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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 methylerythritol 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 spectrometry 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

### REFERENCES

- Gutensohn, M., Orlova, I., Nguyen, T. T. H., Davidovich-Rikanati, R., Ferruzi, M. G., Sitrit, Y., Lewinsohn, E., Pichersky, E., & Dudareva, N. (2013) Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. *The Plant Journal*, 75(1), 351-363.
- Nagegowda, D. A., Gutensohn, M., Wilkerson, C. G., & Dudareva, N. (2008) Two nearly identical terpene synthases catalyze the formation of nerolidol and linalool in snapdragon flowers. *The Plant Journal*, 55(1), 224-239.
- Wu, S., Schalk, M., Clark, A., Miles, R. B., Coates, R., & Chappel, J. (2006) Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. *Nature Biotechnology*, 24(11), 1441-1447.
- Gutensohn, M., Nagegowda, D. A., & Dudareva, N. (2013) Involvement of compartmentalization in monoterpene and sesquiterpene biosynthesis in plants. *Isoprenoid Synthesis in Plants and Microorganisms: New Concepts and Experimental Approaches*, Bach, T.J., Rohmer, M. (eds.) Springer Science+Business Media, 2013 New York. (11), 155-169

- Davidovich-Rikanati, R., Lewinsohn, E., Bar, E., Iijima, Y., Pichersky, E., & Sitrit, Y. (2008) Overexpression of the lemon basil  $\alpha$ -zingiberene synthase gene increases both mono- and sesquiterpene contents in tomato fruit. *The Plant Journal*, 56(1), 228-238.
- Schnee, C., Kollner, T. G., Held, M., Turlings, T. C. J., Gershenzon, J., & Degenhardt, J. (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *The Proceedings of the National Academy of Science U S A*, 103(4), 1129-1134.
- Dudareva, N., Andersson, S., Orlova, I., Gatto, N., Reichelt, M., Rhodes, D., Boland, W., & Gershenzon, J. (2005) The nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. *The Proceedings of the National Academy of Science U S A*, 102(3), 933-938.
- Aharoni, A., Giri, A. P., Deurlein, S., Griepink, F., de Kogel, W.-J., Verstappen, F. W. A., Verhoeven, H. A., Jongsma, M. A., Schwab, W., & Bouwmeester, H. J. (2003) Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. *Plant Cell*, 15(1), 2866-2884.
- Enfissi, E. M.A., Fraser, P. D., Lois, L.-M., Boronat, A., Schuch, W., & Bramley, P. M. (2004) Metabolic engineering of the mevalonate and non-mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. *Plant Biotechnology Journal* 3(1), 17-27.