

Supplementary data

Biochar-dual oxidant composite particles alleviate the oxidative stress of phenolic acid on tomato seed germination

Yuting Tu ^{1,3,4,†}, Jinchun Shen ^{2,†}, Zhiping Peng ^{1,3,4}, Yanggui Xu ^{1,3,4}, Zhuxian Li ^{1,3,4}, Jianyi Liang ^{1,3,4}, Qiufang Wei ⁵, Hongbo Zhao ^{2,*} and Jichuan Huang ^{1,3,4,*}

¹ Institute of Agricultural Resources and Environment, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

² College of Horticulture, South China Agricultural University, Guangzhou 510642, China

³ Key Laboratory of Plant Nutrition and Fertilizer in South Region, Ministry of Agriculture, Guangzhou 510640, China

⁴ Guangdong Key Laboratory of Nutrient Cycling and Farmland Conservation, Guangzhou 510640, China

⁵ College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China

* Correspondence: zhao@scau.edu.cn (H. Zhao); huangjichuan@gdaas.cn (J. Huang)

† These authors contributed equally to this work.

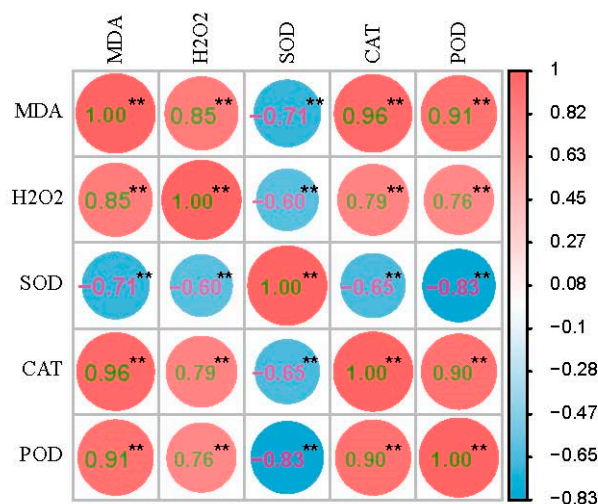


Figure S1. Spearman correlation between content of MDA, H₂O₂, and enzymatic activities of SOD, CAT and POD in tomato seed radicals on the 7th day of culture. Abbreviations: MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase.

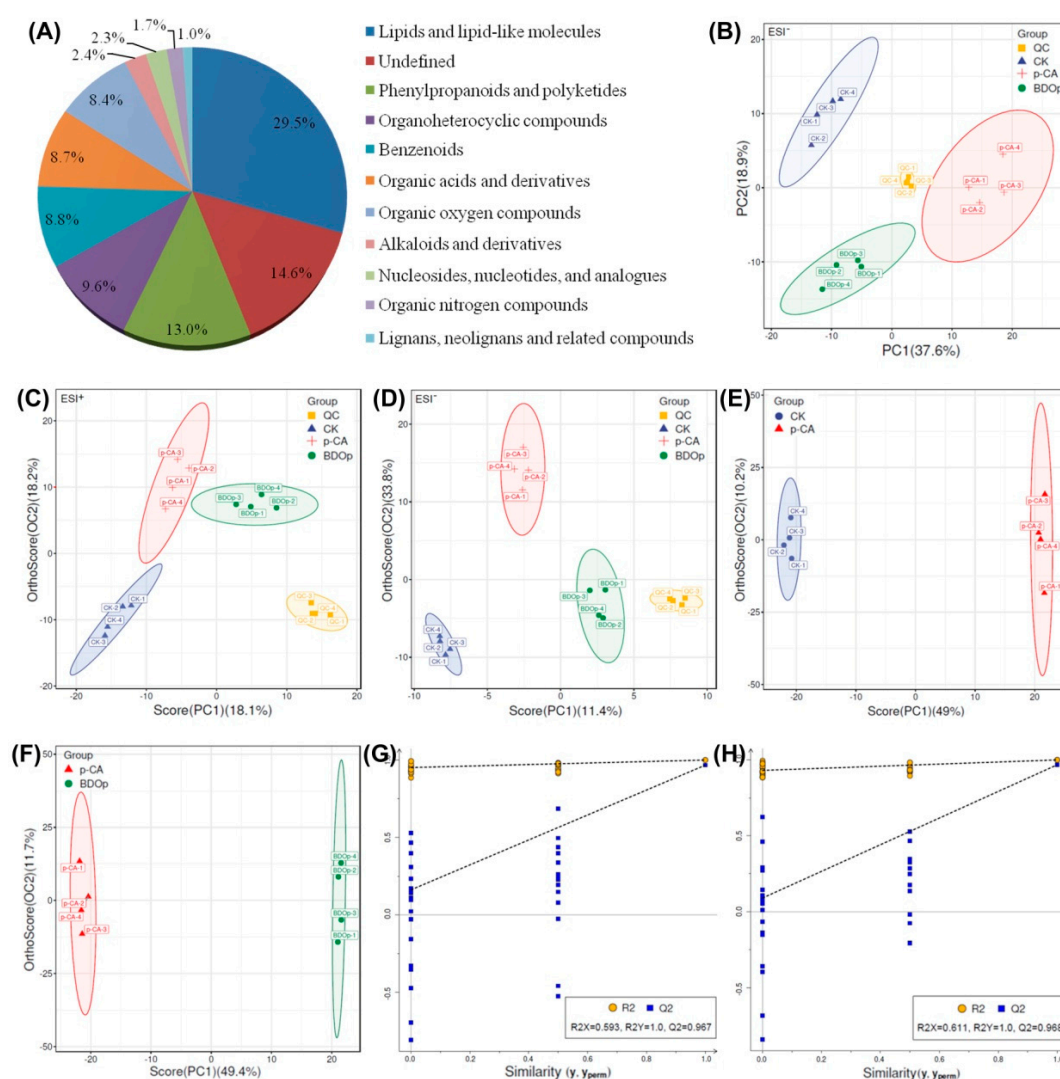


Figure S2. Pie chart of metabolite categorization (A). PCA score plot of metabolites under negative (ESI⁻) ion mode (B). OPLS-DA plots of metabolites in three different treatment groups under positive (ESI⁺) (C) and negative (ESI⁻) (D) ion mode. OPLS-DA score plot of metabolites in CK and *p*-CA group (E) and *p*-CA and BDOP group (F). Permutation test of OPLS-DA for CK vs. *p*-CA (G) and *p*-CA vs. BDOP (H). (QC: quality control; CK: ddH₂O control group; *p*-CA: treatment group with 10 mL 400 mg/L *p*-coumaric acid; BDOP: *p*-coumaric acid with biochar–dual oxidant particles. Each treatment was subjected to four biological replicates)

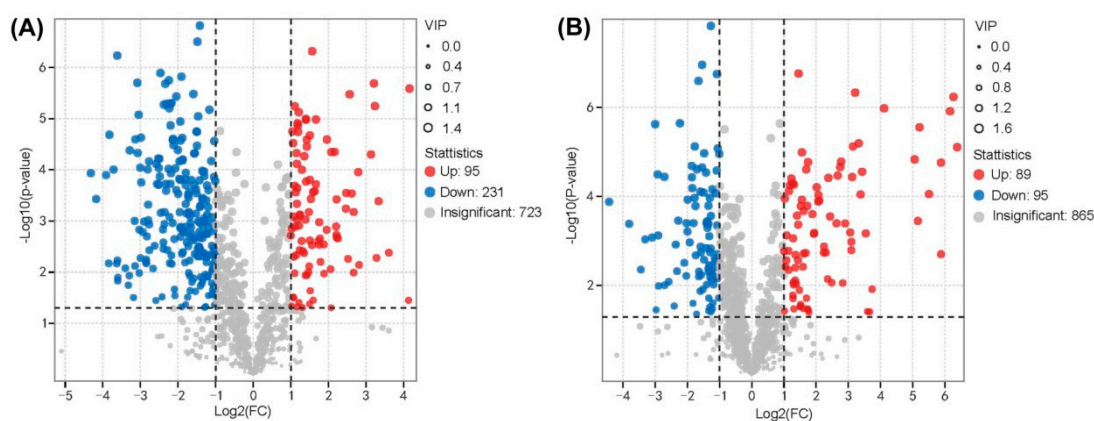


Figure S3. Volcano map of metabolites in *p*-CA vs. BDOP (A) and CK vs. BDOP group (B).

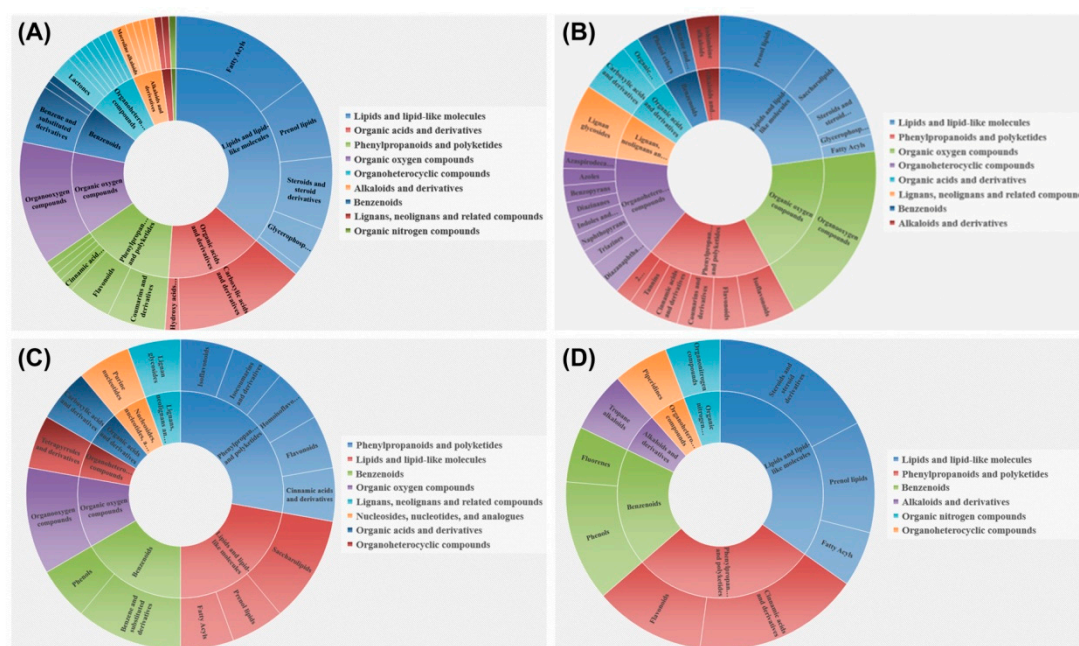


Figure S4. The sunburst chart for classification of the 165 up-regulated (A) and 59 down-regulated (B) DAMs in response to the *p*-CA stress, and 19 up-regulated (C) and 25 down-regulated (D) DAMs specifically regulated by the BDO composite particles.

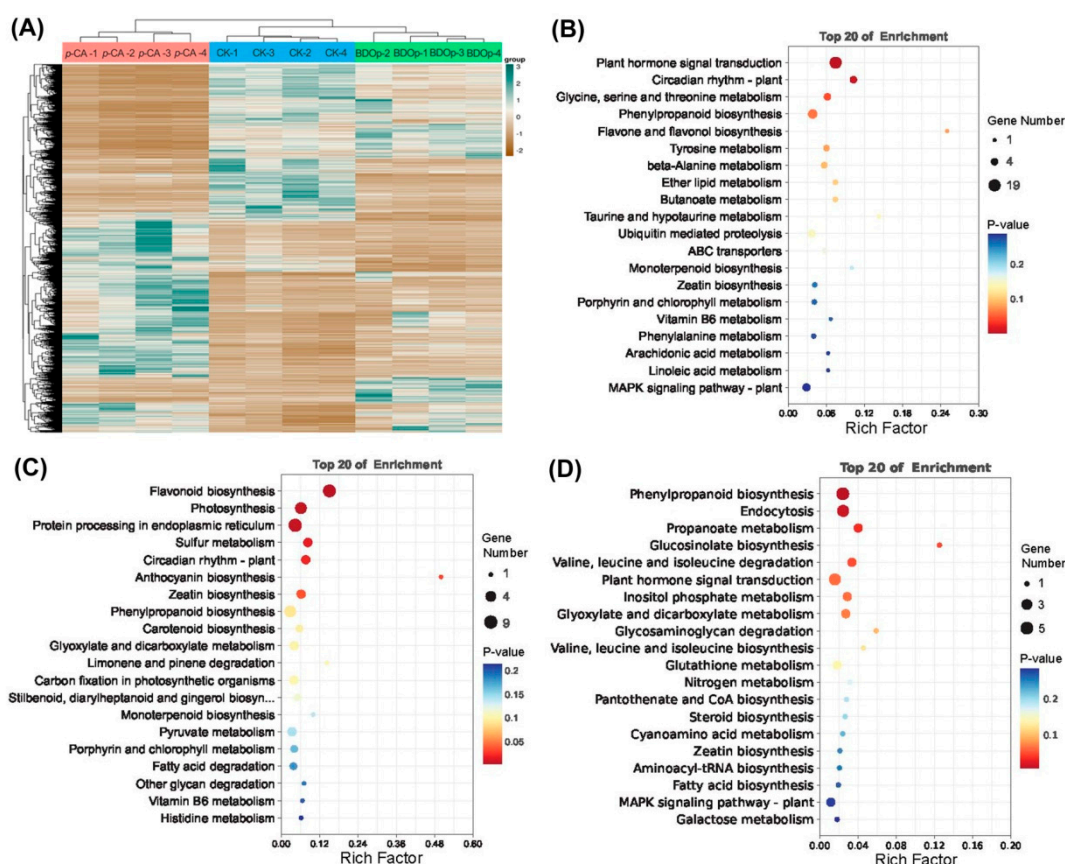


Figure S5. Hierarchical clustering heatmap of FPKM profiles in three treatments groups (A). The bubble diagrams for the top 20 KEGG pathways for 863 down-regulated DEGs in response to the *p*-CA stress (B), and 159 up-regulated (C) and 259 down-regulated (D) DEGs specifically regulated by the BDO composite particles. (CK: ddH₂O control group; *p*-CA: treatment group with 10 mL 400 mg/L *p*-coumaric acid; BDOp: *p*-coumaric acid with biochar-dual oxidant particles. Each treatment was subjected to four biological replicates).

Table S1. Effect of different *p*-coumaric acid concentrations on the germination of tomato seeds.

Concentration (mg/L)	GP (%)	GR (%)	RL (mm)
0	86.5±1.9 a	95.0±2.6 a	61.7±1.9 a
200	47.3±2.2 b	93.0±3.5 a	50.0±1.3 b
400	0.0±0.0 c	92.5±3.4 a	23.9±1.2 c
600	0.0±0.0 c	84.0±4.9 b	19.1±1.0 d
800	0.0±0.0 c	81.0±4.2 b	8.5±0.4 e
1000	0.0±0.0 c	51.0±2.6 c	4.6±0.2 f

Abbreviations: GP, germination potential; GR, germination rate; RL, radical length. Each data presents the mean ± standard deviation ($n = 4$). Different letters indicate statistically significant differences between treatments (One-way ANOVA, LSD test, $P < 0.05$).

Table S2. Specific primers were used for q-PCR.

Primer	Sequence (5'-3')	Product (bp)
CYP73A-F	ACCATAGACTCCTAGCGACCA	188
CYP73A-R	GTCTTGTCATTCCCCGTGA	
LOX1_5-F	GGTTGCCACGTCTGAGTTTG	127
LOX1_5-R	GACGCGGTCTCTGGGATTAC	
LOX2S-F	CAATGGGAAAGGCGAGAGGA	137
LOX2S-R	AGCGCCTGGGATAAGGTTTC	
LCAT3-F	GTGCCATGCAAAGGTTCTCTG	101
LCAT3-R	TGGGTGCATTCTGACTTCCA	
FAD2-F	CCACGAGAGGTGCATGTTCA	169
FAD2-R	CGAATCCGTCAGAAGCAGGA	
GAPDH-F1	AGCCACTCAGAAGACCGTTG	189
GAPDH-R1	AGGTCAACCACGGACACATC	

Table S3. Effects of various treatments on the tomato seed germination indices

Treatment	GP (%)	GR (%)	RL (mm)	RSA (cm ²)	GI (%)	SE
<i>p</i> -CA + BC-CP _P	57.0±2.6	89.0±3.8	37.7±1.7	0.353±0.017	57.2±2.7	-0.30±0.01
<i>p</i> -CA + BC-PS _P	0.0±0.0	31.0±1.1	3.1±0.1	0.079±0.004	1.6±0.1	-0.89±0.00
<i>p</i> -CA + CP-PS _P	53.0±2.6	76.0±1.6	9.1±0.6	0.209±0.010	11.8±0.3	-0.56±0.00

Abbreviations: *p*-CA + BC-CP_P, 10 mL 400 mg/L *p*-coumaric acid with 0.0277 g biochar and calcium peroxide composite particles; *p*-CA + BC-PS_P, 10 mL 400 mg/L *p*-coumaric acid with 0.1230 g biochar and potassium persulfate composite particles; *p*-CA + CP-PS_P, 10 mL 400 mg/L *p*-coumaric acid with 0.0125 g calcium peroxide and potassium persulfate composite particles; GP, germination potential; GR, germination rate; RL, radical length; RSA, radical surface area; GI, germination index; SE, synthetic allelopathic effect index. Each data presents the mean ± standard deviation ($n = 4$). Different letters indicate statistically significant differences between treatments (One-way ANOVA, LSD test, $P < 0.05$).