

## SUPPLEMENTARY INFORMATION

# Effects of Long-Term Nitrogen Fertilization on the Formation of Metabolites Related to Tea Quality in Subtropical China

Yuzhen Chen <sup>1,2,†</sup>, Feng Wang <sup>1,2,†</sup>, Zhidan Wu <sup>1,2</sup>, Fuying Jiang <sup>1,2</sup>, Wenquan Yu <sup>3</sup>, Jie Yang <sup>4</sup>, Jiaming Chen <sup>4</sup>, Guotai Jian <sup>4</sup>, Zhiming You <sup>1,2,\*</sup> and Lanting Zeng <sup>4,\*</sup>

<sup>1</sup> Tea Research Institute, Fujian Academy of Agricultural Sciences, No. 104 Pudang Road, Xindian Town, Jin'an District, Fuzhou 350012, China; taotaoyuzhen@163.com (Y.C.); 82458lin@163.com (F.W.); wzd@163.com (Z.W.); fuyingjiang93@163.com (F.J.)

<sup>2</sup> National Agricultural Experimental Station for Soil Quality, No. 1 Hutouyang Road, Shekou Town, Fu'an 355015, Fujian, China

<sup>3</sup> Fujian Academy of Agricultural Sciences, No. 247 Wusi Road, Gulou District, Fuzhou 350013, China; ywq1972@163.com (W.Y.)

<sup>4</sup> Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement & Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, No. 723 Xingke Road, Tianhe District, Guangzhou 510650, China; yangjie0727@163.com (J.Y.); chenjiaming@scbg.ac.cn (J.C.); jiangt@scbg.ac.cn (G.J.)

\* Correspondence: youzm1964@163.com (Z.Y.); zenglanting@scbg.ac.cn (L.Z.), Tel.: +86-20-3702-1938

† These authors contributed equally to this work.

**Table S1.** The primers used for quantitative real time PCR (qRT-PCR) in the study.

Gene	Accession number	Forward primer 5'-3'	Reverse primer 5'-3'
<i>CsEF1-<math>\alpha</math></i>	KA280301.1	TTGGACAAGCTCAAGGCTGAA CG	ATGGCCAGGAGCATCAAT GACAGT
<i>CsHEMA1</i>	XM_028228202.1	TGCAGCTGACAGGTATACAAA	ATGGCAAGCTTTTCACGCATT
<i>CsHEMA2</i>	-	ATGGCAAGCTTTTCACGCATT	ATGGCAAGCTTTTCACGCATT
<i>CsPOR1</i>	XM_028215906.1	GGCTCCATAACAGGAAACACA	TTCAAGCCTCCTGCAAGTCC
<i>CsPOR2</i>	XM_028228181.1	AGGGAAAGCCTAGTGTTCCTTT GA	GCCATTGGTTTGATTCTTCTT GA
<i>CsPAO</i>	XM_028208343.1	TCGCTCCTCTCTCTGAAGGT	AGGCCTGAGGGATTTCGAGTA
<i>CsTS1</i>	TEA015198.1	GTTGATGTTTCTGGGCAGCA	CTCACCCACACCAGTCAGAT
<i>CsGS1.1</i>	MG778703	CAGCACCAAGTCTACGAGGA	AATCATGGAAGTAACCACA
<i>CsGS1.2</i>	MG778705	TGCAAACCGTGGTGCATCTG	GTTTCCACAGGATGGTGGTAG
<i>CsGS1.3</i>	MG778704	TGGCCGATTGGTTGGCCTG	ACAACCTGATCTCCAGAACT
<i>CsGS2</i>	MG778706	CTGGAACGGTGCAGGATGC	GCCCCACGCGGATTGAACA
<i>CsPDX2.1</i>	MT726050	AAGGAAACTTGCTAGGGACTG C	CAACAATGCTGCTACTTGAGG C
<i>CsPAL</i>	D26596	ATTCCTTGCCAATCCTGTAA	ACTGCCTCGGCTGTCTTTCT
<i>CsAAAT1</i>	MH544095	CGCCGACGAACATCACAATC	TGGGTCTCCATACCCAGAG
<i>CsAAAT2</i>	MH544096	CCAAGGGAACAAAGGGCTGA	CCGTTGAATTTGGCGGATCG
<i>CsNES</i>	KY033151	CAGCACAAACGAAATTTCTT	CATTCCATGACCCAAGAGAA
<i>CsTSA</i>	KX022968	ACCACACCTACTACTCCAACA	CTTACAGATACACGAGCACC AG
<i>CsTSB2</i>	KX022970	CCTTATCTCCACGCCACTA	ACGACTATGCCGACTTGAAG
<i>CsLOX1</i>	EU195885	GCTGACTGGACAACCGATGA	CAACATATGCTTCTATGAAAA TGC
<i>CsMYC2a</i>	KU892079	ATCCCGGTTTTTCAGGTCCAC	ATTCGAATCATCGCGTCCCA
<i>CsMYC2b</i>	KU892080	TTGCCCTTTGGATACCCACC	TTCGCGTGAAAATGCTGCAA
<i>CsMYC2c</i>	KU892081	TGCAACAAGCCAAGTCACTG	AGCTCAGATTCGGCATTGGT

*EF1- $\alpha$* , encoding elongation factor 1- $\alpha$ ; *HEMA*, glutamyl-tRNA reductase; *POR*, protochlorophyllide oxidoreductase; *PAO*, pheophorbide a oxygenase; *TS*, L-theanine synthase; *GS*, L-glutamine synthase; *PDX*, pyridoxal 5'-phosphate synthase subunit; *PAL*, phenylalanine lyase; *AAAT*, aromatic amino acid aminotransferase; *NES*, (E)-nerolidol synthase; *TSA*, tryptophan synthase  $\alpha$ -subunit; *TSB*, tryptophan synthase  $\beta$ -subunit; *LOX*, lipoxygenase; *PPDC*, phenylpyruvate decarboxylase; *MYC*, myelocytomatosis protein.

**Table S2.** Effects of nitrogen application on the contents of aroma compounds in fresh tea leaves collected in spring.

Aroma compound (nmol/g FW)	N0	N1	N2	N3
Benzyl alcohol	25.85 $\pm$ 3.50a	22.71 $\pm$ 4.27ab	18.24 $\pm$ 3.23bc	16.11 $\pm$ 2.99c
Phenylethanol	88.77 $\pm$ 8.91a	80.60 $\pm$ 19.99a	57.53 $\pm$ 11.12b	55.15 $\pm$ 5.97c
Methyl salicylate	25.16 $\pm$ 4.53a	25.49 $\pm$ 3.06a	20.13 $\pm$ 2.52b	18.89 $\pm$ 0.86b
Geraniol	502.25 $\pm$ 61.82a	482.68 $\pm$ 99.80a	347.12 $\pm$ 59.92b	313.18 $\pm$ 29.59b
1-Hexanol	1.23 $\pm$ 0.17a	1.03 $\pm$ 0.47a	0.91 $\pm$ 0.15a	0.90 $\pm$ 0.29a
Benzaldehyde	0.36 $\pm$ 0.08a	0.35 $\pm$ 0.047a	0.34 $\pm$ 0.09a	0.37 $\pm$ 0.11a
Phenylacetaldehyde	1.24 $\pm$ 0.46a	1.13 $\pm$ 0.27a	1.12 $\pm$ 0.18a	1.70 $\pm$ 0.50a
(E)-Nerolidol	0.90 $\pm$ 0.17a	0.86 $\pm$ 0.16a	1.12 $\pm$ 0.30a	0.78 $\pm$ 0.11a

Linalool	147.68±22.31a	140.97±34.72a	117.56±15.22a	110.89±16.45a
Linalool oxide I	24.33±4.95a	28.27±3.48a	23.67±1.02a	22.65±4.63a
Linalool oxide II	71.02±13.17a	84.86±9.41a	72.23±2.64a	70.75±12.71a
Hexanal	5.44±0.63a	5.78±1.35a	5.40±1.73a	5.26±0.96a
<i>cis</i> -3-Hexenyl acetate	3.18±0.50a	2.86±0.92a	2.83±1.22a	2.24±0.17a
Indole	0.22±0.12a	0.12±0.04a	0.11±0.08a	0.09±0.01a

Values followed by different letters (a–c) mean significant at the 5% level among different treatments.