

Analytical Platform 4 (cuticular wax profiling) was conducted by the Nikolau group at the W.M. Keck Metabolomics Research Laboratory. Fresh plant material (approximately 100 mg) was weighed immediately following harvesting. Prior to extraction 1 μ g of hexadecane (Sigma Chemical Co., St. Louis, MO, USA) was applied directly to leaf tissue as an internal standard. The plant material was completely immersed in HPLC-grade chloroform (Fisher Scientific C606-1) for 60 seconds. Upon removal of the plant material the chloroform was concentrated by evaporation and quantitatively transferred to a 1.5mL glass vial and dried under a stream of nitrogen gas. Samples were derivatized in 0.5mL HPLC-Grade acetonitrile (Fisher Scientific A998-4) with 35 μ L BSTFA+TCMS (Sigma 15239) at 60°C for 20 minutes. The derivatized sample is filtered with Iso-dis Filters (Supelco, Iso-dis filters PTFE 13mm•0.45 μ m Cat. #54131-U) and dried under nitrogen gas. The dried sample was reconstituted in 200 μ L of HPLC-grade chloroform and subjected to GC-MS as described previously (Dietrich et al., 2005)

GC-MS analyses were performed on an Agilent 6890N GC coupled to an Agilent 5973 inert MSD detector. Samples were loaded (injection volume 1 μ L) with an Agilent 7683 G2613A auto-sampler onto a HP-5MS fused silica column (30 m x 250 μ m; 0.25 μ m film thickness). The initial temperature of the injector and MSD interface were set at 275°C and 280°C, respectively. Metabolites were separated at a flow rate of 1.0 mL/min using He as carrier gas, and using a thermal gradient that started at 80°C (2 min), ramped first to 260°C at 4°C/min, held at this temperature for 10 min, and then ramped to 320°C at 5°C/min. Data were acquired using MSD Chemstation (D.00.01) from Agilent Technologies. Metabolites were identified based on their mass fragmentation patterns by comparison with those of authentic standards using the NIST (National Institute of Standards and Technology) Mass Spectral Search Program (Version D.05.00) and GLOSSY library (Fatland et al., 2002). Peak areas were obtained from the Total Ion Chromatogram (TIC), raw data were exported to Microsoft Excel and peak areas were normalized to fresh tissue mass and the internal standard.

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