SUPPORTING INFORMATION for

Regulation on iron and cadmium uptake in rice roots by iron(III) oxide nanoparticles: Insights from iron plaque formation, gene expression, and nanoparticle accumulation

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Contents:

S1. Dynamic dissolution of Fe_2O_3NPs in the solution

A dynamic experiment was conducted to investigate the amount of Fe ion released from the Fe_2O_3 NPs in the laboratory conditions. Briefly, 1000 mg L^{-1} Fe_2O_3 NPs was prepared with 1/2 strength Kimura B nutrient solution without Fe in the triangular bottles with the stoppers and then sonicated for 1 h. After shaking on the water bath oscillator for 1d, 2d, 4d, 6d, 10d, and 14d, an accurate 10 mL mixture was sampled and then centrifuged with 10, 000 r min^{-1} at 4 °C for 30 minutes. The supernate was filtered with a 0.45 μm filter and then acidized with 10% HCl. The Fe in the solution was measured with an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7500, U.S.A.). As shown in Fig. S1B, the concentration of Fe ion in the solution significantly increased along with the incubation time, and it reached 5.78 mg L^{-1} on the 10th day, and then it tended to be equilibrium since no increase was found on the 14th day.

S2. Scanning electron microscopic investigations

Samples were prepared for SEM analysis according to our previous study.[1] Briefly, the fresh roots of the 35 old seedlings were washed with deionized water and then cut down with a blade. The root tips were fixed in a fixative consisting of 2.5% glutaraldehyde in 0.05 mol L^{-1} phosphate buffer (pH 7.2) overnight. After that, the root segments were dehydrated in a series of ethanol rinses (25, 50, 75, 95, and 100%) and then processed for freeze-drying. The dried samples were viewed under Hitachi SU8000 field emission scanning electron microscope (Hitachi, Tokyo, Japan), and energy dispersive spectroscopy (EDS) was performed simultaneously to detect the nature of depositions on the root surface.

S3. Determination of photosynthetic pigment in leaves

The Chlorophyll a (Chl a) and Chlorophyll b (Chl b) of the leaves were measured according

to the method described by Huang et al. (2018) with modification.[2] Briefly, the fresh leaves were cut into pieces and mixed thoroughly. Accurate 0.200 g fresh leaves were weighted into the tube contained with 95% ethanol alcohol. All the mixture was placed in the dark at 25 °C for three days. The absorbance of supernate at wavelengths of 665 nm, 649 nm, and 470 nm was measured, and the 95% ethanol was recorded as the blank.

Chlorophyll a: $C_a=(13.95A_{665}-6.88A_{649})$

Chlorophyll b: $C_b=(24.96A_{649}-7.32A_{665})$

Carotenoid$=(1000A_{470}-2.05C_a-114.8C_b)$

S4. Determination of malondialdehyde (MDA) content

Accurate 0.5 g of fresh plant tissues were ground with 5 mL extraction solution containing 20% (w/v) trichloroacetic acid and 0.5% (w/v) thiobarbituric acid (TBA). The mixture was transferred into the 10 mL centrifuge and then centrifuged at 12, 000×*g* at 4 °C for 10 min. The supernatant was incubated in a water bath at 100 °C for 20 min. After that, the tubes were centrifuged at 3000 r/min for 10 min, and then the supernatant was recorded at 450, 532, and 600 nm with a UV spectrophotometer.[2]

S5. Soluble protein assay

Fresh plant tissues were used to extract enzymes and measure antioxidant enzyme activities.[2-4] Briefly, approximately 0.5 g fresh shoot and root tissues samples were homogenized with 5mL 50 mM pH 7.8 phosphate buffer solution in a prechilled mortar on the ice. The mixtures were transferred into the10 mL centrifuge tubes and then were centrifuged at 12, 000×g for 15 min at 4 °C. Soluble protein in plant tissues was determined by coomassie brilliant blue G-250 staining-colorimetry, and the absorbance value was determined at 595 nm. The standard curve was made of bovine serum albumin.[5]

S6. Results of photosynthetic pigments, soluble protein, and MDA

As shown in Fig.S3 (A), the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid in the rice leaves in the Cd treatment significantly decreased by 57.1%, 42.8%, and 40.2% compared to that in CK treatment, respectively, while the application of 50 mg L^{-1} Fe_2O_3NPs elevated the concentration of Chl a in rice leaves. Compared to Cd treatment, the concentrations of Chl a, Chl b, and carotenoid of rice leaves in Cd+FeNPs treatment significantly increased by 22.9%, 16.8%, and 21.1%, respectively. Similarly, a previous study also reported that 10 μM Cd significantly reduced the concentrations of photosynthetic pigments in rice grown in hydroponics, but it was mitigated by the applied nano-scale zero-valent iron at 100 mg L^{-1}.[6] The application of 50 mg L^{-1} Fe_2O_3 NPs alone significantly increased the chlorophyll-a concentration in rice plant (Fig. S3), which was in line with that addition of 10 and 1000 mg/kg of Fe_2O_3 NPs increased the chlorophyll content in peanut (*Arachis hypogaea*) evaluated by SPAD.[7]

Soluble protein is a critical osmotic regulatory substance and nutrient. As shown in Fig. S3, the soluble protein concentration in roots significantly increased by 32.1%, 80.8%, and 46.6% in the FeNPs, Cd, and Cd+FeNPs treatment compared with the CK treatment, respectively. There was no significant difference in the soluble protein concentration of shoots between FeNPs and CK treatments (Fig. S3). However, the concentration of shoots soluble protein significantly increased by 18.0% and 14.4% in the Cd and Cd+FeNPs treatment compared with the CK treatment, respectively.

Cd stress caused the membrane lipid peroxidation in rice plants, thus significantly increasing the MDA content of roots and shoots of rice plant in the Cd treatment (Fig. S3C). The concentrations of MDA in roots and shoots in the Cd treatment were significantly increased by 16.3% and 33.5% as compared to that in CK treatment, respectively. Compared

to CK, no significant difference was found in the MDA concentrations of roots and shoots in FeNPs treatment. However, the application of Fe_2O_3 NPs had a significant effect on the mitigation of the toxic effects of Cd and significantly decreased the MDA contents in the plant tissues of rice plant. The data showed that the roots and shoots MDA concentrations of rice plant in Cd+FeNPs treatment significantly decreased by 11.1% and 32.5%compared to that in Cd treatment, respectively (Fig. S3). To sum up, the application of Fe_2O_3 NPs alleviated cellular oxidative stress induced by the Cd and maintained the concentrations of the soluble protein of plants, primarily attributed to the inhibition of Cd uptake and accumulation in plant tissues.

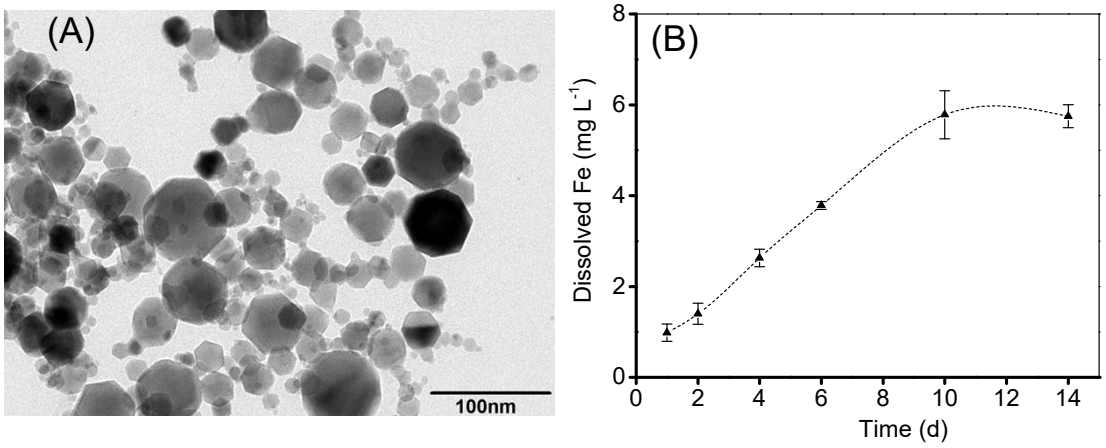

Figure S1. Transmission electron microscopic (TEM) image of Fe_2O_3 NPs (A) and its dynamic dissolution in the Kimura B nutrient solution (B).

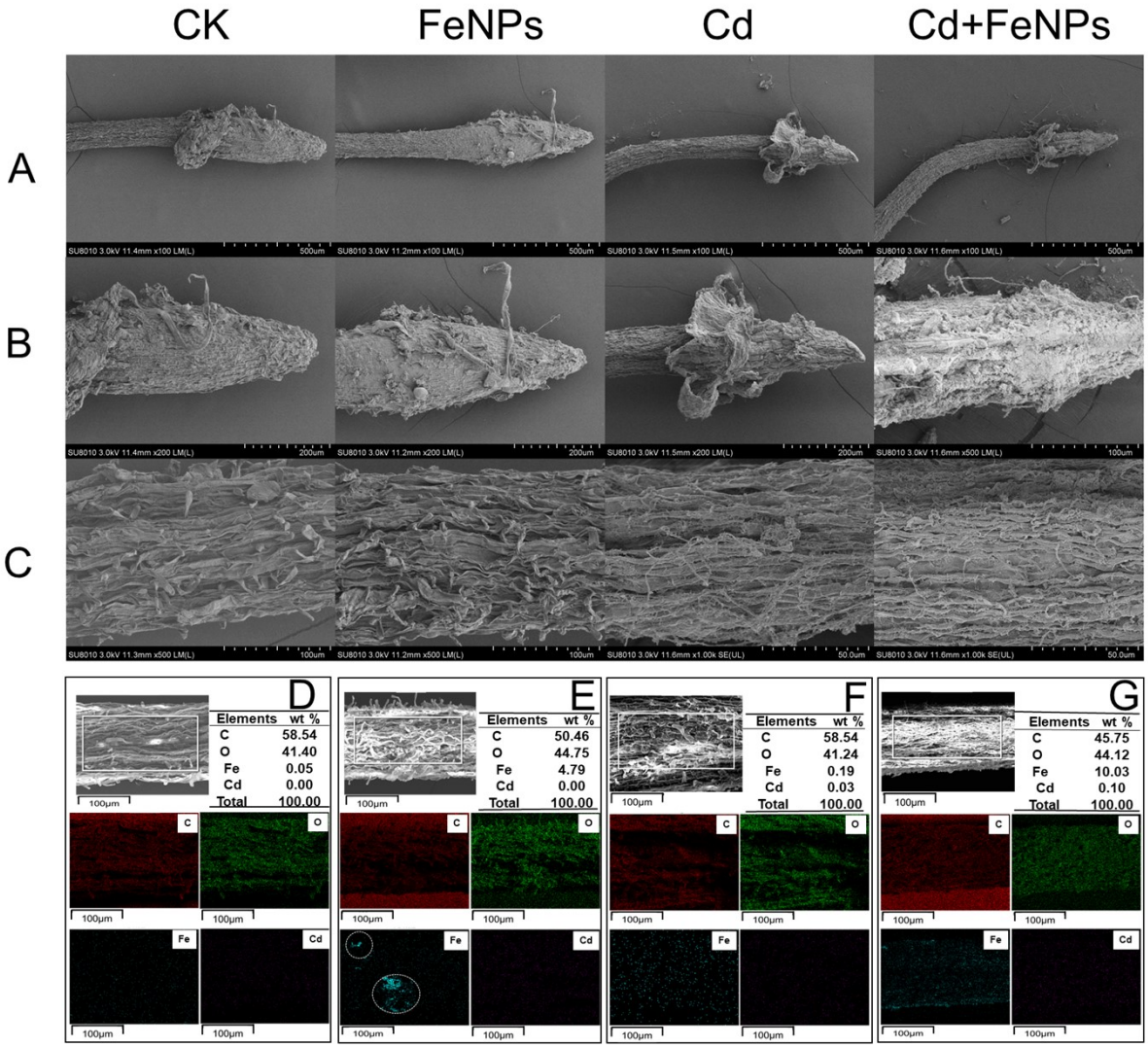

Figure S2. Scanning electron microscopic (SEM) image of the rice seedlings (A, B, and C) and EDS analysis of the elements on the root surface including C, O, Fe, and Cd (D, E, F, G) in CK, FeNPs, Cd, and Cd+FeNPs treatments, respectively.

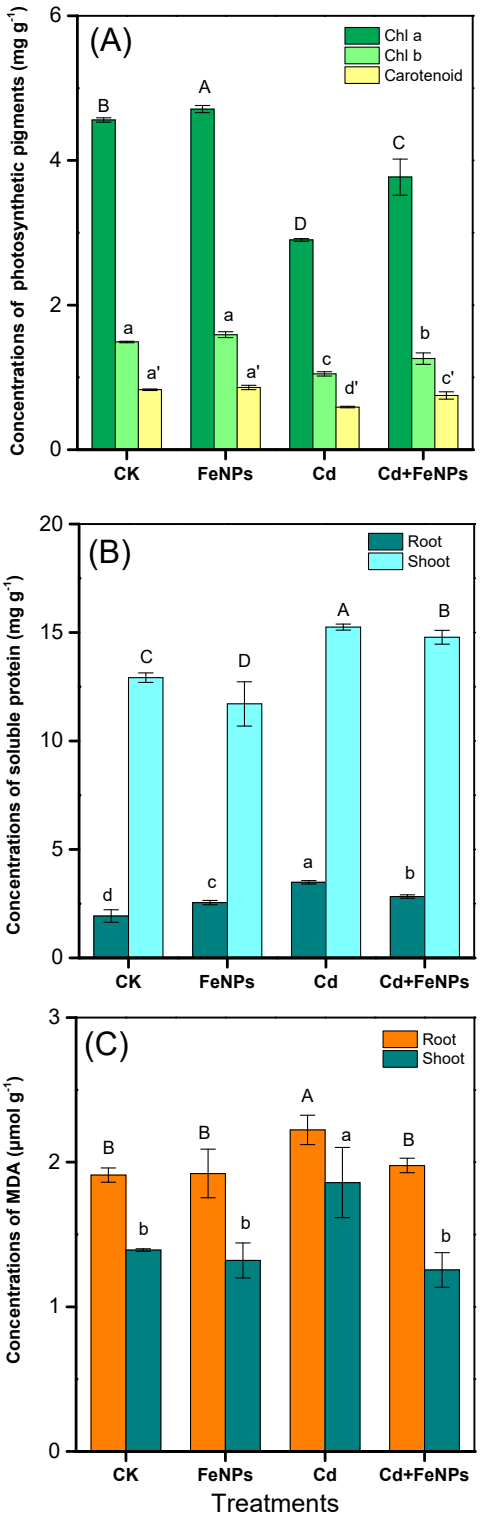

Figure S3. Concentrations of photosynthetic pigments (A) in leaves, and soluble protein (B) and MDA (C) in plant tissues in CK, FeNPs, Cd, and Cd+FeNPs treatments, respectively. Different capital or small low case letters above the columns indicate a significant difference among the treatments ($P \leq 0.05$).

Table S1. Sequences of the primers used in this study.

Gene		Sequences 5' to 3'	Reference
OsYSL15	Forward	AACATAAGGGGGACTGGTAC	6
	Reverse	TGATTACCGCAATGATGCTTAG	
OsIRT1	Forward	CGTCTTCTTCTTCTCCACCACGAC	6
	Reverse	GCAGCTGATGATCGAGTCTGACC	
OsIRT2	Forward	CGTTCCACACGCGGGGCAGCAAG	6
	Reverse	CCCATCCCCTCGAACATCT	
OsNRAMP1	Forward	GGAAACTGGAGGTTGTGGTC	8
	Reverse	TTTGCTGATGCGGGTGTATT	
OsNRAMP5	Forward	AGCAGTAAGAGCAAGATGGGGC	9
	Reverse	TTGGGGAGGTCGTTGTGGATGA	
OsCd1	Forward	ATGGAGGTGTTCTACTACCTCGTG	10
	Reverse	TTAAGGATTCAGTGGCTCATCTTCATCATC	
OsVIT2	Forward	GGCTGCAGGCATCCAAGTAAATGT	9
	Reverse	CACTACAAGCACGCAGCAAACGTA	
OsHMA3	Forward	GGGTGGAGATGTTCTTGAATCACTTGG	8
	Reverse	CCATTGGGTGGCTTGACTTGCTCT	
OsHMA2	Forward	CGCCATCTCCCAATCCCAAAT	11
	Reverse	TTCTCACTGCCGTTCCCATAA	
Actin	Forward	GACTCTGGTGATGGTGTCAGC	12
	Reverse	GGCTGGAAGAGGACCTCAGG	

Table S2. Total contents of Fe and Cd in Fe plaque, shoot, root, and whole plant.

Treatments	$Fe_{Fe\ plaque}$ (μg)	Fe_{shoot} (μg)	Fe_{root} (μg)	$Fe_{whole\ plant}$ (μg)	$Fe_{Fe\ plaque}/Fe_{whole\ plant}$	$Cd_{Fe\ plaque}$ (μg)	Cd_{shoot} (μg)	Cd_{root} (μg)	$Cd_{whole\ plant}$ (μg)	$Cd_{Fe\ plaque}/Cd_{whole\ plant}$	$Cd_{Fe\ plaque}/Fe_{Fe\ plaque}$
CK	112.20	69.97	36.26	106.23	1.06	-	-	-	-	-	-
FeNPs	5543.20	89.37	1056.20	1145.57	4.84	-	-	-	-	-	-
Cd	115.50	36.93	11.36	48.29	2.39	2.82	30.37	97.31	127.68	0.022	0.0244
Cd+FeNPs	8460.90	34.73	351.89	386.62	21.88	6.74	24.52	107.39	131.91	0.051	0.0008

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