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The effect of transient HMG-CoA Reductase and 1-Deoxy-D-Xylulose-5-Phosphate Synthase overexpression on terpene production in transgenic tomato fruits

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ABSTRACT

Isoprenoids are secondary metabolites that control numerous plant functions including signaling, growth, photosynthesis, and membrane structure. The bioengineering of isoprenoid synthesis could produce plants with a variety of beneficial traits. Plants form isoprenoids using two different pathways, the mevalonate (MVA) pathway and the methylerithritol phosphate (MEP) pathway, which cooperate via metabolic cross-talk. Transgenic tomato lines expressing both the plastidic and cytosolic forms of the snapdragon nerolidol/linalool terpene synthase under a fruit ripening specific promoter were transiently transformed to overexpress key enzymes in the two isoprenoid pathways. Hydroxymethylglutaryl-coenzyme A reductase (HMGR) is the rate limiting enzyme in the MVA pathway that was selected for overexpression. 1-deoxy-D-xylulose-5-phosphate synthase (DXPS) in the plastid was targeted as it is the first committed step in the MEP pathway. HMGR and DXPS coding regions were cloned into binary vectors under a constitutive promoter and introduced into Agrobacterium tumefaciens which were then injected into ripening tomato fruits for transient expression. Additionally, untransformed fruits were incubated with either the MVA-inhibitor mevinolin or the MEP-inhibitor fosmidomycin. Terpene production was characterized by gas chromatography and mass spectrometery of fruit volatiles collected at the ripe stage. Inhibitor treatment is expected to decrease terpene synthesis in the same compartment as the inhibited pathway. The overexpression of early MVA and MEP pathway genes is expected to significantly increase the formation of terpenes.

KEYWORDS

HMGR, DXPS, mevalonate pathway, MVA pathway, non-mevalonate pathway, MEP pathway, terpene, isoprenoid

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