## **Supplementary Material**

Copper oxide nanoparticles alleviate cadmium toxicity in cereal crops

Liangbo Fu,<sup>a</sup> Tingting Su,<sup>a</sup> Dongming Wei,<sup>a</sup> Dezhi Wu,<sup>a</sup> Guoping Zhang<sup>a,b</sup> and

Qiufang Shen<sup>a,b,\*</sup>

aInstitute of Crop Science, College of Agriculture and Biotechnology, Zhejiang

University, Hangzhou 310058, China

<sup>b</sup>Zhejiang University Zhongyuan Institute, Zhengzhou 450000, China

\*Corresponding author: Qiufang Shen E-mail: shenqf@zju.edu.cn Tel & Fax: (86)-571-88982115

## **Supplementary Material**

**Fig. S1.** Relative dry weight of four rice and barley genotypes at 7 days after CuO NPs treatment.

Fig. S2. Effects of CuO NPs on MDA content, SOD, POD and CAT activities in shoots of rice and barley after 7 days Cd treatment.

**Fig. S3.** Relative expression level of Cd transporter genes in shoots of rice and barley after 4 days Cd exposure.

**Fig. S4.** Relative expression level of ROS scavenging related genes in roots of rice and barley after 4 days Cd exposure.

**Table S1.** All primer sequences in the qRT-PCR experiments.

**Table S2.** Microelement concentration in the roots and shoots of rice and barley under treatment conditions.



**Figure S1.** Relative dry weight of four rice and barley genotypes at 7 days after CuO NPs treatment. (a) Relative shoot and (b) root dry weight of Nipponbare, 9311, Golden Promise and XZ26 after 7 days of 0, 5, 10, 50, 100 and 200 mg/L CuO NPs treatment. Data are shown as mean  $\pm$  SD of four biological replicates (n=4) and different small letters indicate significant difference at p < 0.05 by One-way ANOVA test.



**Figure S2.** Effects of CuO NPs on MDA content, SOD, POD and CAT activities in shoots of rice and barley after 7 days Cd treatment. (a) Accumulation of malondialdehyde (MDA) and the (b) SOD, (c) POD, (d) CAT activities in shoots of four rice and barley genotypes after 7 days of 0 and 10  $\mu$ M CdCl<sub>2</sub> treatments (mean ± SD, n=3). Different small letters indicate significant difference at p < 0.05 by One-way ANOVA test.



**Figure S3.** Relative expression level of Cd transporter genes in shoots of rice and barley after 4 days Cd exposure. Relative expression level of (a-d) *Nramp5*, (e-h) *HMA2*, (i-l) *IRT1* and (m-p) *LCT1* in shoot of Nipponbare, 9311, Golden Promise and XZ26, respectively. Data are shown as mean  $\pm$  SD of three biological replicates (n=3) and different letters indicate significant difference at p < 0.05 by One-way ANOVA test. +NP and -NP mean plants with and without CuO NPs pretreatment, respectively.



**Figure S4.** Relative expression level of ROS scavenging related genes in roots of rice and barley after 4 days Cd exposure. Relative expression level of APX, SOD and CAT in roots of Nipponbare (A-C), 9311 (D-F), Golden Promise (G-I) and XZ26 (J-L), respectively. Data are shown as means  $\pm$  SD of three biological replicates (n=3) and different letters indicate significant difference at p < 0.05 by One-way ANOVA test. +NP and -NP mean plants with and without CuO NPs pretreatment, respectively.

Species	Gene ID	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')		
H. vulgare	HvNramp5	TCCCTCGCCTACCTGGAT	GCTTCGGATACTCGCTCTT		
	HvHMA2	CGTGGATTCGTCTGCAGGTA	CAGCCATGCTTGTCTACGGA		
	HvHMA3	CTTGTCGACGCCTCCGTAAT	GCACGCTACCCTTGCTACAT		
	HvIRT1	CGTCTTCTTCTTCT	GGGGCTGTTGTCCTT		
	HvLCT1	CAAGAGTTGGAGACCGCCTT	GGTCTTCCCTGAACCACCAG		
	HvAPX1	CCCATGGTTCAAATGCTGGC	CAACTACTCCGGCAAGCTGA		
	HvSOD	CCGAAGATGAAATCCGCCAT	CGGCCAATGATTGAATGTGG		
	HvCAT2	CCATAAGGGACCATGCATGCCAGCTAC	GCTCGAAGCACCCACTTTAGTTTAAGC		
O. sativa	OsNramp5	TTCGTTTATATTTGTGCGGTCC	CACCTCCCCTCAAATGCTTATA		
	OsHMA2	ATACTCATGCTGATTGCTGGTA	CAAGCCAAAATGCATGCATTAG		
	OsHMA3	CAATGGTGTTGGTCGTTGC	CTCCCATTTCTGCAGTCTTTC		
	OsIRT1	GCAATTCGCTGCATTGTTAGAT	GAGAAGTCACAGTCACTGTACA		
	OsLCT1	AGCACATCTCTGGCTTCCAC	CGGCTCATTGCATTCTGCTC		
	OsAPX1	ATCAAGGAGGAGATACCCACCAT	TGGTCAGAACCCTTGGTAGCAT		
	OsSOD2	ATGCAAGCCATCCTCGCCGC	TTGAGCACGGCGACGGCCTT		
	OsCAT3	AGCTTGCACAGTTTGACAGGGA	ACGACTGTGGAGAACCGAACA		
Reference gene	GAPDH	CAACGACCCCTTCATCACCA	CGGCCTTGTCCTTGTCAGT		

 Table. S1 All primer sequences in the qRT-PCR experiments.

Genotype	Treatments -	Root			Shoot			
		Mn	Fe	Zn	Mn	Fe	Zn	
Nipponbare	-NPs	66.68 a <sup>1</sup>	245.87 a	265.67 a	504.08 a	100.92 a	290.18 a	
	+NPs	65.13 a	239.35 ab	278.11 a	517.03 a	102.03 a	290.61 a	
	Cd-NPs	41.6 b	215.4 b	151.01 b	190.66 c	79.41 c	149.68 c	
	Cd+NPs	55.1 ab	237.28 ab	198.41 b	290.44 b	89.75 b	181.44 b	
9311	-NPs	77.7 a	258.43 a	261.45 a	521.89 a	103.6 a	297.56 a	
	+NPs	68.31 ab	237.71 b	259.57 a	513.63 a	101.48 a	290.68 a	
	Cd-NPs	39.24 c	206.51 c	151.79 c	189.5 c	78.89 c	149.47 c	
	Cd+NPs	52.81 bc	236.74 b	195.8 b	287.63 b	90.3 b	181.44 b	
Golden Promise	-NPs	300.05 a	494.83 a	382.78 a	142.22 a	79.53 a	136.64 a	
	+NPs	293.57 a	479.97 a	359.7 a	146.77 a	82.55 a	130.36 a	
	Cd-NPs	163.45 c	371.5 b	194.83 b	73.07 b	50.69 c	66.14 c	
	Cd+NPs	209.45 b	398.23 b	239.57 b	90.3 b	59.82 b	77.02 b	
XZ26	-NPs	322.21 a	506.42 a	393.56 a	140.48 a	78.73 a	126.39 a	
	+NPs	298.64 a	482.52 a	380.61 a	138.56 a	77.82 a	125.27 a	
	Cd-NPs	168.86 b	358.89 b	183.41 c	71.63 b	47.88 c	61.41 c	
	Cd+NPs	203.59 b	385.22 b	243.23 b	84.35 b	59.74 b	73.96 b	

Table S2. Microelement concentration in the roots and shoots of rice and barley under treatments.

 $^1$  Different letter indicate significant difference at p < 0.05 by One-way ANOVA test.