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Diverse responses of tanshinone biosynthesis to biotic and abiotic elicitors in hairy root cultures of *Salvia miltiorrhiza* and *Salvia castanea* Diels f. *tomentosa*

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Abstract *Salvia miltiorrhiza* (*S. miltiorrhiza*) and *Salvia castanea* Diels f. *tomentosa* (*S. castanea*) are both used for treatment of cardiovascular diseases. They have the same bioactive compound tanshinones, but whose contents are hugely different. This study illustrated diverse responses of tanshinone biosynthesis to yeast extract (YE) and Ag^+ in hairy roots of the two species. YE enhanced both the growth and tanshinone biosynthesis of two hairy roots, and contributed more to tanshinone accumulation in *S. castanea* than that in *S. miltiorrhiza*. Genes encoding 1-deoxy-D-xylulose 5-phosphate synthase (*DXS2*), geranylgeranyl diphosphatesynthase (*GGPPS1*), copalyl diphosphate synthase (*CPS1*), and two cytochromes P450 (*CYP76AH1* and *CYP76AH3*) were also more responsive to YE in *S. castanea* than those in *S. miltiorrhiza*. Accumulations of dihydrotanshinone I and tanshinone I, and most biosynthetic genes in *S. miltiorrhiza* were more responsive to Ag^+ than those in *S. castanea*. Accumulations of dihydrotanshinone I and cryptotanshinone were more responsive to YE, while tanshinone IIA accumulation was more responsive to Ag^+ in *S. miltiorrhiza*. However, accumulations of other four tanshinones and related genes in *S. castanea* were more responsive to YE than Ag^+. This study provides foundations for studying diverse specialized metabolism between the related species.

Abbreviations

AACT, acetyl-CoA C-acetyltransferase; CPS, copalyl diphosphate synthase; CT, cryptotanshinone; CYPs, cytochromes P450; DMAPP, dimethylallyl diphosphate; DT-I, dihydrotanshinone I; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DW, dry weight; GGPP, geranylgeranyl diphosphate; GGPPS, geranylgeranyl diphosphate synthase; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; HPLC, high performance liquid chromatography; KSL, *ent*-kaurene synthase like; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MVA, mevalonate; PDA, photodiode array detector; qRT-PCR, Real-time quantitative PCR; ROS, reactive oxygen species; *S. castanea*, *Salvia castanea* Diels f. t*omentosa* Stib; *S. miltiorrhiza*, *Salvia miltiorrhiza* Bunge; T-I, tanshinone I; T-IIA, tanshinone IIA; YE, yeast extract.

Keywords Yeast extract; Ag^+; tanshinone; gene expression