

**Title** Ethylene-regulated (methylsulfanyl)alkanoate ester biosynthesis is likely to be modulated by precursor availability in *Actinidia chinensis* genotypes

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### Abstract

The limiting steps of ethylene-dependent (methylsulfanyl)alkanoate ester biosynthesis have been investigated in this study, using closely related *Actinidia chinensis* genotypes and the commercial cultivar 'Hort16A'. Quantification of methylsulfanyl-compounds from the headspace of ethylene-producing kiwifruits revealed little variation in their volatile composition but remarkable differences in the magnitude of the fruit volatile levels. To test whether the variations in fruit volatile levels can be correlated with the genotype-specific apparent catalytic efficiency, the initial slope of the substrate response curve ( $V'_{\text{Max}}K_M^{-1}$  where  $V'_{\text{Max}}$  is the apparent  $V_{\text{Max}}$  in a crude extract) was evaluated for total alcohol acyltransferase (EC 2.3.1.84) activity. The  $V'_{\text{Max}}K_M^{-1}$  values of different (methylsulfanyl)alkyl-CoAs were in a similar range for most genotypes, which suggests substrate availability as the limiting factor for (methylsulfanyl)alkanoate ester synthesis in these kiwifruit. Furthermore, gene expression analysis of acyltransferase expressed sequence tags points towards the action of multiple isozymes for (methylsulfanyl)alkanoate ester synthesis, emphasizing the central role of substrate levels on final ester concentrations. Volatile levels of the potential precursor methional were increased in ethylene-producing *A. chinensis* kiwifruit and a close connection between (methylsulfanyl)alkanoate ester formation and ethylene synthesis in plants is proposed. Finally, a possible biosynthetic pathway is presented.