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Metabolic engineering for prenylated flavonoids in transgenic plants using bacterial and plant prenyltransferases

(Laboratory of Plant Gene Expression, RISH, Kyoto University)

Akifumi Sugiyama, Kojiro Takanashi and Kazufumi Yazaki

Plants produce a large number of secondary metabolites that do not appear to be relevant for primary biological activity. Among these secondary metabolites, polyphenols are common to all plant species and are known as anti-oxidants and UV protectants. Polyphenols modified with prenyl residues have a variety of biological activities, such as anti-tumor, anti-bacterial, anti-virus, anti-oxidant, anti-tyrosinase and estrogenic. Although prenylated polyphenols are attractive natural products because of their various biological activities, their limited occurrence in plants as well as the difficulty of purification from complicated mixtures has limited the potential applications of these compounds for many years; therefore, metabolic engineering with prenyltransferase genes has been of particular interest. The identification of prenyltransferase genes from Streptomyces and S. flavescens enabled us to synthesize prenylated polyphenols via metabolic engineering. In this study, Streptomyces genes (NphB, SCO7190, and NovQ) and S. flavescens genes (N8DT and G6DT) were used for the targeted production of prenylated polyphenols. We modified the subcellular localization of prenyltransferases to express them in the cytosol, plastids, and mitochondria for soluble-type prenyltransferases from Streptomyces (NphB, SCO7190, and NovQ). A total of 624 transgenic L. japonicus were generated and analyzed for the production of prenylated polyphenols, and the effect of modification of the subcellular compartmentation on the production of prenylated polyphenols was evaluated.

Leaves of transgenic plants were extracted with methanol for chemical analysis using liquid chromatography/mass spectrometry (LC/MS). Unfortunately, none of the transgenic lines accumulated a detectable level of prenylated polyphenol in the leaves. We then supplemented naringenin (100 μM) or genistein (100 μM) to transgenic leaves and incubated for 24 hours before extraction. 7-O-geranylgenistein was detected in transformants with plastid-localized NphB, but not detected in transformants with cytosol-localized or mitochondria-localized NphB. Both 6-dimethylallylnaringenin and 6-dimethylallylgenistein were detected in plastid-localized SCO7190 transformants; however, these products were not detected in transformants with cytosol-localized and mitochondria-localized SCO7190. Following incubation with 100 μM naringenin and genistein, N8DT transformants produced 8-dimethylallylnaringenin, the direct enzyme reaction product, and G6DT transformants produced 6-dimethylallylgenistein. The overexpression of prenyltransferases in plants yielded only low levels of prenylated polyphenols even after supplementation of the substrate. For in vivo prenylation, plastids are the most suitable subcellular compartment for prenyltransferases, both for soluble and membrane bound types. At the same time the problems to be solved are also pointed out, e.g. higher supply of both substrates (i.e. DMAPP and polyphenols). One new strategy will be plastid transformation for the higher production of prenylated polyphenols.

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References