Supplementary Information (SI) for Environmental Science: Nano.

Supporting Information

Small-sized selenium nanoparticles reduce the bioavailability of selenium in rice (*Oryza sativa* L.) by stimulating the formation of more iron plaque

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Text S1: Fluorescent Detection of Superoxide Radical and H_2O_2 in the roots of rice seedlings

Detection of superoxide radical ($O_2^{\cdot -}$) was performed using the fluorescent probe dihydroethidium (DHE)[1]. Rice root tips were carefully removed and incubated with DHE (10 μM) in a cell culture plate for 30 minutes in the dark. After immersion, the dye was washed thoroughly and then washed with Tris-HCl buffer (50 mM, pH 7.4) for 5 minutes. Examination and photographing of stained samples using a laser confocal microscope (Nikon, A1, JPN). Based on similar procedures, to observe the presence of H_2O_2 in rice root tips, 2,7-dichlorodihydrofluorescein diacetate (DCFDA) was used according to the method described by Morina et al[2].

Text S2: Determination of malondialdehyde (MDA) content in rice roots

Malondialdehyde (MDA) content was determined as described by Hu et al.[3] Briefly, a 0.1g aliquot of powdered sample was homogenized in 1 mL of a solution containing 20% (v/v) trichloroacetic acid (TCA) and 0.5% (v/v) thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 30 min, followed by cooling to room temperature. After centrifugation at 8000g for 10 min, the absorbance of the supernatant was measured at 532 nm and 600 nm. Malondialdehyde (MDA) concentration was calculated using the formula: MDA (nmol g^{-1} FW) = $[(A_{532} - A_{600})] / (\varepsilon \times FW) = [53.763 \times (A_{532} - A_{600})]/FW$, where FW represents fresh weight and ε is the molar extinction coefficient (155 mM^{-1} cm^{-1}). Results are expressed as nmol g^{-1} FW.

Table S1 Component of a half-strength nutrient solution

Serial number	Mass element	Concentration(mM/L)
1	K_2SO_4	0.094
2	KCl	0.1
3	KH_2PO_4	0.1
4	$MgSO_4·7H_2O$	0.294
5	$Ca(NO_3)_2·4H_2O$	0.183
6	EDTA·2Na	0.2
Serial number	Trace element	Concentration(μM/L)
1	H_3BO_3	1.0
2	$MnSO_4·H_2O$	1.0
3	$ZnSO_4·7H_2O$	1.0
4	$CuSO_4·5H_2O$	0.1
5	$(NH_4)_6Mo_7O_{24}·5H_2O$	0.05
6	$FeSO_4·7H_2O$	6.0

Table S2 Physical and chemical properties of soil tested

Indicators	Numerical value
PH	7.5 ± 0.1
TOC (g/kg)	67.3 ± 1.2
Mg (g/kg)	6.5 ± 1.2
N (g/kg)	10.5 ± 1.3
P (g/kg)	0.7 ± 0.1
K (mg/kg)	30.3 ± 0.2
S (g/kg)	0.9 ± 0.1
Ca (g/kg)	7.2 ± 0.1
Na (g/kg)	5.9 ± 1.0
Fe (mg/kg)	29.8 ± 1.5
Mn (mg/kg)	487.9 ± 48.4
Zn (mg/kg)	48.5 ± 5.6
Cu (mg/kg)	31.1 ± 3.7
Mo (mg/kg)	1.8 ± 0.3
Se (μg/kg)	309.1 ± 42.8

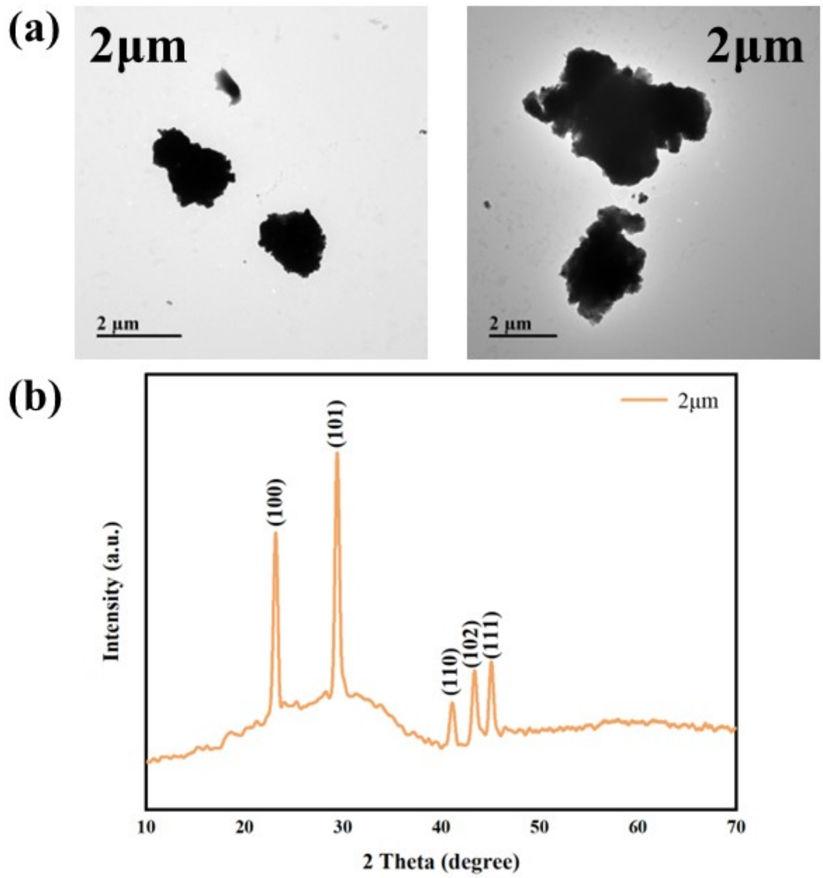

Fig. S1 (a) TEM images of Se NPs (2 μm), (b) Powder XRD pattern of Se NPs (2 μm).

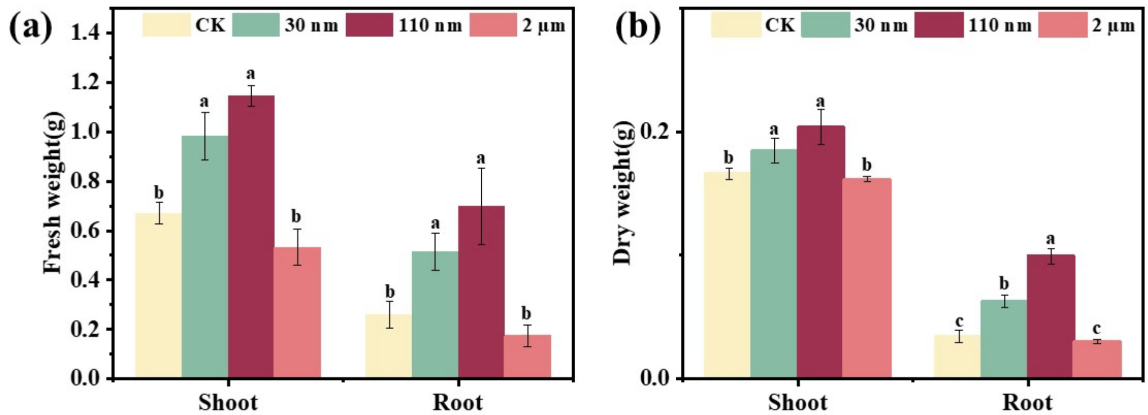

Fig. S2 Maturity biomass under different treatments: (a) Fresh weight, (b) Dry weight.

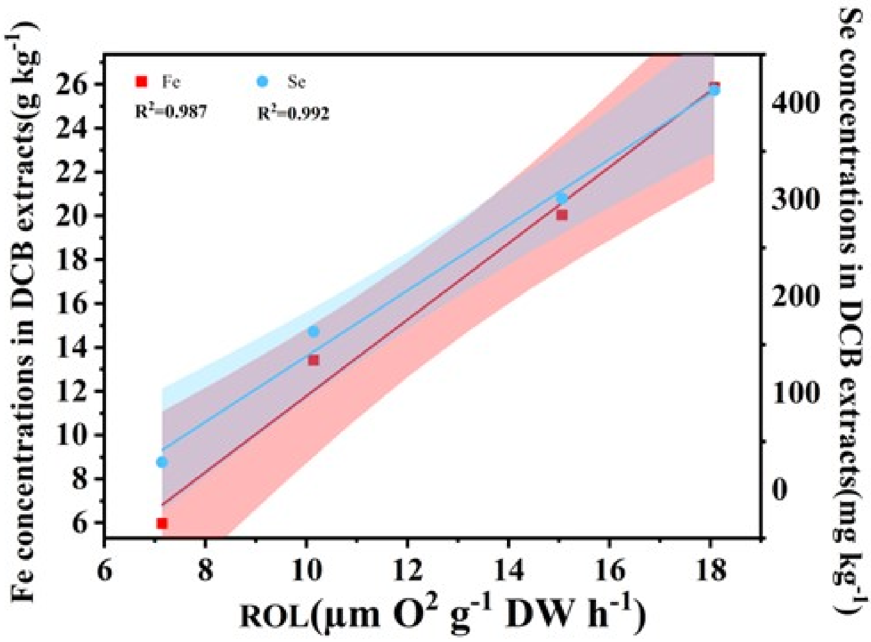

Fig. S3 Relationship between Fe and Se content in IP and ROL of rice.

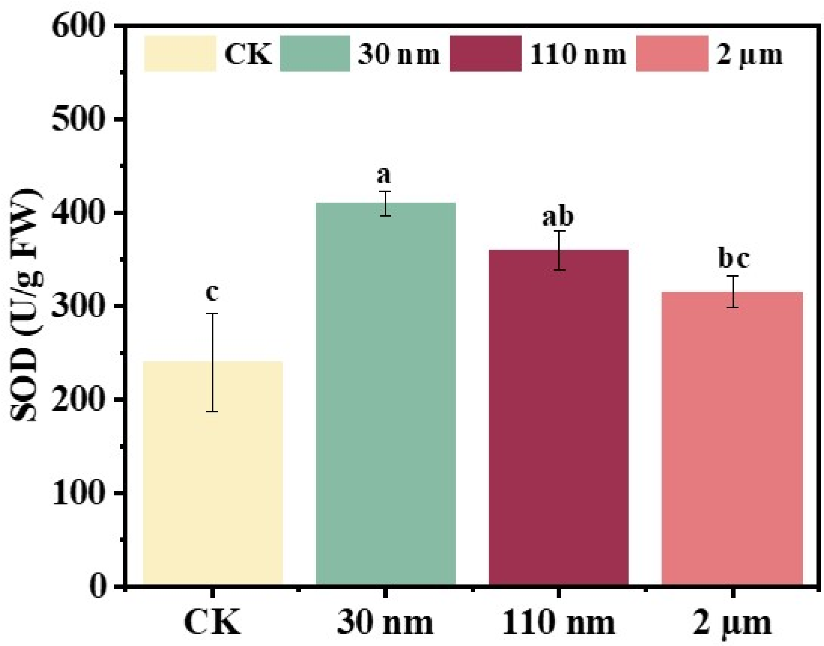

Fig. S4 Activity of SOD in rice roots at maturity under different treatments.

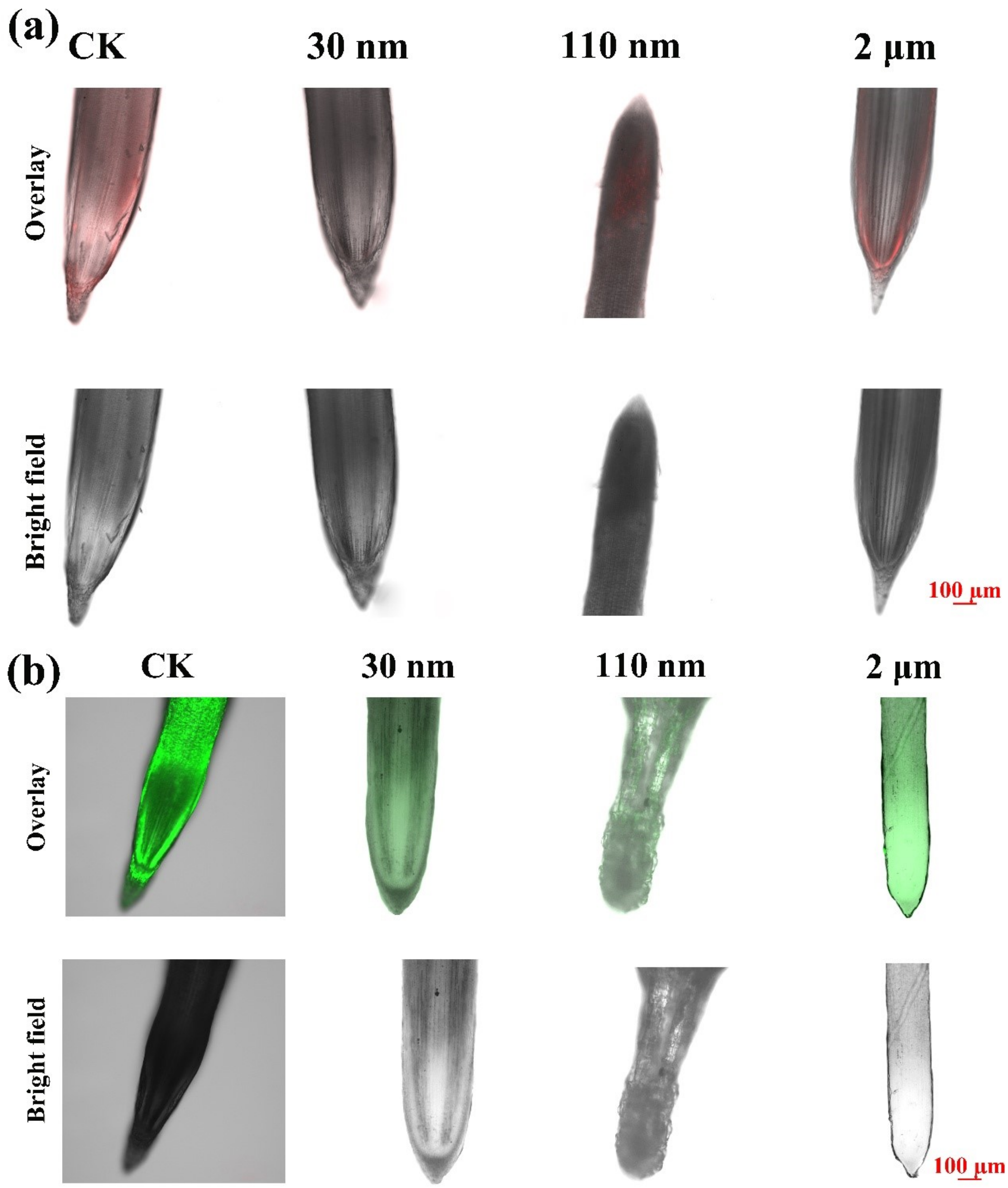

Fig. S5 Fluorescent histochemical staining of roots with fluorescent superimposed bright field and bright smooth images for (a) superoxide radical ($O_2^{\cdot -}$), (b) hydrogen peroxide (H_2O_2).

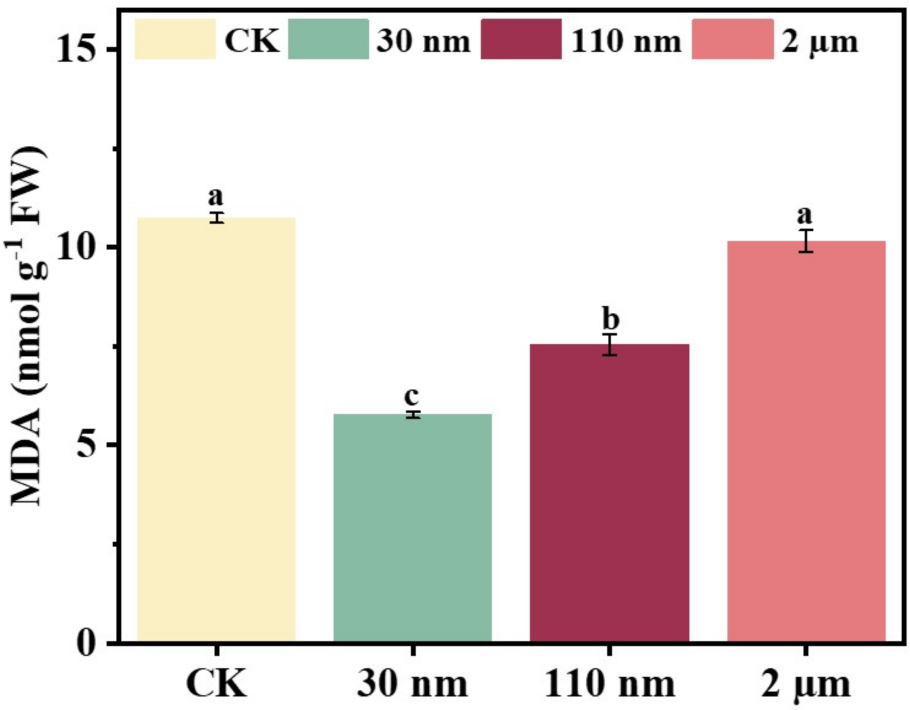

Fig. S6 Malon-dialdehyde (MDA) content in roots of hydroponically grown rice.

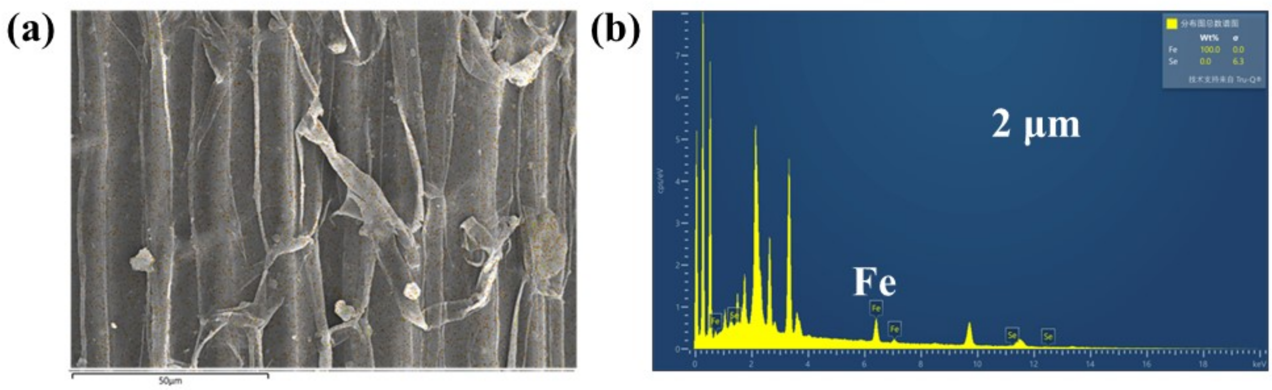

Fig. S7 (a) SEM image of rice roots treated with Se NPs (2 μm), (b) Elemental distribution of Se and Fe (2 μm).

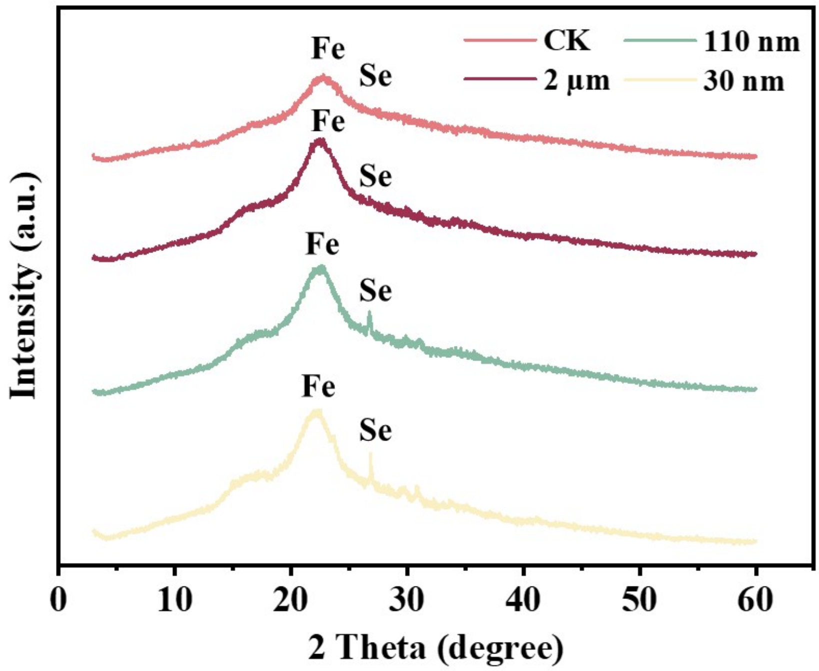

Fig. S8 XRD pattern of iron plaque under different treatments.

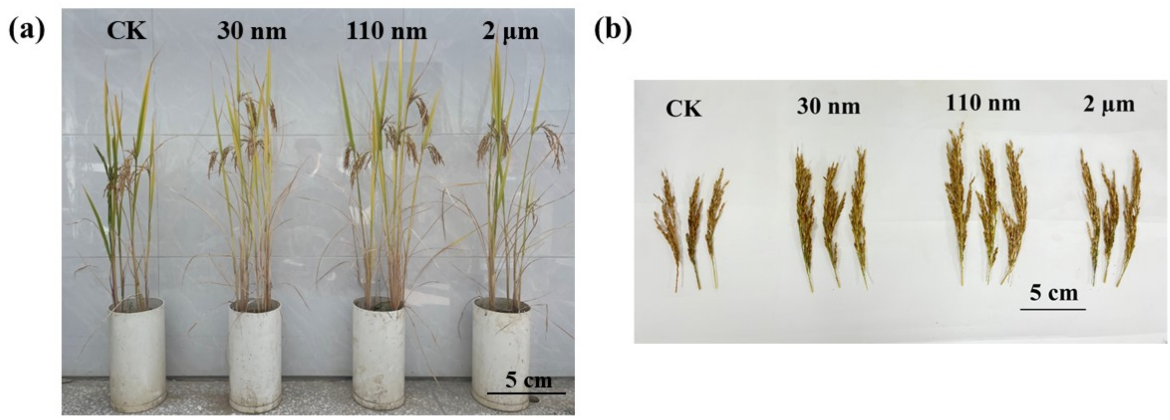

Fig. S9 (a) Photo of rice at maturity under different treatments, (b) Representative photo of rice panicle under different treatments.

References

(1) Y. Li, H. Y. Sun, Z. W. Liu, Y. T. Chu, Y. Z. Huang and Q. L. Bao, Foliar jasmonic acid application reduces Cd and As accumulations in rice grains by regulating physiological, biochemical, and ROS scavenging attributes., *Environ. Technol. Innov.*, 2024, **34**, 103596.

(2) F. Morina, L. Jovanovic, M. Mojovic, M. Vidovic, D. Pankovic and S. V. Jovanovic, Zinc-induced oxidative stress in Verbascum thapsus is caused by an accumulation of reactive oxygen species and quinhydrone in the cell wall., *Physiol. Plant*, 2010, **140**, 209-224.

(3) Z. Hu, J. Fan, K. Chen, E. Amombo, L. Chen and J. Fu, Effects of ethylene on photosystem II and antioxidant enzyme activity in Bermuda grass under low temperature. *Photosynth. Res.*, 2016, **128**, 59–72.