IDENTIFICATION AND FUNCTIONAL VALIDATION OF METHYL KETONE SYNTHASE 2 IN WOODLAND STRAWBERRY

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Abstract: Woodland strawberry (*Fragaria vesca*, 2x) is the diploid closest ancestor of the cultivated strawberry (*F.* × *ananassa*, 8x) and the model species for genetic studies in the Fragaria genus. It is naturally distributed across Europe and it is appreciated for its delicate aroma and flavor.

Methyl ketones are compounds with demonstrated insect repellent effects (Antonious *et al*. 2003). They are highly abundant in the glandular trichomes of wild tomato (*Solanum habrochaites*), where their pathway was first described, but not in the cultivated species (*S. lycopersicum*). Their synthesis derives from fatty acids in a two-step process mediated by a thioesterase (ShMKS2) and a decarboxylase (ShMKS1) (Ben-Israel *et al.* 2009, Yu *et al.* 2010).

Higher diversity and quantity of methyl ketones are present in the volatilome of woodland strawberry ripe fruits than in those of *F.* × *ananassa* (Ulrich *et al.* 2007, Ulrich and Olbrich 2013, Zorrilla-Fontanesi *et al*. 2012, Urrutia *et al.* 2017). The aim of this study is to reveal the genetic basis of methyl ketone production in strawberry fruit.

We quantified methyl ketones (2-heptanone, 2-nonanone, 2-undecanone), their secondary alcohols (2-heptanol, 2-nonanol, 2-undecanol) and the methyl esters of their fatty acid precursors (methyl octanoate, methyl decanoate, methyl dodecanoate) by GC-MS in a natural collection of European woodland strawberry, that comprises 199 accessions fully genotyped with >1.8 M SNPs representing the continental diversity. Conducting a Genome-Wide Association Study (GWAS), we identified a candidate region linked to methyl ketones accumulation harbouring three homologues of *ShMKS2*: *FvMKS2A*, *FvMKS2B* and *FvMKS2C*. Interestingly, *FvMKS2A*, which presented two alleles in the European collection (*FvMKS2A-1* and *FvMKS2A-2*), is the only *FvMKS2* paralog expressed in woodland strawberry fruit, being up-regulated during ripening.

Functional validation of all candidate genes and alleles by transient over-expression and silencing in both *Nicotiana benthamiana* leaves and *F. vesca* fruits has revealed that FvMKS2A and FvMKS2B, but not FvMKS2C, are capable of synthesizing methyl ketones, and point to a single SNP in FvMKS2A as responsible for the enzymatic substrate specificity, supporting FvMKS2A as the main MKS2 paralog responsible for methyl ketones in woodland strawberries.

Key words: Methyl ketone synthase 2 (MKS2), Woodland strawberry, Secondary metabolism, Volatiles.

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