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Metabolic flux analysis of diterpene biosynthesis pathway in rice

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Phytoalexins are low-molecular-weight compounds produced as a plant defence system. Fourteen diterpenes have been identified as phytoalexins from rice (*Oryza sativa* L.) and grouped into four structurally distinct types, momilactones A and B, oryzalexins A-F, oryzalexin S, and phytocassanes A-E. Two distinct families of diterpene cyclases are involved in this conversion of geranylgeranyl pyrophosphate (GGPP) to their hydrocarbon precursors. The first cyclization reaction producing *ent*- or *syn*-copalyl pyrophosphate (CPP) is initiated by protonation of GGPP (CPS-type) and the second cyclization reaction by elimination of the pyrophosphate group (KS-type). Using rice genome information and *in vitro* assay with recombinant enzymes, 2 CPS-type and 4 KS-type diterpene cyclases have been identified and characterized for the biosynthesis of rice diterpenoid phytoalexins. For gibberellins biosynthetic pathway, *ent*-copalyl diphosphate synthase and *ent*-kaurene synthase are encoded by single genes. In this work, competition between two or three enzymes using the same substrate was investigated at the branch points of isoprenoid biosynthetic pathway. Relative expression levels of 8 diterpene cyclase genes using reverse transcription quantitative PCR technique (RT Q-PCR) were modified to present the metabolism flux changes in UV-irradiated rice. The pool of GGPP is supposed to be distributed according to the relative expression of *eCPS1* for gibberellin biosynthesis, and *eCPS2* and *sCPS* for phytoalexin biosynthesis, providing that the specific activity of each enzyme is equal. As the relative expression ratio between 9 beta-pimara-7,15-diene synthase (PMS) and stemar-13-ene synthase (STS) was 2:1, for example, in control, 67% of *syn*-CPP might be used as a substrate for PMS and the rest 33% for STS. Out of 24% of total GGPP pool used for *syn*-CPP formation, therefore, 16% was used for pimaradiene and 8% for stemarene intermediate. To our knowledge, this is the first report that RT Q-PCR was used for the metabolism flux analysis of plant, in stead of time-consuming metabolite measurements.

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