Supporting information

Alleviation of arsenic stress in *Brassica chinensis L.* by Fe(II): Decreased arsenic bioavailability and activation of antioxidant-flavonoids defense system

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Text S1 Details of the growing process of *Brassica chinensis L.* cultivation

Seeds of *Brassica chinensis L.* were surface-sterilized by soaking in 10% H_2O_2 for 15 min. After sterilization, the seeds were rinsed thoroughly with deionized water five times to remove residual H_2O_2 and prevent potential phytotoxicity. To promote germination, the seeds were soaked overnight at 32 °C. They were then germinated in darkness at 25 °C for three days. After germination, seedlings were transferred to a hydroponic system containing 50% Hoagland nutrient solution and cultured in a sterile growth chamber. The plants were maintained under a photoperiod of 18 h light / 6 h dark at 23/21 °C (day / night). The nutrient solution was renewed every two days to ensure sterility and nutrient sufficiency.

Text S2 The sample preparation procedure of TEM

Fresh leaves of *Brassica chinensis L.* from CK, 50 μM As(V), 50 μM Fe^{2+} and 50 μM As(V) + 50 μM Fe^{2+} treatment groups were collected for transmission electron microscopy (TEM) analysis. Leaf tissues of approximately 1 mm^3 were immediately immersed in 2.5% glutaraldehyde at room temperature for 1 h, followed by fixation at 4 ℃ for 24 h. The samples were rinsed three times with 0.1 mol/L PBS buffer (pH 7.4), each for 15 min, and then fixed with 1% osmium tetroxide for 2 h. After osmium fixation, samples were rinsed three times with PBS, each for 10 min. Dehydration was carried out through a graded ethanol series (30%, 50%, 70%, 80%, 95%, and 100%), followed by three changes of 100% propylene oxide, each for 15 min. The dehydrated samples were infiltrated and embedded in resin, and ultrathin sections of 60 - 70 nm were obtained using an ultramicrotome. Sections were mounted on copper grids, stained with uranyl acetate for 25 min, rinsed with water, and then stained with lead citrate for 7 min. After drying, samples were examined with a transmission electron microscope operating at 80 kV.

Text S3 The sample preparation procedure of root metabolites

Accurately weigh 100 ± 5 mg of the sample into a 2 mL centrifuge tube, add a 6 mm grinding bead, and then add 400 μL extraction solution (methanol : water = 4:1, v/v) containing four internal standards [e.g., L-2-chlorophenylalanine (0.02 mg/mL)]. The mixture is homogenized for 6 min using a tissue grinder (-10 °C, 50 Hz), followed by low-temperature ultrasonic extraction for 30 min (5 °C, 40 kHz). The samples are then kept at -20 °C for 30 min, centrifuged for 15 min (13,000 rpm, 4 °C), and the supernatant is transferred to an autosampler vial with an insert for instrumental analysis. Additionally, 20 μL of supernatant from each sample is pooled to prepare a quality control (QC) sample.

Text S4 Determination of As species in plant tissues

A total of 0.5 g of *Brassica chinensis L.* tissue was freeze-dried for 48 h using a freeze dryer (SCIENTZ-18N, Ningbo Xinzhi, China). The lyophilized samples were ground into a fine powder, and 10 mg of the powder was extracted with 5 mL of 50% (v/v) methanol solution. Ultrasonic extraction was performed for 2 h using an ultrasonic cleaner (SB-5200DTD, Ningbo Xinzhi, China). The supernatant was collected after centrifugation, and the extraction was repeated once. The combined supernatants were diluted and subsequently analyzed. Arsenic speciation was determined using liquid chromatography coupled with atomic fluorescence spectrometry (LC-AFS6500, Beijing Haiguang, China).

Fig. S1. Effects of Fe^{2+} on the *Brassica chinensis L.* planted in soil after 14 days. (a-d) Application of 50 μM Fe^{2+} during the planting process; (e-h) no Fe^{2+} were added during the planting process.

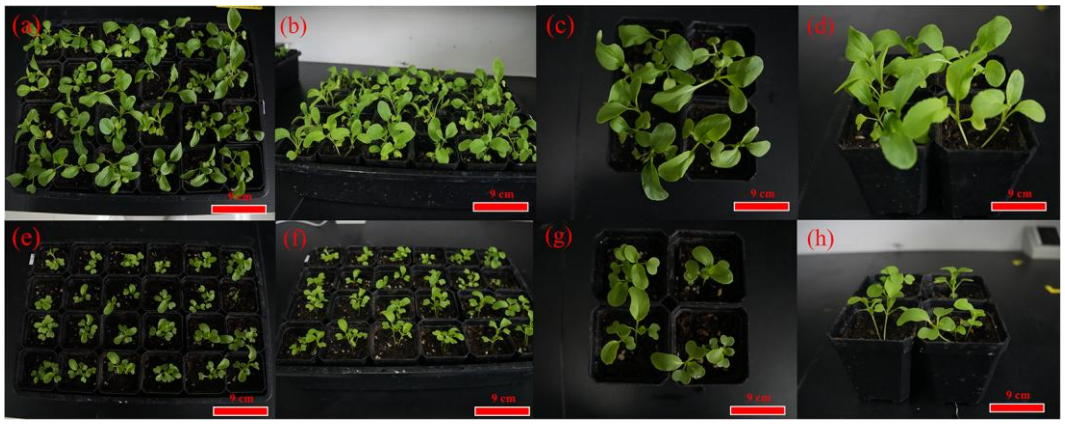

Fig. S2. Root surface attachments under different treatments (CK, 50 μM Fe^{2+}, 50 μM As(V) and 50 μM As(V) + 50 μM Fe^{2+}) were observed using a research-grade inverted fluorescence microscope at different magnifications (2x, 10x, 20x).

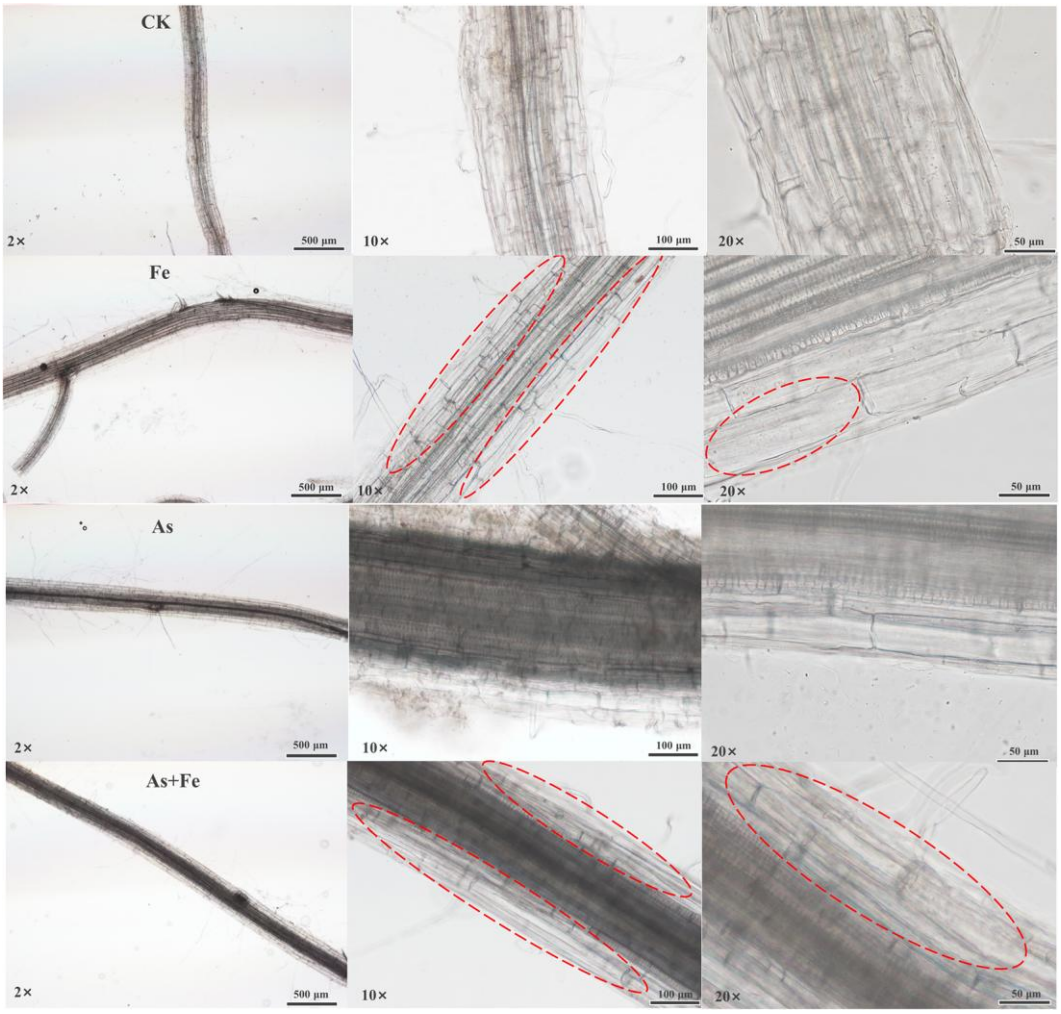

Fig. S3. Effects of different concentrations of Fe^{2+} on As(III) content and total As content in roots (a) and leaves (b) exposed to different As(V) treatments.

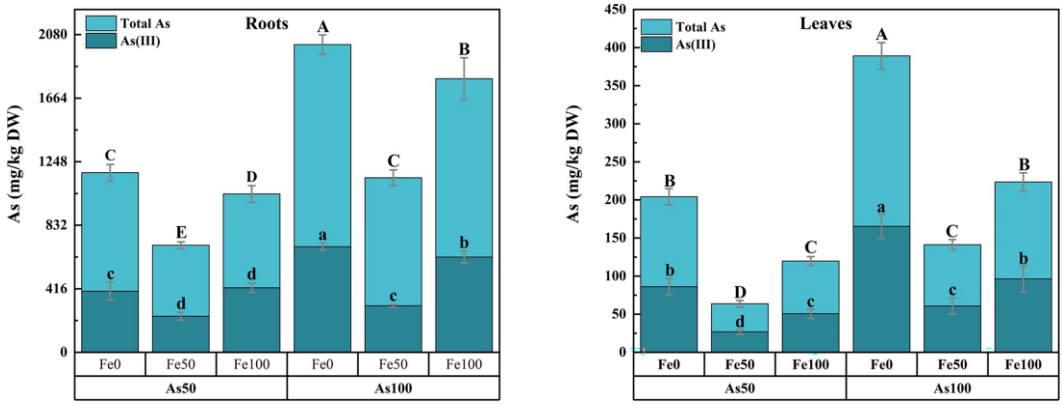

Fig. S4. Effects of quercetin on the (a) total fresh weight, (b) chlorophyll content and (c) carotenoids content after 7 days in *Brassica chinensis L.* treated with As(V).

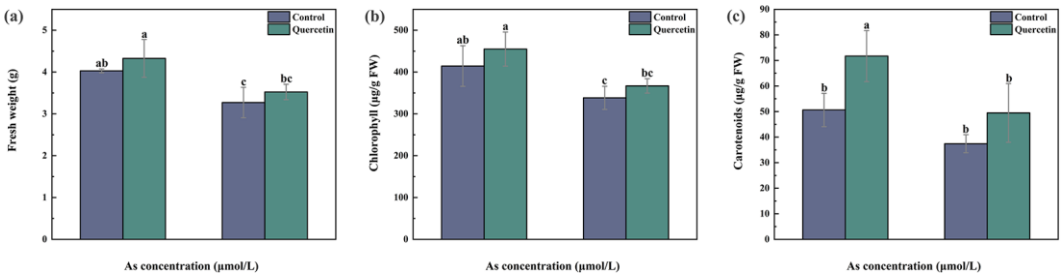

Fig. S5. The metabolomic analysis in roots of *Brassica chinensis L.* exposed to different Fe^{2+}/As(V) treatments. (a) Distribution of metabolites at various levels in plant compound. (b) Specific distribution of various differentially expressed secondary metabolites (DEMs).

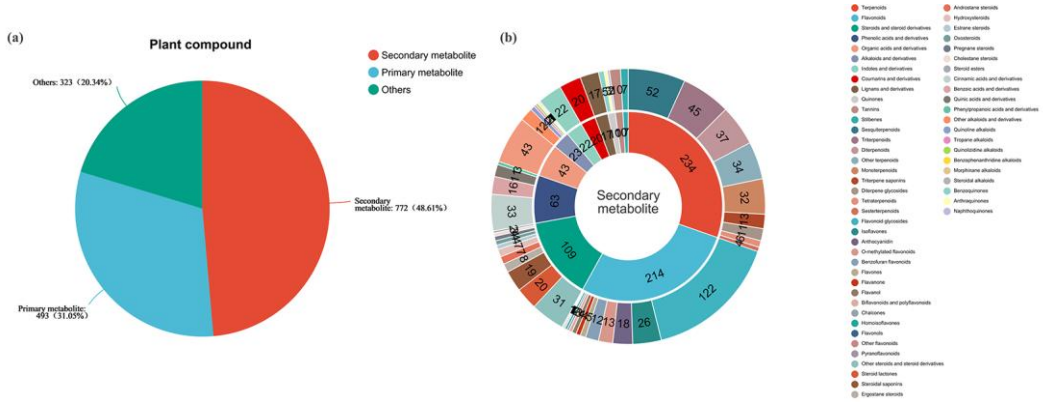

Table S1 The molecular mass, catalog numbers and purity of the chemicals used in this research.

Chemicals	Molecular mass	CAS	Purity
$Na_2HAsO_4·7H_2O$	312.01	10048-95-0	≥ 98%
$FeSO_4·7H_2O$	278.01	7782-63-0	≥ 99%
H_2O_2	34.01	7722-84-1	≥ 30%
C_2H_5OH	46.07	64-17-5	≥ 99.9%
HNO_3	63.01	7697-37-2	65%-68%
$C_6H_5Na_3O_7·2H_2O$	294.1	6132-04-3	≥ 99%
$NaHCO_3$	84.01	144-55-8	≥ 99.5%
$Na_2S_2O_4$	174.11	231-890-0	≥ 90%
CH_3OH	32.04	67-56-1	≥ 99.9%
$C_{15}H_{10}O_7$	302.24	117-39-5	≥ 98.5%

Table S2 The primers sequences used in RT-qPCR reactions.

Gene name	Forward/Reverse	Primer Sequences (5′-primer-3′)
PHT1;1	F	CGCCGGTATGGGTTTCTTTA
	R	CGCTGACTCCGGATTGAAATA
PHT1;4	F	GGTCTCTCTTTCGGACACAAG
	R	GATCGTAGCGGACAAAGGATAG
Actin	F	GGAGCTGAGAGATTCCGTTG
	R	GAACCACCACTGAGGACGAT

Table S3 Specific reaction mechanisms of Fe(III) with As(V) at different pH.

pH	Reaction mechanism
< 1.99	No $Fe(OH)_3$ is generated and As is not removed.
1.99 ~ 3.20	$Fe(OH)_3$ is generated, and the positively charged $Fe(OH)_3$ will adsorb some H_3AsO_4.
3.20 ~ 6.61	$Fe(OH)_3$ flocculates to form a large number of precipitates, which will adsorb a large amount of H_3AsO_4 in the solution during the sedimentation process.
6.61 ~ 10	The main process is the flocculation and precipitation of $Fe(OH)_3$, but as the pH increases, $Fe(OH)_3$ gradually becomes negatively charged and is converted into $Fe(OH)_4^-$, which gradually dissolves into the solution.
> 10	$Fe(OH)_3$ reacts with AsO_4^{3-} to form iron arsenate precipitate.

Table S4 The pH in the different treatment groups.

Items	Concentration			Concentration		
As(μM)		50			100	
Fe(μM)	0	50	100	0	50	100
pH	6.00±0.01	5.82±0.06	5.98±0.02	6.09±0.04	5.98±0.26	6.19±0.01