Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information for

Physiological, Transcriptomic and Metabolomic Analyses Reveal Zinc Oxide Nanoparticles Modulate Plant Growth in Tomato

Liangliang Sun[a], Yibo Wang[b], Ruling Wang[c], Ruting Wang[a], Ping Zhang[a], Qiong Ju[a], Jin Xu[a,*]

[a] *College of Horticulture, Shanxi Agricultural University, Taigu 030801, China.*

Email: xujin@sxau.edu.cn

[b] *GanSu Key Laboratory for Utilization of Agricultural Solid Waste Resources, College of Bioengineering and Biotechnology, Tianshui Normal University, Tianshui, GanSu 741000, China*

[c] *CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China*

This file includes:

Supporting Tables

Table S1 List of primers.

	Forwards	Reverse	
Solyc01g104780.3	GTGCATCTGCTTTTGCGTTTG	TTCCCATAGCCAACCATCCAC	VIT4-like
Solyc04g078250.3	CGACCTTTACCAGGGAGTGC	CTCTTGCTGTTGAGGGGAGC	Nramp2-like
Solyc07g043230.3	TCCAAGTCTTTCTTGGTTTCCTCT	CCCCTAATACTATCGCGGGAA	ZIP5-like
Solyc03g031920.3	TGTGGATTCGGGAGCTTTCC	GTATGCCGTGAAGGCTGGTA	YSL7
Solyc11g072570.2	TGCACCACACTTTTCCCCAT	GCGTATTGGTGTGTTGACGG	NRT1
Solyc09g075820.3	ACGTTCTCTCCACCGTTGTC	ACCATGCGAAAGCCGATACA	STP 13 isoform X1
Solyc09g082660.3	GCCTCGAGATGAAGGCCAAT	TCTTATGCTCCACACCAGCC	flavonoid 3',5'-methyltransferase
Solyc10g076670.2	CCATCGGAGAGGCCAAGTTC	AGACACTACGTGCGTTAGCC	leucoanthocyanidin dioxygenase
Solyc05g052680.1	GGAAACGGCTGAGATTTCGT	TAGCCAGAACTTCCCCTGCT	BAHD acyltransferase DCR
X14449.1	TGGATATGCTCCAGTGCTTG	TTCCTTACCTGAACGCCTGT	EF1a

Table S2. Differentially expressed genes in the leaves and roots of tomato seedlings.

(See EXCEL file)

Table S3 Metal element contents in leaves and roots of tomato. Twenty-day-old tomato seedlings were transferred to fresh Hoagland solution with sufficient (control) or 1/100 Fe (Fe-), and the seedlings were foliar sprayed with 20 mg/L ZnO NPs for 12 days. The ± represent the SEs. Different letters indicate that values were significantly different at $P < 0.05$ according to Tukey's test.

Metal element	control	Fe-	ZnO NPs	ZnO NPs + Fe-
Leaf				
Cu (mg.kg^{-1} DW)	35±2.84a	35.5±2.9a	32±0.35a	34±2.53a
Fe (g.kg^{-1} DW)	0.13±0.01b	0.11±0.004d	0.15±0.003a	0.12±0.005c
Mg (g.kg^{-1} DW)	9.8±1.09b	10.3±0.21a	9.08±0.4ab	11.4±0.24b
Mn (mg.kg^{-1} DW)	212.2±12.9b	310.4±10.23a	200±7.9b	305±6.83a
Zn (mg.kg^{-1} DW)	31.3±1.4d	48.9±1.56b	38.9±0.6c	58.4±1.09a
K (g.kg^{-1} DW)	68.5±1.5b	67.2±2.38b	65±1.77b	74.3±2.58a
Ca (g.kg^{-1} DW)	25.1±0.69c	30.6±0.45a	27.2±0.16b	30.6±0.28a
Root				
Cu (mg.kg^{-1} DW)	150.3±6.2a	146.2±3.18ab	139.3±2.93b	144.7±2.13ab
Fe (g.kg^{-1} DW)	1.56±0.15a	0.33±0.04c	1.42±0.12a	0.5±0.11b
Mg (g.kg^{-1} DW)	7.99±0.31d	8.9±0.04c	9.52±0.3b	10.6±1.98a
Mn (mg.kg^{-1} DW)	2988±89.9a	2294.9±23.4b	2922.5±24.8a	2921.3±13.1a
Zn (mg.kg^{-1} DW)	208.6±3.68c	253.9±3.44a	155±4.9d	219.1±1.12b
K (g.kg^{-1} DW)	76.8±1.75a	76.1±0.91a	72.2±2.63a	77.9±0.66a
Ca (g.kg^{-1} DW)	4.4±0.15b	4.8±0.12a	4.84±0.08a	4.7±0.17a

Table S4 Sugar contents in leaves and roots of tomato. Twenty-day-old tomato seedlings were transferred to fresh Hoagland solution with sufficient (control) or 1/100 Fe (Fe-), and the seedlings were foliar sprayed with 20 mg/L ZnO NPs for 12 days. The ± represent the SEs. Different letters indicate that values were significantly different at $P < 0.05$ according to Tukey's test.

Suagrs	control	Fe-	ZnO NPs	ZnO NPs + Fe-
Leaf				
Sucrose (mg g^{-1} FW)	2.3±0.12c	2.19±0.21c	3.2±0.26b	4.2±0.31a
Starch (μmol Glc g^{-1} FW)	1.4±0.1b	0.73±0.045d	1.96±0.11a	1.21±0.08c
Glucose (μmol g^{-1} FW)	1.56±0.1b	0.89±0.07c	1.78±0.12a	1.47±0.2b
Root				
Sucrose (mg g^{-1} FW)	1.45±0.08c	1.56±0.09c	2.12±0.22b	3.1±0.25a
Starch (μmol Glc g^{-1} FW)	0.79±0.07b	0.61±0.04c	0.88±0.06a	0.78±0.04b
Glucose (μmol g^{-1} FW)	0.89±0.07b	0.62±0.04c	1.04±0.05a	0.9±0.06b

Table S5 Amino acid contents in leaves and roots of tomato. Twenty-day-old tomato seedlings were transferred to fresh Hoagland solution with sufficient (control) or 1/100 Fe (Fe-), and the seedlings were foliar sprayed with 20 mg/L ZnO NPs for 12 days. The ± represent the SEs. Different letters indicate that values were significantly different at $P < 0.05$ according to Tukey's test.

Amino acids	control	Fe-	ZnO NPs	ZnO NPs + Fe-
Leaf				
Gly (μg g^{-1} FW)	5.7±0.42c	6.1±0.71bc	6.4±0.71ab	7.1±0.57a
His (μg g^{-1} FW)	2.34±0.18b	2.43±0.25b	3.3±0.41a	3.43±0.51a
Ser (μg g^{-1} FW)	8.73±0.68c	9.8±0.78b	9.21±0.88bc	10.33±1.1a
Arg (μg g^{-1} FW)	7.44±0.55b	5.7±0.43c	8.7±0.9a	7.97±0.77b
Ala (μg g^{-1} FW)	4.3±0.28a	3.33±0.24b	4.7±0.55a	3.89±0.27a
Pro (μg g^{-1} FW)	3.88±0.23b	4.4±0.37a	4.1±0.34b	4.67±0.53a
Met (μg g^{-1} FW)	0.31±0.041c	0.28±0.017c	1.2±0.08a	0.88±0.067b
Thr (μg g^{-1} FW)	6.4±0.47b	5.1±0.45c	8.1±0.77a	7.44±0.66a
Leu (μg g^{-1} FW)	9.74±0.88b	8.78±0.91c	11.2±0.89a	10.2±0.93ab
Lys (μg g^{-1} FW)	1.89±0.21b	1.78±0.23b	2.91±0.3a	3.12±0.25a
Ile (μg g^{-1} FW)	2.1±0.11a	1.88±0.17a	2.23±0.33a	1.78±0.31a
Val (μg g^{-1} FW)	4.1±0.46b	2.89±0.31c	5.6±0.71a	3.87±0.45b
Tyr (μg g^{-1} FW)	1.89±0.2b	2.3±0.31b	2.12±0.17b	2.67±0.4a
Phe (μg g^{-1} FW)	3.4±0.28a	2.7±0.19b	3.56±0.24a	3.2±0.44a
Cys (μg g^{-1} FW)	0.89±0.056a	0.91±0.07a	0.8±0.072a	0.94±0.078a
Asp (μg g^{-1} FW)	14.8±2.4c	21.3±3.1a	17.8±2.6b	24.5±2.2a
Glu (μg g^{-1} FW)	11.2±1.8c	9.8±2.3d	14.5±1.89a	12.9±1.45b
Root				
Gly (μg g^{-1} FW)	6.45±0.51a	4.2±0.33c	6.2±0.48a	5.5±0.45b
His (μg g^{-1} FW)	3.16±0.33a	2.89±0.31a	3.22±0.28a	3.34±0.21a
Ser (μg g^{-1} FW)	8.21±0.77bc	7.9±0.67c	8.89±0.76b	9.23±0.83a
Arg (μg g^{-1} FW)	6.21±0.45a	4.5±0.48b	5.97±0.6a	4.67±0.31b
Ala (μg g^{-1} FW)	5.1±0.49a	4.2±0.34b	5.33±0.47a	5.34±0.61a
Pro (μg g^{-1} FW)	4.32±0.36b	5.6±0.47a	4.11±0.34b	5.3±0.41a
Met (μg g^{-1} FW)	0.22±0.017b	0.17±0.023b	0.3±0.041a	0.34±0.019a
Thr (μg g^{-1} FW)	5.51±0.61a	4.5±0.61b	3.77±0.2c	4.3±0.32b
Leu (μg g^{-1} FW)	8.43±0.71b	8.12±0.77b	9.64±0.83a	8.51±0.77b
Lys (μg g^{-1} FW)	2.5±0.18b	2.34±0.19b	2.78±0.31b	3.3±0.24a
Ile (μg g^{-1} FW)	1.87±0.25b	2.1±0.25b	2.67±0.38a	2.89±0.4a
Val (μg g^{-1} FW)	3.2±0.22a	2.67±0.37b	2.41±0.19b	3.04±0.27a
Tyr (μg g^{-1} FW)	1.67±0.17b	1.34±0.21b	3.56±0.46a	1.54±0.21b
Phe (μg g^{-1} FW)	4.1±0.37a	2.87±0.33b	4.12±0.51a	2.58±0.22b
Cys (μg g^{-1} FW)	0.71±0.067a	0.67±0.081a	0.69±0.055a	0.73±0.08a
Asp (μg g^{-1} FW)	9.8±0.78b	7.8±0.91c	11.3±1.34a	11.6±0.98a
Glu (μg g^{-1} FW)	7.8±0.69b	6.5±0.52c	9.8±0.78a	10.2±1.1a

Supporting Figures

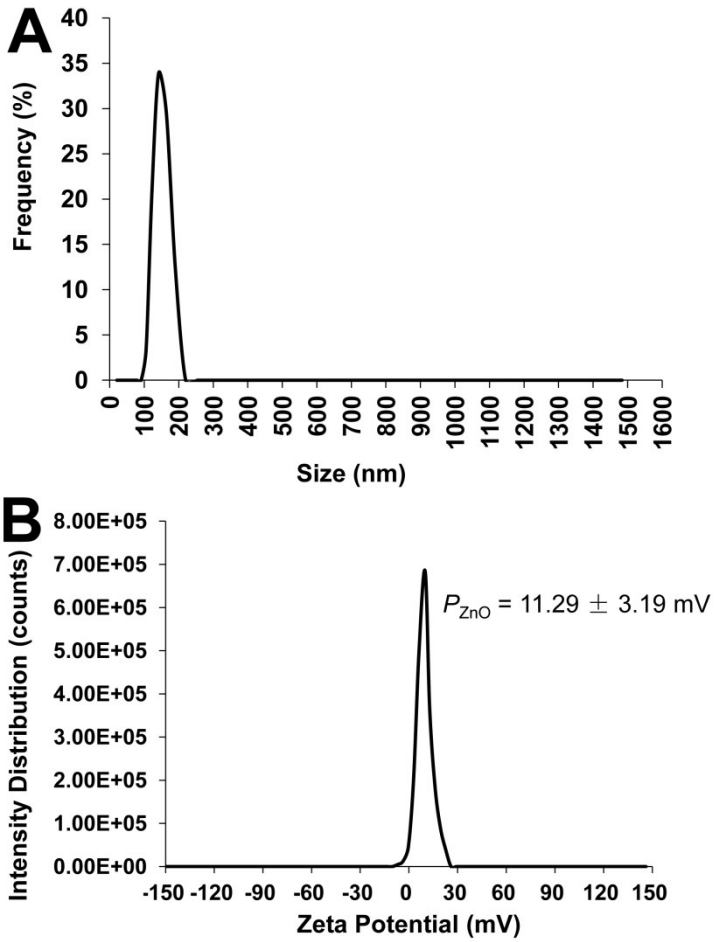

Fig. S1 (A) The hydrodynamic diameter distribution of ZnO NPs in water and (B) the Zeta potential of ZnO NPs.

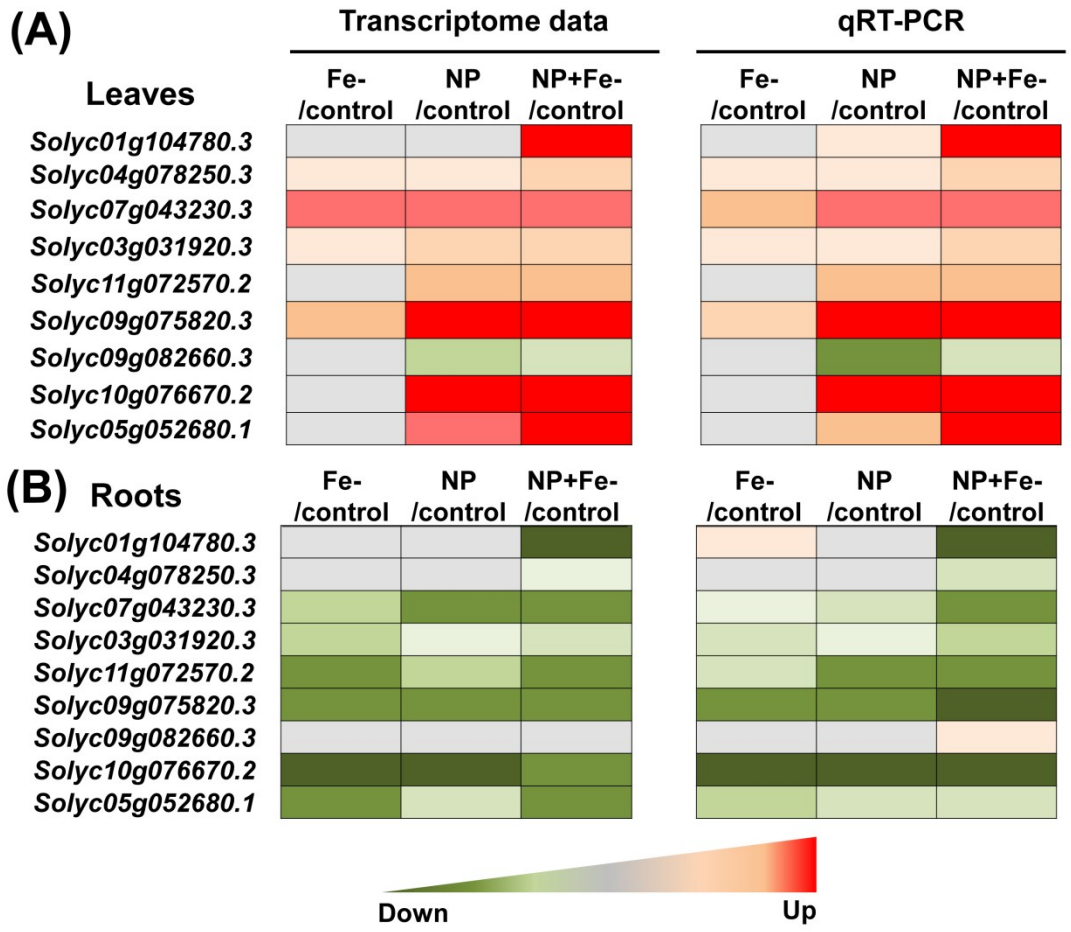

Fig. S2 The relative expression levels of nine genes in the (A) leaves and (B) roots of tomato seedlings compared with those in untreated control were measured by qRT-PCR.

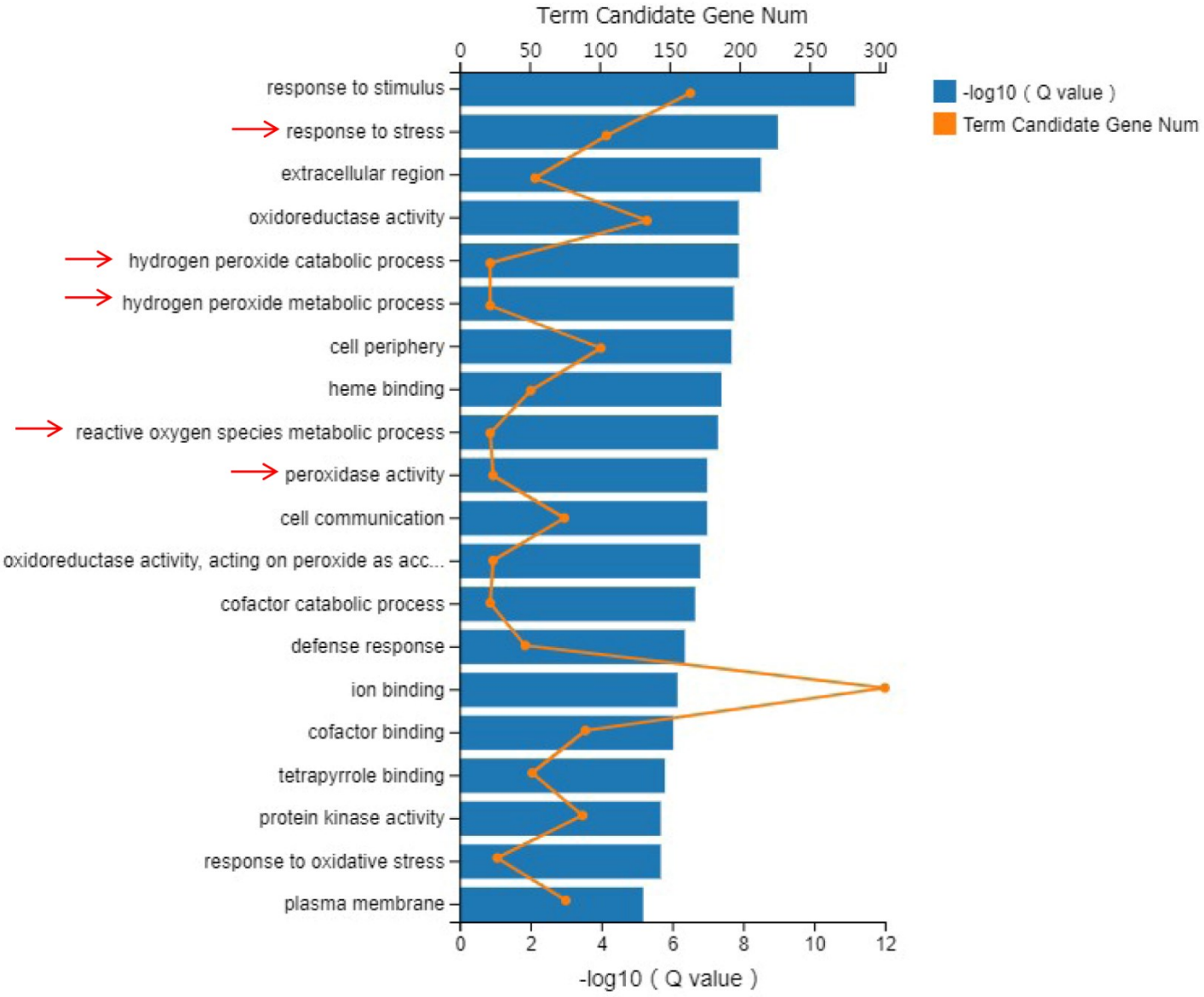

Fig. S3 Gene Ontology (GO) biological process analysis of the differentially expressed genes in ZnO NP-treated leaves. Q value, adjusted *P* value.

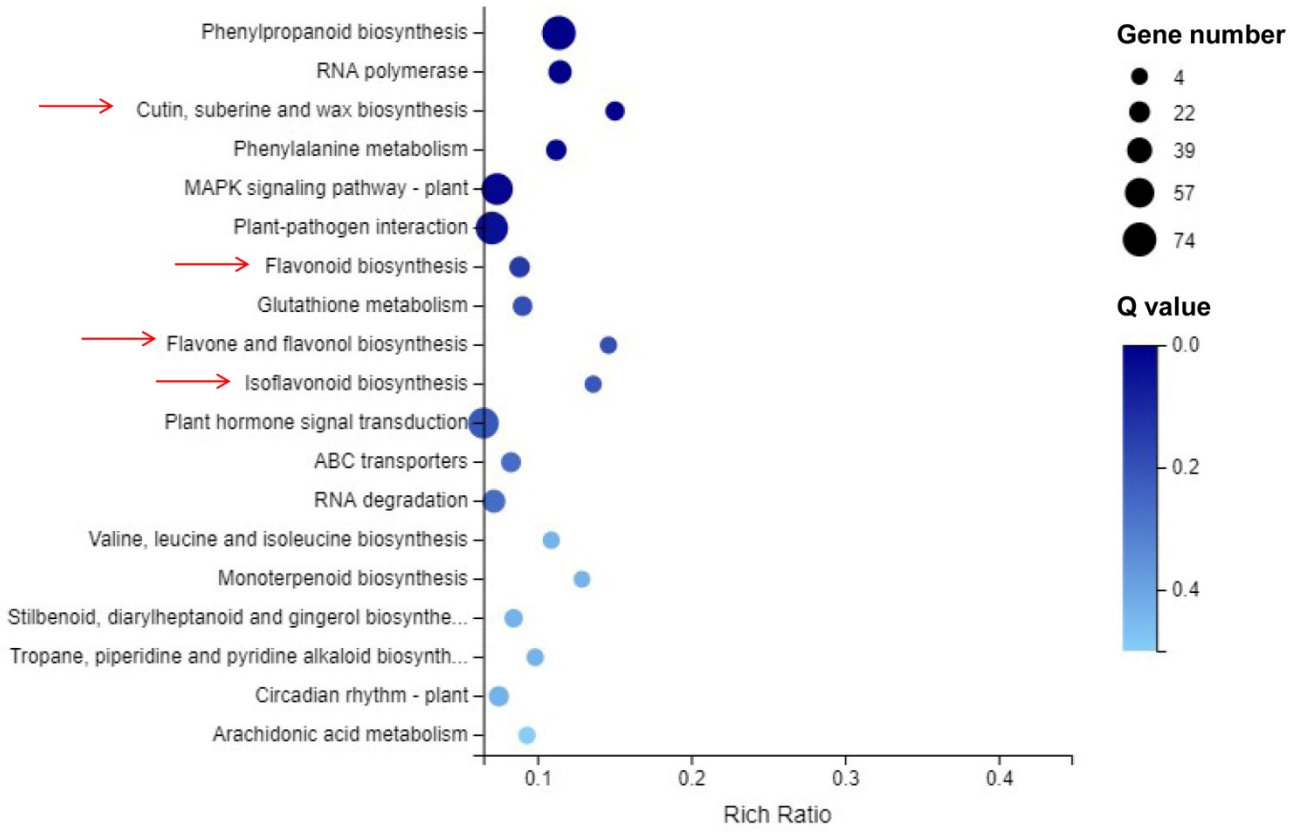

Fig. S4 KEGG pathway enrichment analysis of the differentially expressed genes in ZnO NP-treated tomato roots. Q value, adjusted *P* value.

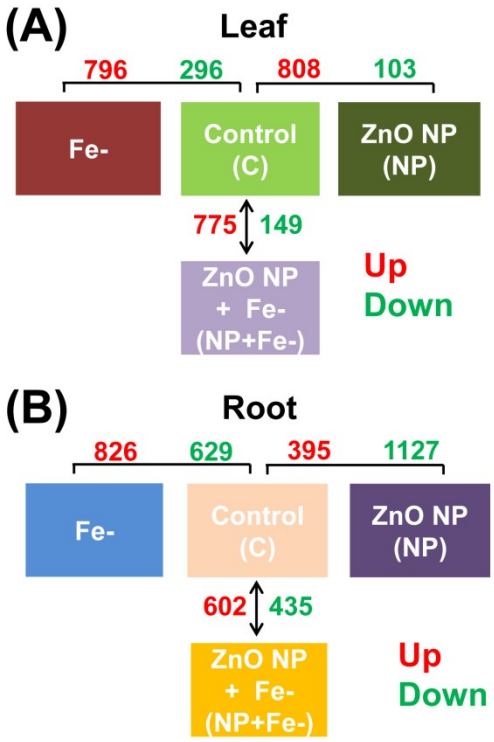

Fig. S5 Experimental design and comparisons between the treatments in leaves (A) and roots (B). Numbers of differentially expressed genes from each comparison are noted in red (upregulated) or green (downregulated). Twenty-day-old tomato seedlings were transferred to fresh Hoagland solution with sufficient (control) or 1/100 Fe (Fe-), and the seedlings were foliar sprayed with 20 mg/L ZnO NPs for 12 days.

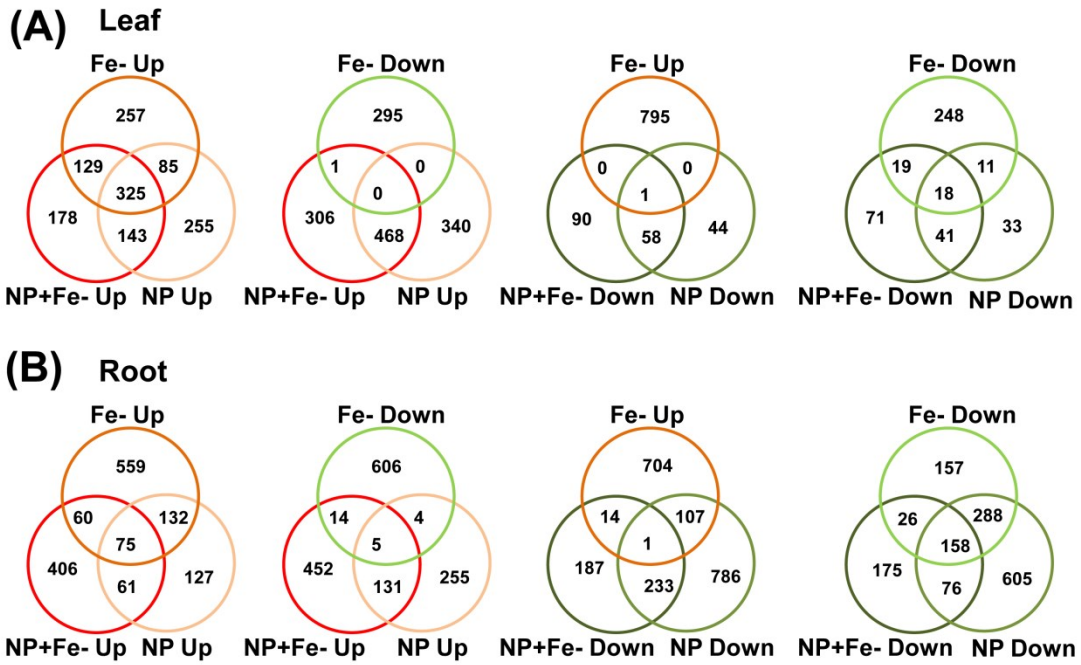

Fig. S6 Comparative transcriptome analysis of tomato plants in response to Fe deficiency, ZnO nanoparticles (NP) treatment and their combination. Twenty-day-old tomato seedlings were transferred to fresh Hoagland solution with sufficient (control) or 1/100 Fe (Fe-), and the seedlings were foliar sprayed with 20 mg/L ZnO NP for 12 days. (A,B) Venn diagram analysis showing the overlapping upregulated (Up) or downregulated (Down) genes among the comparisons in leaves (A) and roots (B).

Supporting Materials and methods

Determination of Antioxidative Enzyme Activity

After treatment, the fresh sample (0.5 g) was ground in liquid nitrogen to extract total protein. The powder was suspended in protein extraction buffer (3 mL, 50 mM, pH 7.8). For the determination of APX activity, the powder was suspended in HEPES buffer (pH 7.5). The extract was centrifuged at a speed of 12,000 rpm for 30 min, and the supernatant was taken to determine the enzyme activity. SOD activity was measured by recording the inhibition of formazan formation by enzymes.[43,44] The reaction mixture was subjected to a temperature of 25°C and a light intensity of 120 mol m^{-2} s^{-1} for 15 minutes. The absorbance value at a wavelength of 560 nm was determined. The amount of enzyme required to inhibit the reduction rate of 50% NBT was defined as one unit of SOD activity. CAT activity was determined by measuring the decrease in H_2O_2 absorbance at 240 nm. The activity was estimated with an extinction coefficient of 39.4 mM^{-1} cm^{-1} and is expressed as μmol H_2O_2 min^{-1} mg $protein^{-1}$. POD activity was measured as described by Teisseire and Guy (2000).[44] APX activity was estimated by the absorbance decrease at 290 nm, and the absorbance decrease followed the H_2O_2-dependent oxidation of ascorbate. The activity was estimated with an extinction coefficient of 2.8 mM^{-1} cm^{-1} and is expressed as nmol ascorbate oxidized min^{-1} mg $protein^{-1}$. The experiments were performed with three independent biological replicates, with 6 seedlings used in each biological replicate.

Determination of the Contents of Sugars and Amino Acids

After treatment, the samples were collected after 8 h of exposure to light and were then

ground in 80% ethanol and extracted twice at 80°C. The supernatants were used to measure soluble sugars. The contents of soluble sugars and starch were measured as described by Eimert *et al.*.[45] The free amino acid contents were determined using high-performance liquid chromatography (HPLC) as described previously.[46] The assays were repeated three times, with 6 seedlings in each replicate.

Comparative transcriptomics

Total RNA was extracted using RNAiso Plus (TaKaRa, Japan) from 24 samples (the roots and leaves of control, Fe-deficient, ZnO NP-treated and ZnO NP-treated Fe-deficient tomato seedlings with three biological replicates). The cDNA was synthesized using a PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa). The mRNA was purified using Oligo (dT) magnetic bead and then was randomly fragmented using fragmentation buffer. Random hexamers were used to synthesize the first- and second-strand cDNA. The cDNA was purified and subjected to end repair and addition of the A tail, and then was linked to a sequencing adaptor and then subjected to fragment size selection using AMPure XP beads. The cDNA library was subsequently obtained through PCR amplification.

Comparative transcriptome analysis was performed using the BGISEQ-500 platform. The raw sequences were transformed into clean tags after certain steps of data processing were performed, including the removal of adaptor sequences, empty reads, and low-quality tags; clean tags were ultimately generated. Sequences from tomato (ITAG4.0, https://solgenomics.net/) were used to obtain the reference genome sequence. All of the clean tags were mapped to the reference sequences. The

differentially expressed genes (DEGs) were identified by comparison with the control plants ($\log_2$ fold change (FC) ≥ 1 or ≤ -1 and adjusted *P* value (Q value) ≤ 0.001). Venn diagram (http://www.omicshare.com/tools/Home/Soft/venn) was carried out to analysis the DEGs.