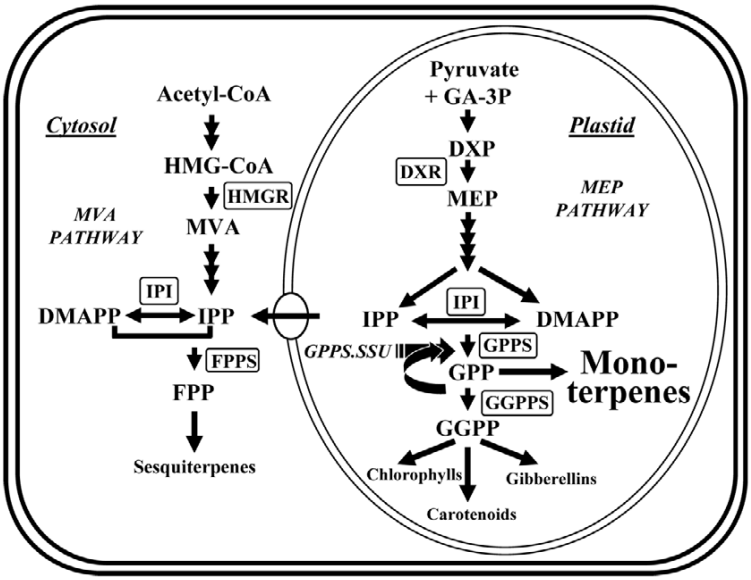

Figure 1. Overall effects of *GPPS.SSU* over-expression on the levels of monoterpenes, sesquiterpenes and GGPP derived products in tobacco plants.
DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, DXP reductoisomerase; FPP, farnesyl diphosphate; FPPS, FPP synthase; GA-3P, glyceraldehyde-3-phosphate; GGPP, geranyl geranyl diphosphate; GGPPS, GGPP synthase; GPP, geranyl diphosphate; GPPS, GPP synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, HMG-CoA reductase; IPI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MVA, mevalonic acid. Names of enzymes are boxed. Bigger and smaller font size of end products indicates increase and decrease in the content, respectively.

Keywords. Geranyl diphosphate synthase; metabolic engineering; monoterpenes

subunit (SSU) and a large subunit (LSU). The SSU by itself is catalytically inactive whereas the LSU alone could be either inactive (Burke *et al.,* 1999), or function as geranylgeranyl diphosphate synthase (GGPPS) on its own (Tholl *et al.* 2004; Wang and Dixon 2009) and only the interaction between the two subunits leading to the formation of a heterodimer results in an active GPPS. It has been reported that in both snapdragon and hop, the spatial and temporal expression of *GPPS.SSU*, but not *GPPS.LSU*, correlated with monoterpene emission, suggesting that SSU plays an important role in regulating the GPP formation and thus monoterpene biosynthesis (Tholl *et al.* 2004; Wang and Dixon 2009). In spite of the importance of GPPS in monoterpene biosynthesis, not much is known on the function of GPPS enzymes *in planta* and the mechanisms that regulate the flux to GPP.

A recent publication in the *Plant Cell* by Orlova *et al.* (2009) addresses how the catalytically inactive snapdragon GPPS.SSU when overexpressed in phylogenetically distant tobacco, finds an interacting partner *in planta* to form active chimeric GPPS and thus increases monoterpene emission. The authors show how overexpression of snapdragon SSU in tobacco modifies the chain length specificity of phylogenetically distant tobacco GGPPSs *in planta* to produce GPP for monoterpene formation, providing the first genetic evidence that the formation of GPPS activity and the control of GPP flux to monoterpene biosynthesis is accomplished by the regulation of GPPS.SSU gene expression and the recruitment of *bona fide* GGPPS proteins. In this work, the authors generated transgenic tobacco plants overexpressing snapdragon *GPPS.SSU* under the control of flower specific *C. breweri* linalool synthase (*LIS*) promoter (Cseke *et al.* 1998) in order to restrict the expression of GPPS.SSU to flowers and thus to avoid any deleterious effects on plant growth and development. Analysis of transgenic plants for transgene expression revealed that the mRNA and protein were expressed in both flowers and leaves despite the use of flower-specific promoter. The transgenic plants showed strong chlorosis and a reduction in stature. Analysis of the effect of snapdragon GPPS.SSU overexpression on the level of terpenoid emission showed a large increase in emitted monoterpenes (figure 1) in both leaves and flowers, suggesting that the endogenous level of the substrate GPP available to monoterpene synthases is limiting in control plants. Leaves of GPPS.SSU transgenic plants showed a marked increase in (*E*)-*β*-ocimene emission than controls and started emitting a new monoterpene myrcene, which was absent in control plants. Flowers of transgenic plants produced significant amount of (*E*)-*β*-ocimene, which was not detected in control plants. However there was no significant change in the level of linalool emission both in leaves and flowers, indicating that linalool could be formed in the cytosol by a bifunctional monoterpene/sesquiteprene synthase (Aharoni *et al.,* 2004; Nagegowda *et al.,* 2008). The increase in monoterpene production was accompanied by a decrease in the total emission of sesquiterpene compounds (figure 1), suggesting the presence of metabolic crosstalk between the plastidic methyl-erythritol-phosphate (MEP) pathway and cytosolic mevalonic acid (MVA) pathway in tobacco and that the introduced GPPS.SSU in transgenic plants increases the flux towards GPP formation in plastids thereby reducing the IPP pool and its transport to the cytosol resulting in reduced sesquiterpene formation. Also, the overexpression of GPPS.SSU in tobacco plants resulted in reduced levels of GGPP-derived metabolites including chlorophyll, carotenoids and gibberillic acids (GAs) (figure 1) leading to leaf chlorosis and stunted growth. Analysis of GGPPS activities and GGPP-derived metabolites in transgenic and control plants suggested that the limitation of IPP due to flux redirection toward GPP formation likely plays a major role in the observed effects on GGPP-derived metabolites.

Further, to identify the endogenous tobacco partner(s) for snapdragon GPPS.SSU, the authors searched the publicly available tobacco EST database for sequences with similarity to snapdragon GPPS.LSU, which revealed four potential GGPPS like sequences. Using bimolecular fluorescence complementation, it was shown that of the four candidates, the two, designated as NtGGPPS1 and NtGGPPS2 having highest similarity to snapdragon GPPS.LSU, interacted with snapdragon GPPS.SSU. The biochemical characterization indicated that the *NtGGPPS1* and *NtGGPPS2* encode bona fide GGPPS proteins, but when co-expressed with SSU, they function as GPPS. Both NtGGPPS1 and NtGGPPS2 contain one conserved CxxxC motif that was shown to be critical for interaction with the small subunit (Wang and Dixon 2009).

In summary, the paper by Orlova *et al*. (2009) provides new information about the role of GPPS.SSU in monoterpene biosynthesis in plants. It is evident that by overexpressing GPPS.SSU, one can increase the total monoterpene biosynthesis, provided the negative effects caused by the overexpression of SSU are addressed. This knowledge paves a new way for metabolic engineering of flower scent and fruit

aroma, and also to increase the GPP derived compounds of medicinal importance (e.g. monoterpenoid indole alkaloids) in medicinal and aromatic plants. Therefore by simultaneous overexpression of SSU and a monoterpene synthase of interest in transgenic plants, it could be possible to obtain a desirable scent/aroma profile. The negative effects such as chlorosis and stunted growth can be overcome by using a stringent tissue specific or trichome specific promoter.

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