

Title: Biosynthesis of Jasmine Lactone in Tea (*Camellia sinensis*) Leaves and Its Formation in Response to Multiple Stresses

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Supplementary Information

Identification of Jasmine Lactone Authentic Standard

Jasmine lactone standard was identified based on mass spectra, NMR, and HRMS data. Mass spectra were recorded on a GC-MS QP2010 SE (Shimadzu Corporation, Kyoto, Japan). NMR spectra was recorded by a Bruker DRX-400 instrument (Bruker BioSpin, Ettlingen, Germany) using TMS as an internal standard and CDCl₃ as solvent. The chemical shifts are given in δ (ppm) and coupling constants in Hz. HRMS was determined by a UPLC-QTOF-MS (Waters Corporation, Milford, MA, USA). MS m/z (%): 108 (9), 99 (75), 71 (100), 55 (49), 41 (49). ¹H NMR (500 MHz, CDCl₃): δ 0.97 (t, $J=7.57$ Hz, 3H), 1.45-2.62 (m, 10H), 4.26-4.31 (m, 1H, CH-O), 5.32-5.60 (m, 2H, CH=CH); ¹³C NMR (125 MHz, CDCl₃): δ 171.6, 135.1, 122.3, 80.2, 33.3, 29.4, 27.1, 20.7, 18.4, 14.0. HRMS: m/z 169.1226 [M+H]⁺, calcd for C₁₀H₁₆O₂, 169.1229; m/z 191.1052 [M+Na]⁺, calcd for C₁₀H₁₆O₂, 191.1048.

Table S1 Primers of qRT-PCR Used in This Study

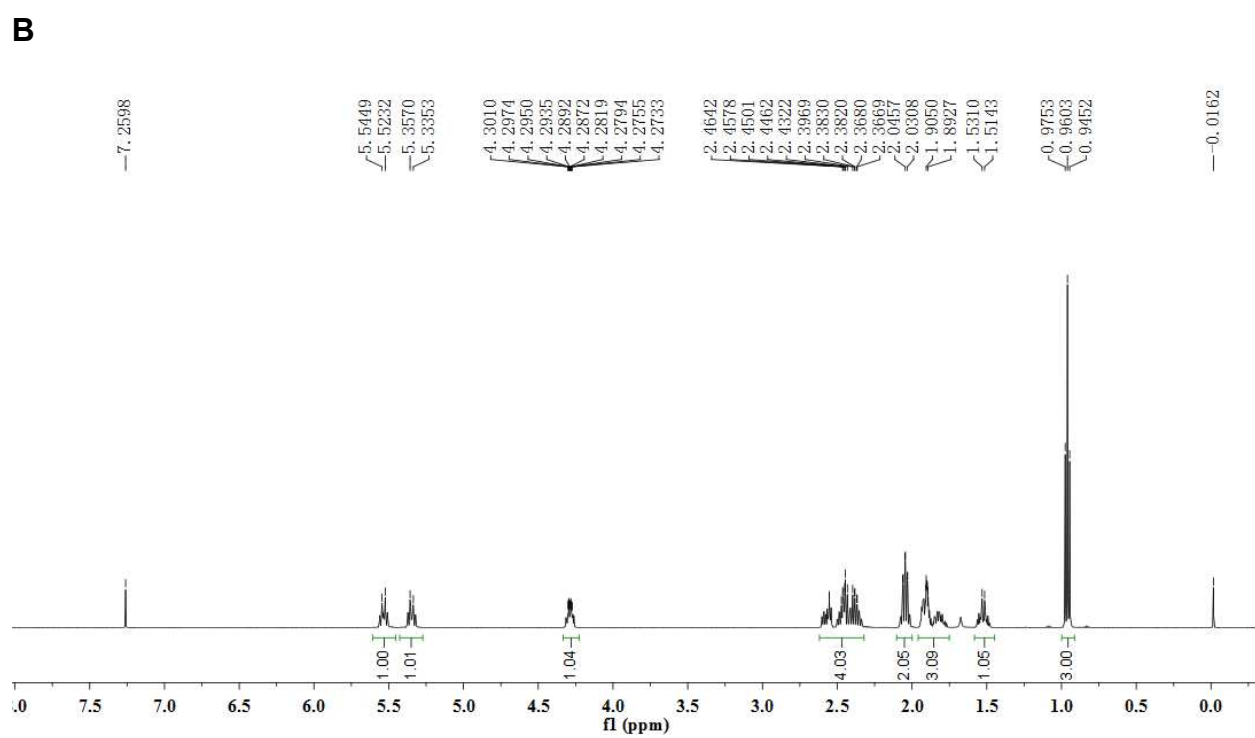
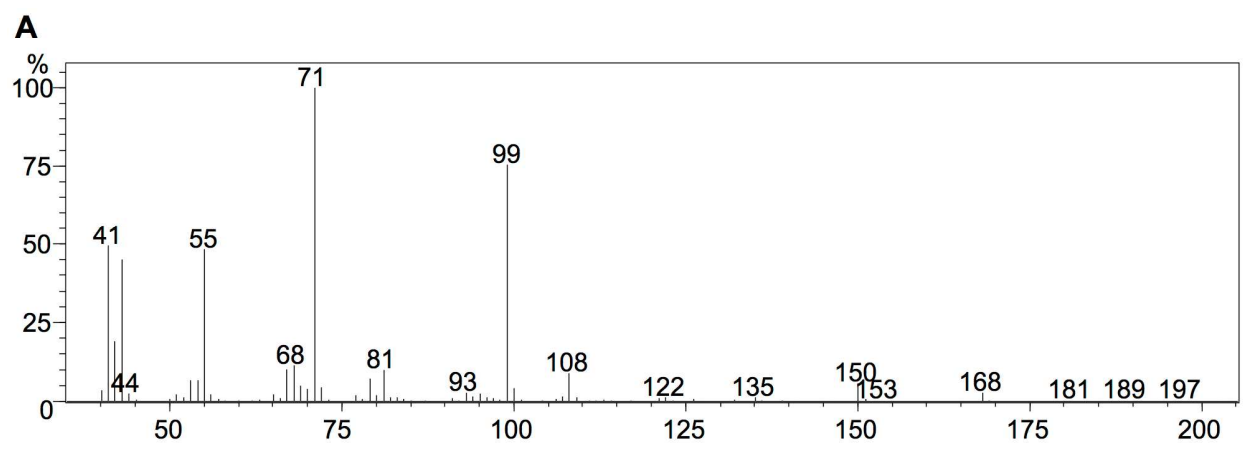
Gene	Accession number	Forward primer 5'-3'	Reverse primer 5'-3'
<i>CsEF1</i>	KA280301.1	TTGGACAAGCTCAAGGCTGAACG	ATGGCCAGGAGCATCAATGACAGT
<i>CsLOX1</i>	EU195885	GCTGACTGGACAACCGATGA	CAACATATGCTTCTATGAAAATGC
<i>CsLOX2</i>	FJ418174	GTTTTGTCAAATCATTCGGT	TTCTTCAAACCTCAAGTTTG
<i>CsLOX3</i>	FJ794853	GGGACAACACTGTATGGG	CCAGAGTCATGAGCAAGGG
<i>CsLOX4</i>	HM370508	TTCTTGGCATTTCCTCGTA	TCATCCTCGTTCATCCTTGT
<i>CsPGX1</i>	GFMU01020278	AAAGGCAAACAGGGTCCCAAAGG	GGCTTCCATCATAAACGGCTCGG
<i>CsPGX2</i>	GAAC01013502.1	TTGCGTCATCATTGAATTGAAACT	TAGACAAGAAGTAGAGCCTCCATT

EF1, encoding elongation factor 1; *LOX*, lipoxygenase; *PGX*, peroxygenase.

Table S2 Primers of PCR for cDNA Cloning Used in This Study

Gene	Accession number	Forward primer 5'-3'	Cutting site	Reverse primer 5'-3'	Cutting site
<i>CsLOX1</i>	EU195885	CGCGGATCCATGTTGC ACAGGGTTGTG	BamHI	GACGTCGACTCAAATTG AAACACTATTAG	Sall

LOX, lipoxygenase.



C

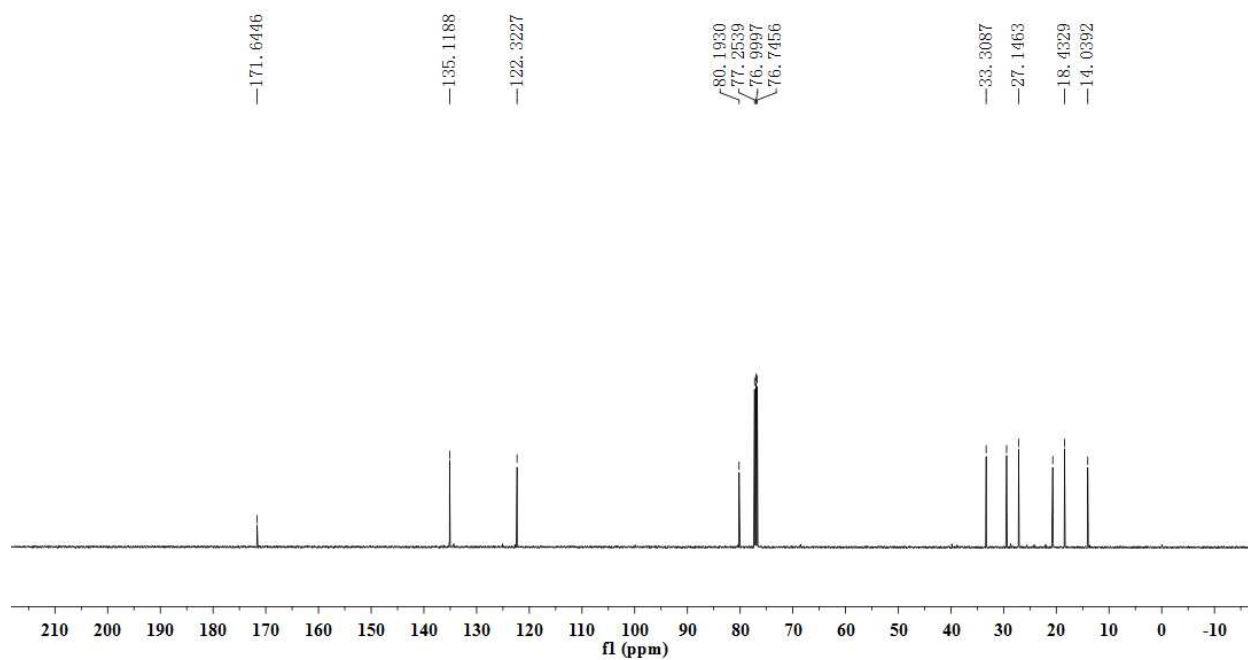


Figure S1 Identification of Jasmine Lactone Authentic Standard.

(A) Mass spectrum, (B) ^1H NMR spectrum, and (C) ^{13}C NMR spectrum of jasmine lactone authentic standard.

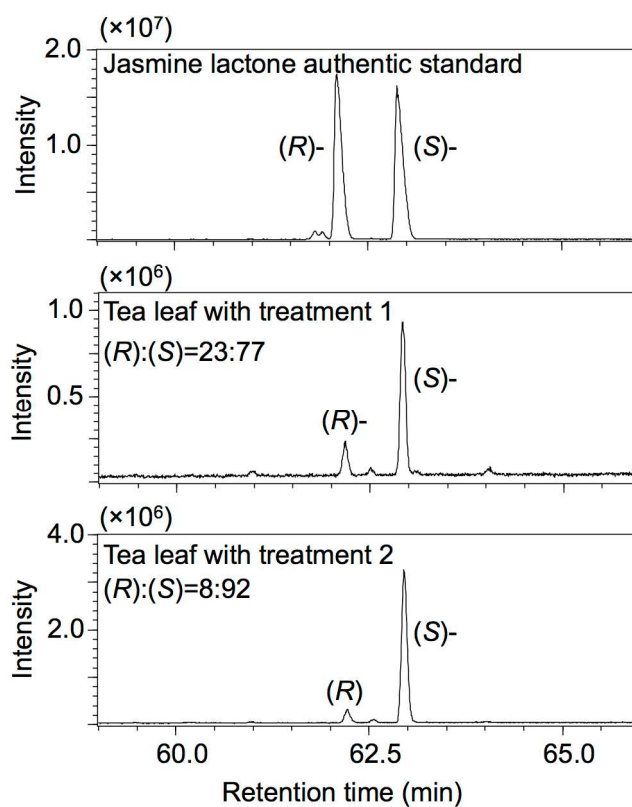


Figure S2 Determination of The Enantiomeric Ratio of Jasmine Lactone in *Camellia sinensis*.

Treatment 1, the tea leaves were shaken using a shaking table at 25 °C for 12 h; Treatment 2, the tea leaves were damaged using a needle to cause mechanical damage, and then exposed to 15 °C temperatures for 16 h.

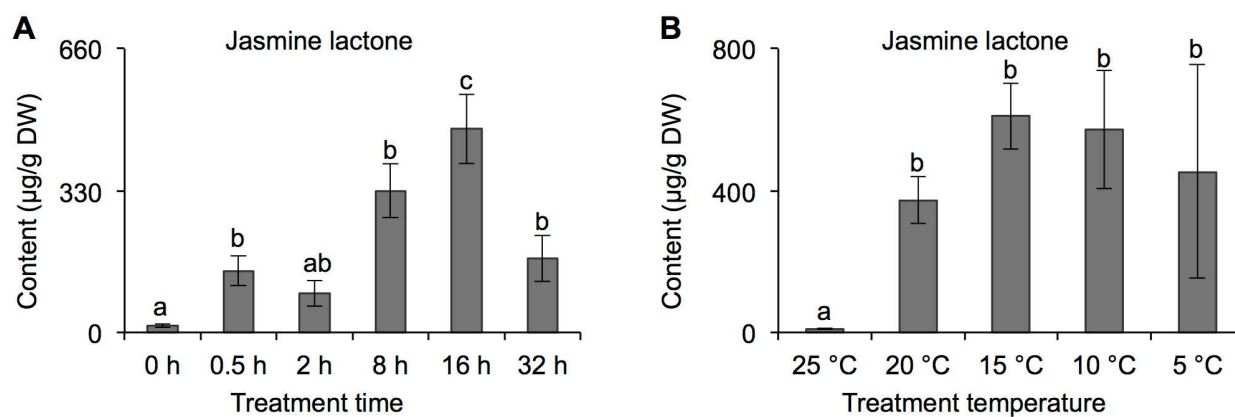


Figure S3 Effect of Treatment Time and Temperature After Mechanical Damage Caused by Needle on Jasmine Lactone Formation.

The characteristic fragment ions of jasmine lactone are m/z 71 and 99. The content of jasmine lactone was calculated based on corresponding calibration curves. DW, dry weight. Means distinguished with different letters are significantly different from each other ($p \leq 0.05$). All data are expressed as mean \pm S. D. ($n = 3$).