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# RNA interference targeting *CYP76AH1* in hairy roots of *Salvia miltiorrhiza* reveals its key role in the biosynthetic pathway of tanshinones

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## Abstract

Plant cytochrome P450s (CYPs) are well known as the largest family of enzymes that contribute to both primary metabolism and the chemical diversity of plant secondary metabolites. It is important to elucidate the *in vivo* role of CYPs in secondary metabolism, in order to apply them in the production of valuable metabolites in medicinal plants via metabolic engineering. *CYP76AH1* has been suggested to catalyze the conversion of the carbon skeleton miltiradiene into the intermediate ferruginol, which is involved in the biosynthesis of tanshinones, the chief bioactive ingredients of *Salvia miltiorrhiza*. However, its role *in planta* remains to be elucidated. In this work, we constructed a *CYP76AH1* RNAi system for hairy roots. Metabolic analysis of RNAi-AH1 hairy root lines showed a significantly increased accumulation of miltiradiene compared to the control lines. At the same time, the concentration of ferruginol decreased revealing the *in vivo* catalytic activity of

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