Supplementary Information (SI) for Environmental Science: Nano.

Supplementary material

Multi-omics analyses reveal the mechanisms of developmental toxicity of a covalent organic framework to the roots of rice (*Oryza sativa*) seedlings

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Characterization

The Fourier Transform Infrared (FT-IR) spectra were recorded in the 500-4000 cm^{-1} range using the potassium bromide pellet method on a Thermo Scientific Nicolet 6700 FT-IR spectrometer (Massachusetts, USA). Solid-state ^{13}C NMR spectra were acquired using a Bruker AVANCE III 500 MHz solid-state NMR spectrometer (Massachusetts, USA). X-ray diffraction (XRD) and powder X-ray diffraction (PXRD) measurements were conducted on a Rigaku D/MAX2550 diffractometer (Tokyo, Japan) with Cu-Kα radiation (λ = 1.5418 Å) over a 2θ range of 0-20°, operating at 50 kV and 200 mA, with a scanning rate of 0.1 °/s. Thermogravimetric analysis (TGA) was performed using a Perkin Elmer Pyris Diamond thermogravimetric analyzer (California, USA) from 30 °C to 700 °C at a heating rate of 10 °C/min under a nitrogen atmosphere.

The Brunauer-Emmett-Teller (BET) surface area measurements were performed using a Micromeritics ASAP 2020 surface area analyzer (Georgia, USA). Before gas adsorption, the synthesized EB-COFs were dried. Nitrogen adsorption/desorption measurements were carried out at 77 K under ultra-high-purity nitrogen gas (99.99%) and analyzed by nonlocal density functional theory.

Transmission electron microscopy (TEM) images were obtained with a Tecnai G2 F20 S-TWIN microscope (Oregon, USA) operating at 200 kV. Scanning electron microscopy (SEM) images were acquired using a Zeiss Ultra Plus Field Emission Scanning Electron Microscope (Thuringia Land, Germany) at 1 kV. For sample preparation, 1 mg of EB-COFs was dispersed in 10 mL of isopropanol and sonicated for 5 minutes. The suspension was then coated on a carbon-coated copper grid for SEM or Si-wafer for TEM imaging, followed by drying at room temperature before imaging.

Reactive oxygen species (ROS) production detection

Rice seedling root samples were collected after 14 days of treatment with EB-COFs at concentrations of 1000 mg/L, as well as a control group (CK, no treatment). A 2′, 7′-dichlorodihydrofluorescein diacetate

(DCFH-DA) kit (Suzhou Grace Biotechnology Co. Ltd, Suzhou, China) was used to quantify the production of reactive oxygen species (ROS), following the manufacturer's instructions.

Captions of tables and figures

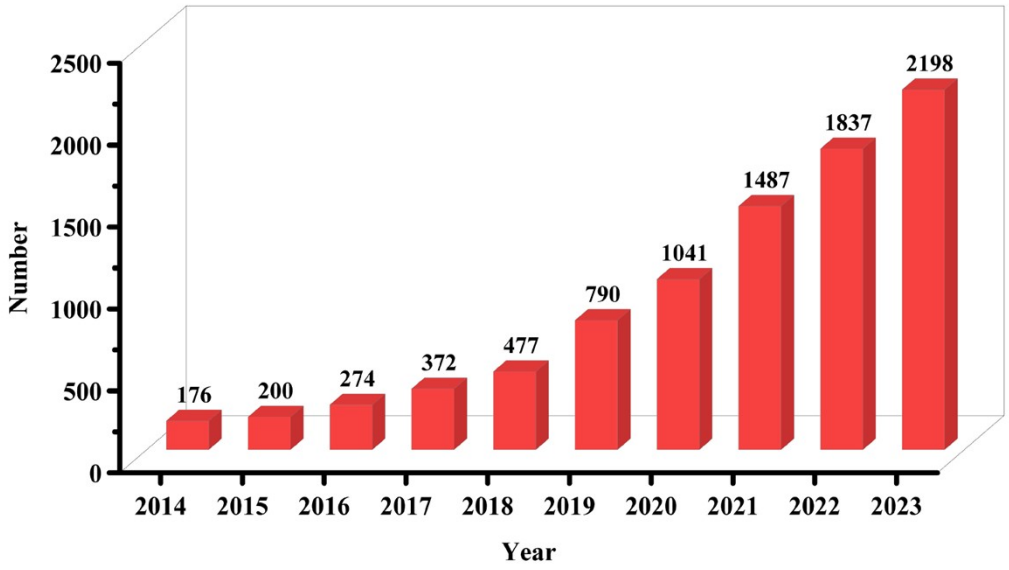


Figure S1. COF materials-related papers published in the last ten years.

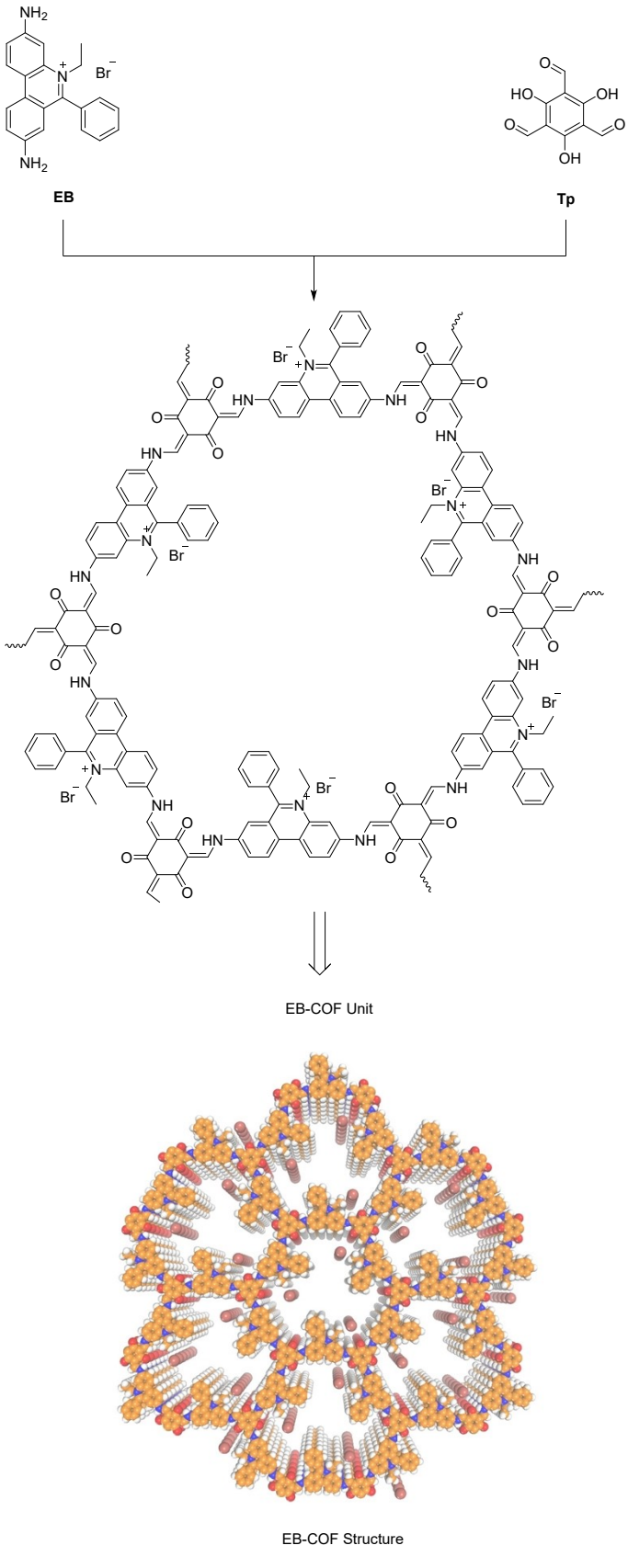
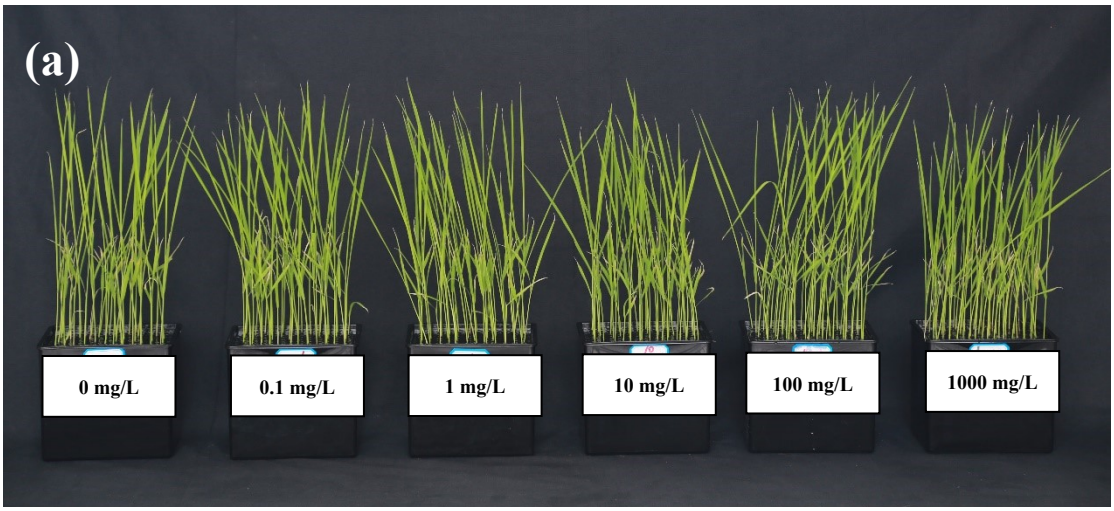
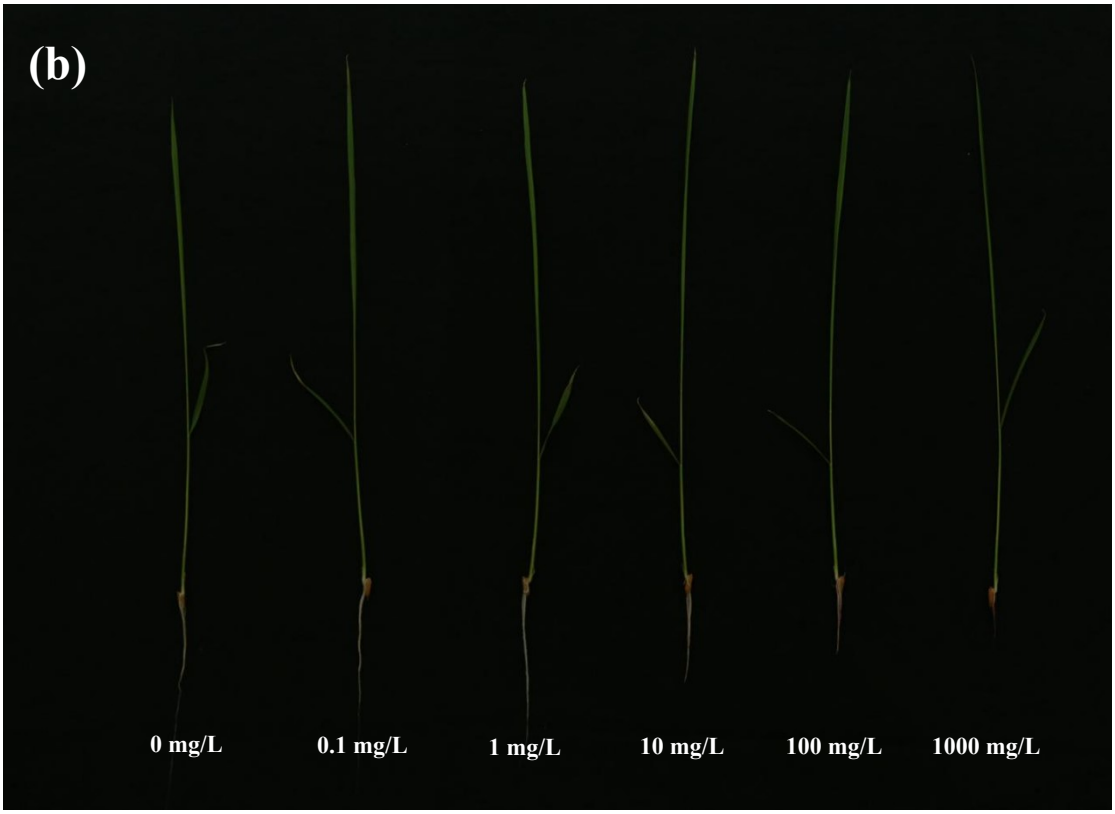


Figure S2. Synthetic route of EB-COF materials (yellow spheres represent H atoms; brown spheres represent Br atoms; white spheres represent C atoms; blue spheres represent N atoms, and red spheres represent O atoms).


(a)
0 mg/L
0.1 mg/L
1 mg/L
10 mg/L
100 mg/L
1000 mg/L


(b)
0 mg/L
0.1 mg/L
1 mg/L
10 mg/L
100 mg/L
1000 mg/L

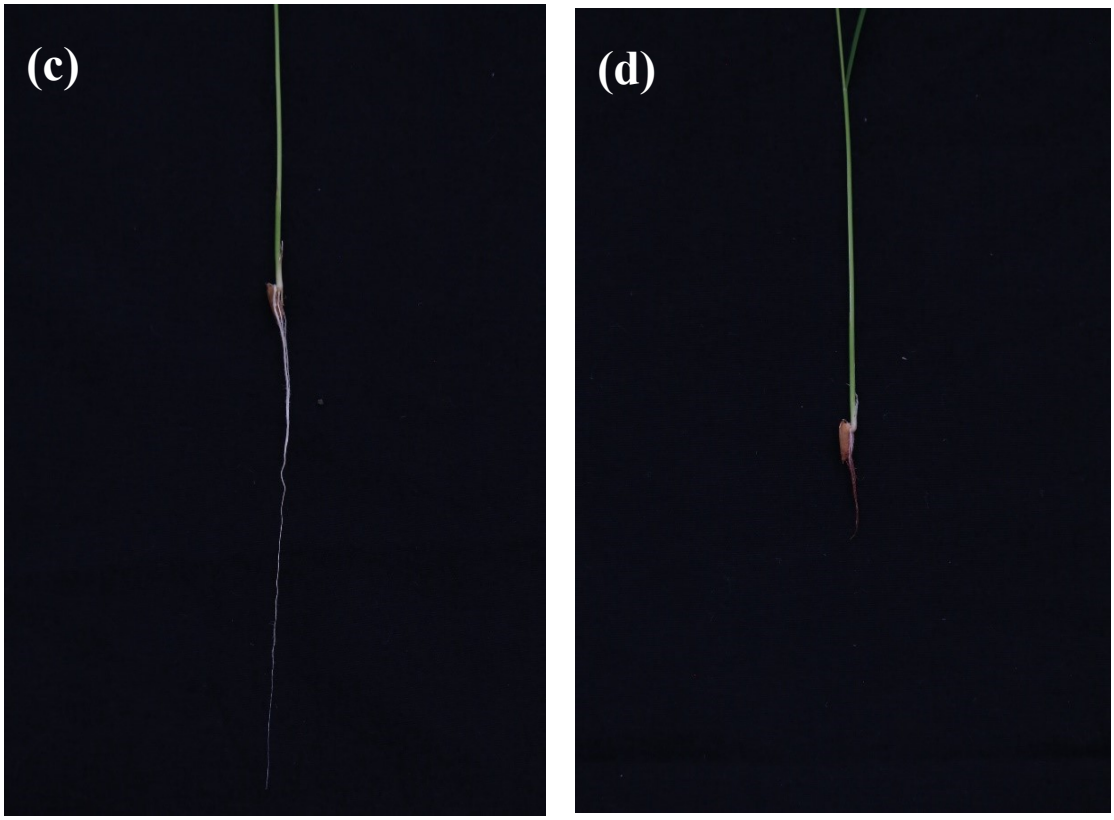


Figure S3. Phytotoxicities of EB-COF materials (a and b) to rice seedlings after 14-day treatments at different concentrations (0 mg/L as control, 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L, and 1000 mg/L). Roots of the control group (c) and the 1000 mg/L treatment group (d) after the 14-day exposure.

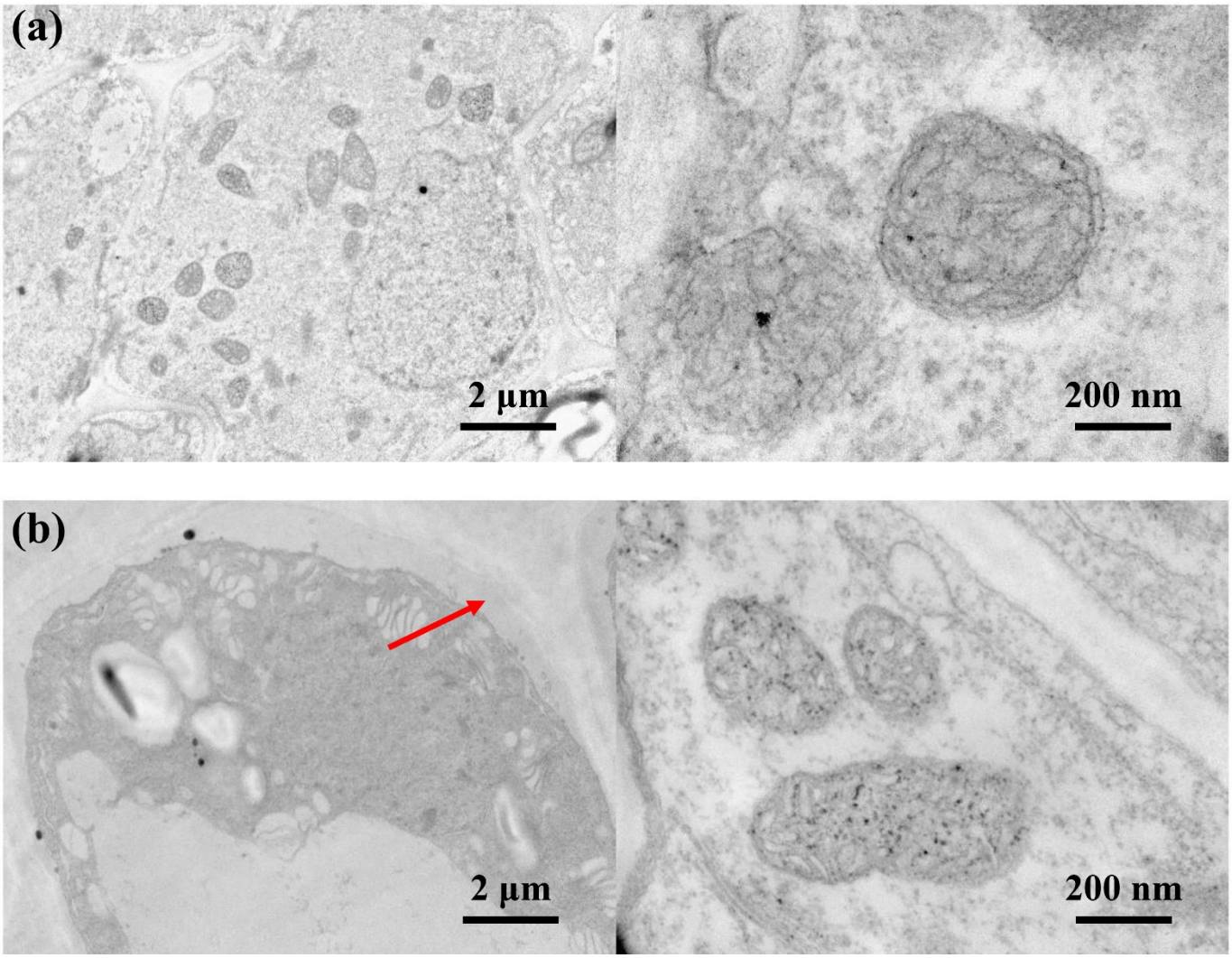


Figure S4. Ultrastructural changes in rice roots after 14-day EB-COFs treatments. (a) control (CK); (b) EB-COFs at 1000 mg/L concentration.

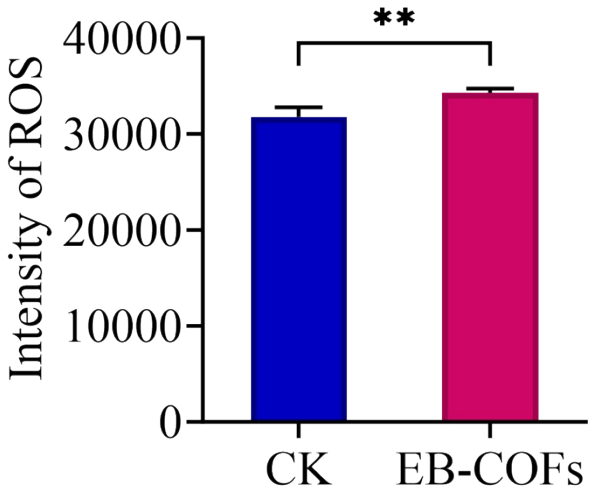


Figure S5. Reactive oxygen species (ROS) levels in rice seedling roots following 14-day exposure to control (CK) and 1000 mg/L EB-COF treatment. Error bars indicate standard deviation (n = 3 biological replicates). The different * mean significant differences among the treatments according to student's t test. * means $p < 0.05$. ** means $p < 0.01$. *** means $p < 0.001$.

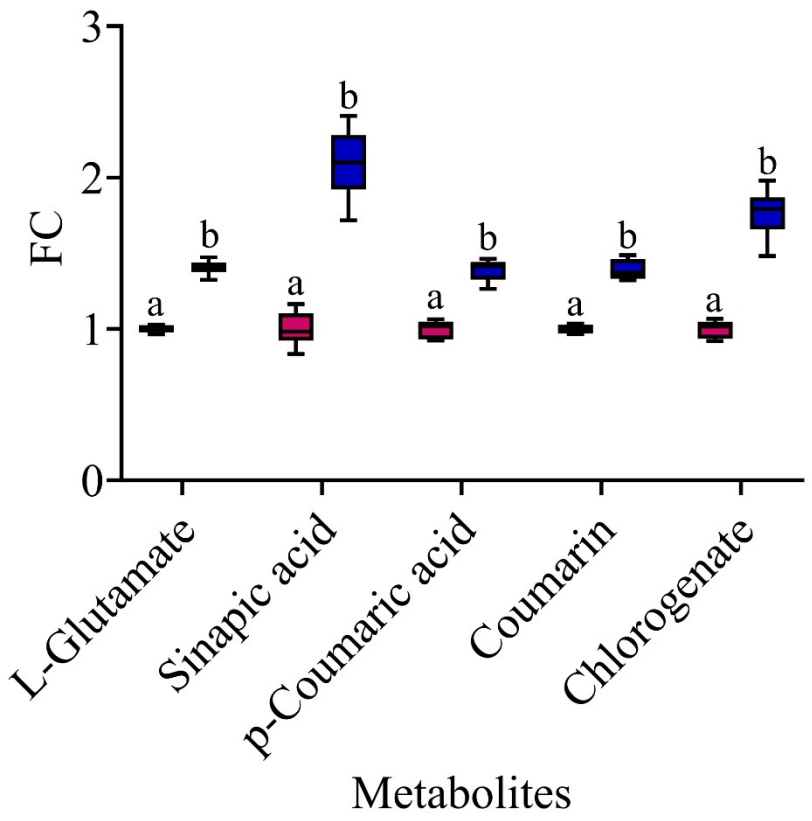


Figure S6. Fold change (FC) of L-glutamate, sinapic acid, p-coumaric acid, coumarin and chlorogenate between CK and EB-COFs. Red indicates CK, and blue indicates EB-COFs.

Table S1. Primer sequences for RT-qRCR.

Gene name	Annotation	Primer	Nucleotide Sequence (5' to 3')	Reference
XR_010735854.1	*18S rRNA*	Forward	CTACGTCCCTGCCCTTTGTACA	1
		Reverse	ACACTTCACCGGACCATTCAA	
NM_001406347.1	*OsWRKY24*	Forward	AGATGGAGGAAAGACGGT	2
		Reverse	GATGTCGATGTCGCTCAT	
NM_001406314.1	*OsCKX2*	Forward	GATAGCCTACAAGCAGTA	3
		Reverse	GCCTTTGGATCATACTTG	
XM_015769432.3	*OsCKX6*	Forward	AAGCAATATCTACCACACTACG	4
		Reverse	TGGGTCATACTTTACCTTTAGC	
NM_001401876.1	*OsRR2*	Forward	GTCATGTCGTCGGAGAAT	5
		Reverse	GCTGGACATCGTTCATCT	
XM_066310065.1	*ERF025*	Forward	GGCTGGCATGATGATGAG	6
		Reverse	ATCACGGTAGCTCCACAG	
NM_001402091.1	*OsGLN1-2*	Forward	CCGACATCAACACCTTCA	7
		Reverse	CCTCCTGTCCTCGAAGTA	
XM_015774263.3	*OsCATC*	Forward	GATGGATCGACGCACTCT	8
		Reverse	AGCCTGAGACCAGTAGGA	
XM_019393518.1	*NAC079*	Forward	GTACTTCTTCTGCCTCAA	9
		Reverse	GAAGATGTCCTTGTCCTT	
XM_026025999.2	*OsERF014*	Forward	AGCAGCAGCATCAGACAGC	10
		Reverse	CGCCGTCTTCACCATGTCAA	
XM_026026810.2	*OsCRL5*	Forward	ACTTTCAGCACGCAGGAG	11

		Reverse	GGGTGATGTCGAAGTTGGT	
NM_001403226.1	*1-Cys Prx B*	Forward	GATCAGGCAGCTCAACAT	12
		Reverse	GCTCAGCTTCACCTTCTT	
NM_001418750.1	*OsGSTU1*	Forward	ATGCAAGGGGAGGGACAG	12
		Reverse	TTGAGGTAGAGCGCGAACT	
NM_001422763.1	*OsWRKY76*	Forward	TTATCGGGCAAGAAGAGGA	12
		Reverse	CTGATGCCTGTTGCTGTT	
XP_004302079.1	*OsERF1B*	Forward	GCAACAACAACAACAAGA	1
		Reverse	TTGGAGATGGAGGAAGAA	
NM_001404443.1	*OsRR9*	Forward	AAGCAACAACAGCAGTAA	5
		Reverse	GCCTTGGTCTTATTGTGT	
NM_001423316.1	*OsCYP90A4*	Forward	AAGCAACAACAGCAGTAA	13
		Reverse	GCCTTGGTCTTATTGTGT	
NM_001403266.1	*PER2*	Forward	CCACCGTGAACCAGGATG	13
		Reverse	GATGTTGGCGTTGTCCGA	
NM_001409015.1	*BGLU5*	Forward	TCTCGCCTTCCATCCTTCA	12
		Reverse	CGTGTGCCCTTACCCAAA	

Table S2. Program settings for the q-PCR experiments.

PCR Program Settings		
Initial denaturation	95 °C	180 s
	95 °C	10 s
	60 °C	30 s, plate read
39 cycles	95 °C	10 s
	60 ~ 95 °C	+1 °C/cycle, holding time 4 s

Table S3. Significant enrichment pathways in combined analyses.

Pathway	DEG	DAM
Phenylpropanoid biosynthesis	71	6
Plant hormone signal transduction	89	1
Starch and sucrose metabolism	64	1
Glycerophospholipid metabolism	25	4
Glycerolipid metabolism	31	1
Glutathione metabolism	45	2
Diterpenoid biosynthesis	26	4
Carbon metabolism	33	4
Biosynthesis of amino acids	28	4

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