

Formation of α -Farnesene in Tea (*Camellia sinensis*) Leaves Induced by Herbivore-Derived Wounding and Its Effect on Neighboring Tea Plants

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Supporting information

1. GC-MS analysis

We adopted a GC-MS QP2010 SE (Shimadzu Corporation, Kyoto, Japan) with utilizing GCMS Solution software (Version 2.72, Shimadzu Corporation, Japan) for GC-MS analysis. The column was SUPELCOWAX 10 column (30 m×0.25 mm×0.25 μm, Supelco Inc., Bellefonte, PA, USA). When injecting samples into the GC injection port, here we applied a splitless mode with holding at 230 °C for 1 min. The heating procedure of GC was started from 60 °C for 3 min, then increased to 240 °C at a speed of 4 °C/min and kept at 240 °C for 30 min. Helium was used as the carrier gas with a rate of 1.0 mL/min. We operated full scan mode in analysis of mass spectrometry (mass range, m/z 40–200). The α -farnesene authentic standard was used for qualitative and quantitative analyses.

2. UPLC-QTOF-MS analysis

We adopted an UPLC-QTOF-MS (Acquity UPLC I-Class/ Xevo® G2-XS QTOF, Waters Corporation, MA, USA) to analyze samples. The column was Waters ACQUITY UPLC HSS T3 C18 column (2.1 mm×100 mm, 1.8 μm). Milli-Q water with 0.1% (v/v) formic acid was Solvent A. Acetonitrile with 0.1% (v/v) formic acid was Solvent B. The solvent gradient was started at 20% B, then increased to 35% within 10 min, later increased to 95% B in 0.1 min and kept for 3 min. The flow rate was 0.4 mL/min. The column temperature was 30 °C. The electrospray ionization was operated in negative mode. The MS conditions were capillary voltage: 1.5 kV; source temperature: 100 °C; desolvation temperature: 300 °C; cone gas flow: 50 L/h; and desolvation gas flow: 600 L/h. The quantitative analyses of phytohormones were based on calibration curves, which were constructed by plotting the concentration of each phytohormone against the peak area of the authentic standard.

3. RT-qPCR analysis

We used a Roche LightCycle 480 (Roche Applied Science, Mannheim, Germany) for analysis. The reaction process was one cycle of 95°C for 60 s, 40 cycles of 95 °C for 15 s, and 60 °C for 30 s in a 20 µL reaction system which contained 10 µL of iTaq™ Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 0.4 µL of each specific forward and reverse primer, 2 µL of cDNA, and 7.2 µL of ddH₂O. A melt curve was performed at the end of each reaction to verify PCR product specificity. The $2^{-\Delta\Delta C_t}$ method was used to calculate the relative expression level. Changes in mRNA level of the test genes for each treatment were normalized to that of *CsEF1*.