Supporting Information

Cerium oxide nanomaterials improve cucumber flowering, fruit yield and quality: the rhizosphere effect

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Text S1 Determination of IAA and tryptophan

The contents of IAA in soil and plant samples were determined by HPLC-MS/MS using the previous method.[1] The fresh samples were homogenized in liquid nitrogen and extracted with ethyl acetate (containing 10 μg L^{-1} butylated hydroxytoluene). The mixture was ultrasonicated in an ice bath for 15 min and centrifuged at 12000 rpm at 4 °C for 15 min. The supernatant was vacuum freeze-dried at room temperature and then re-suspended in 70% ethanol (V/V) and 10 μL was used to determine IAA.

HPLC condition details:

Five μL of extraction was injected in HPLC with a T3 column. Mobile phase parameters were as follows: A, 0.01% formic acid/water (v/v); B, 0.01% formic acid/acetonitrile (v/v). The elution gradient was as follows: 0 min, 5% B; 1.5 min, 5% B; 9 min, 70% B; 10 min 70% B; 10.1 min, 5% B; 15 min, 5% B at the flow rate of 0.35 mL min^{-1}.

MS condition details:

PRM (Parallel Reaction Monitoring) was used for collection. The MS^2 resolution was set to 17500. The isolation window and collision energy were 3.0 m z^{-1} and nce: 10. IAA was detected in negative ion mode.

For the determination of tryptophan, fresh samples were homogenized in liquid nitrogen and extracted with 80% methanol. The mixture was ultrasonicated in an ice bath for 5 min and centrifuged at 14000 rpm at 4 °C for 15 min. The supernatant was used to determine tryptophan.

HPLC condition details:

Five μL of supernatant was injected in HPLC with a T3 column. Mobile phase parameters were as follows: A, 20 mM ammonium formate (in water); B, 20 mM ammonium formate (in 90% acetonitrile, v/v). The elution gradient was as follows: 0 min, 95% B; 1 min, 95% B; 15 min, 65% B; 17 min 65% B; 18 min, 95% B at the flow rate of 0.25 mL min^{-1}.

MS condition details:

PRM was used for collection. The MS^2 resolution was set to 17500. The isolation window and collision energy were 3.0 m z^{-1} and nce: 10, 20 and 40 respectively. Tryptophan was detected in positive ion mode.

Text S2 Effects of CeO_2 NMs on the yield of cucumber

CeO_2 NMs had a positive effect on cucumber growth (Fig. S2). The application of 1, 10 and 50 mg kg^{-1} CeO_2 NMs significantly increased plant height by 11.0%, 14.5% and 4.8% over control at maturity stage, respectively (Fig. S2A). Compared with the control, 10 and 50 mg kg^{-1} CeO_2 NMs significantly enhanced the shoot fresh weight by 29.1% and 24.8%, and the root fresh weight by 33.7% and 39.9%, respectively (Fig. S2C, D). Roots are important organs for plants to absorb water and nutrients.[2] The stimulated hormones may be responsible for increased growth by NMs.[3] Finally, 10 and 50 mg kg^{-1} CeO_2 NMs increased the fresh weight of cucumber fruits by 28.5% and 13.6% (Fig. S2B). The diameter of the cucumber cross section was also induced by CeO_2 NMs and reached maximum at 10 mg kg^{-1} (Fig. S2E). Therefore, 10 mg kg^{-1} CeO_2 NMs promoted the growth of cucumber most and was selected for further investigation.

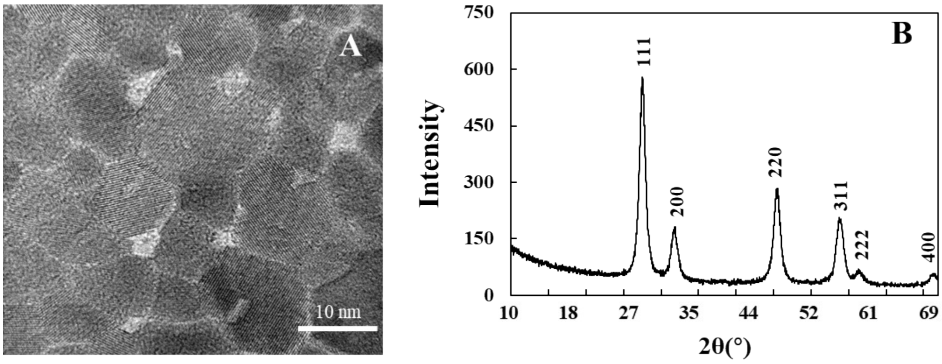

Fig. S1 Characterization of CeO_2 NMs: TEM image (A) and XRD (B).

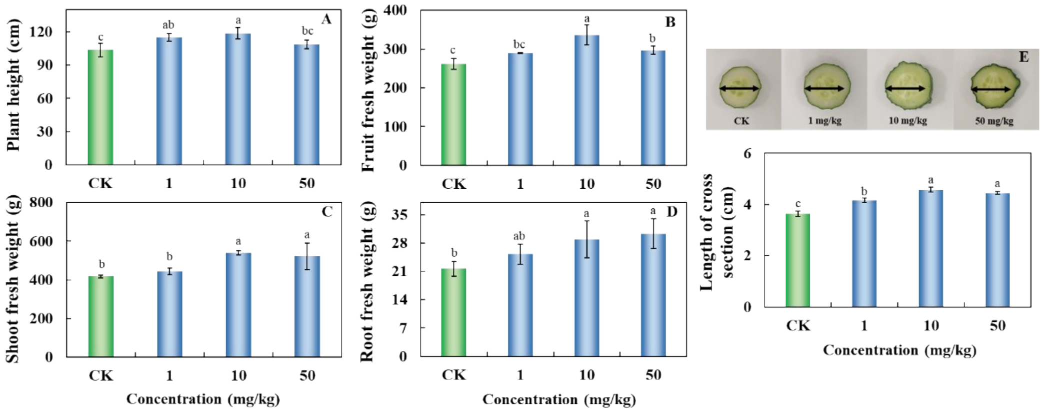

Fig. S2 Cucumber growth as affected by 0, 1, 10 and 50 mg kg^{-1} CeO_2 NMs: plant height (A), total fresh weight of fruit (B), shoot (C) and root (D), cross section of cucumber fruit (E). The error bar with different letters were significantly different corresponding to the standard error of mean value ($p < 0.05$).

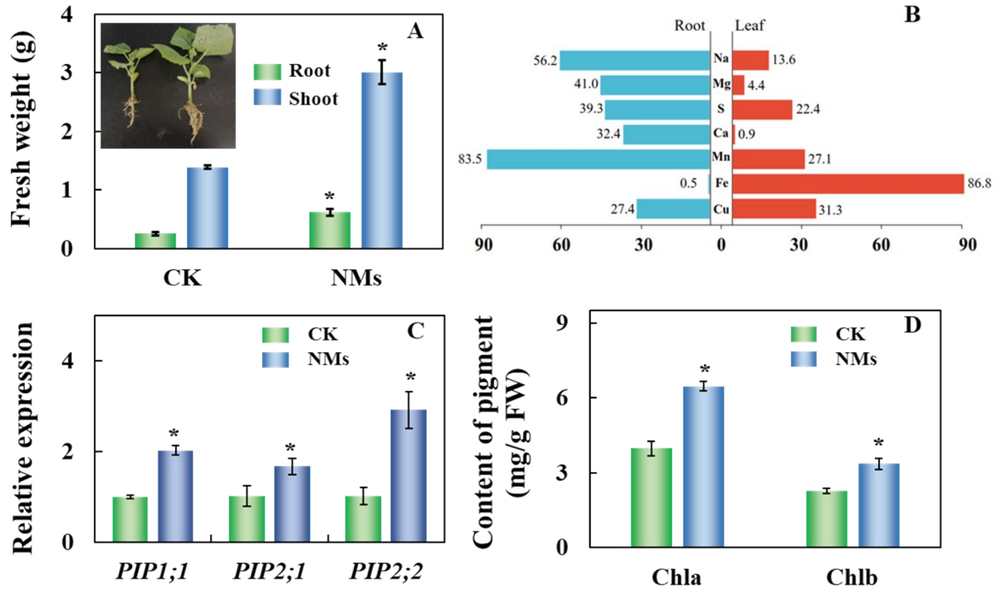

Fig. S3 Cucumber growth as affected by 10 mg kg^{-1} CeO_2 NMs: fresh weight of cucumber root and shoot at seedling stage (A), element content in cucumber root and leaf during flowering (B), relative expressions of aquaporin genes (C) and content of pigment (D) during flowering after exposed to 10 mg kg^{-1} CeO_2 NMs.

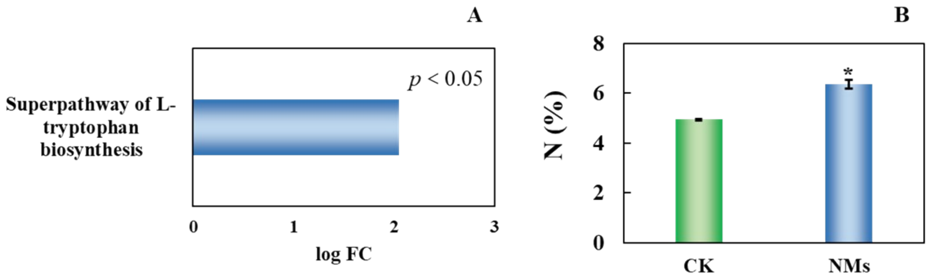

Fig. S4 Microbial tryptophan biosynthesis in rhizosphere soil (A) and total nitrogen content of the apical meristem (B) at seedling stage after exposed to 10 mg kg^{-1} CeO_2 NMs.

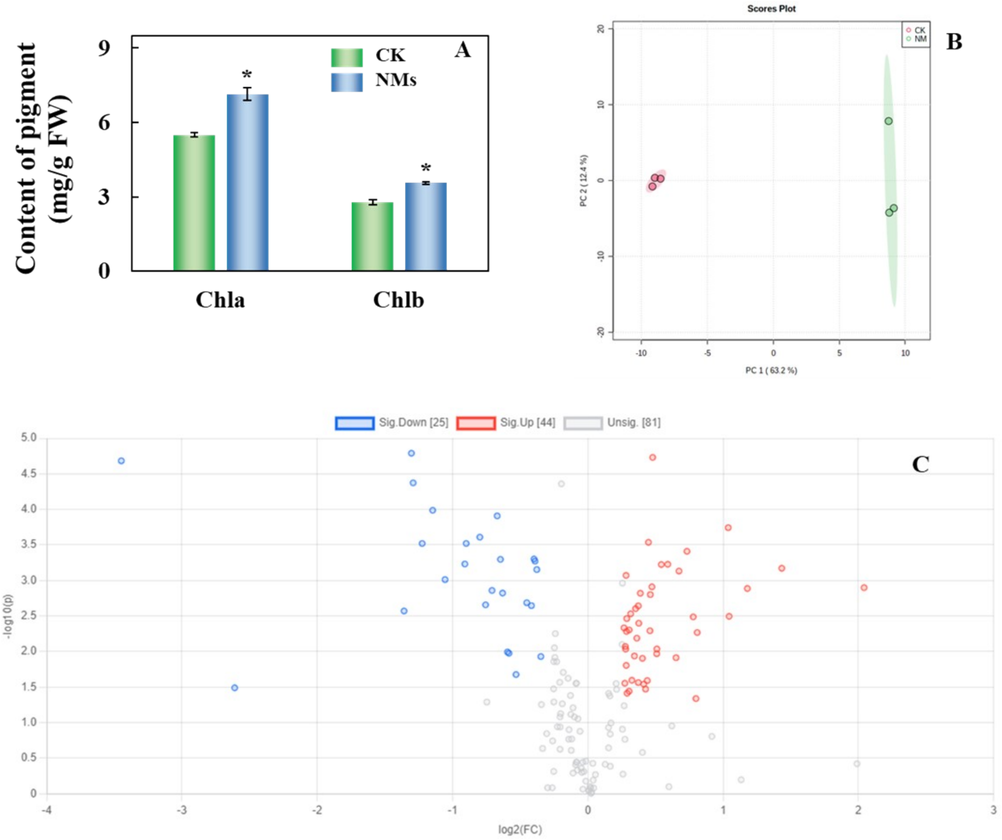

Fig. S5 Chlorophyll content (A), partial least squares-discriminate analysis (PLS-DA) score plots of profiles (B) and volcano plot metabolites of cucumber fruits (C) after exposed to 10 mg kg^{-1} CeO_2 NMs.

Table S1 Zeta potential and hydrodynamic diameter of CeO_2 NMs in deionized water.

NMs	Zeta potential (mV)	Hydrodynamic diameter (nm)
100 mg L^{-1} CeO_2	20.97 ± 0.25	368.51.03 ± 13.64

Table S2 Primer sets list for this study

Gene	Primer	Sequence (5' to 3')
Actin	Forward	GTTCTTACTAATGCTGGTGGTG
	Reverse	TTTACTCACAGTCCCTTGGTCTC
PIP1;1	Forward	TCAAGTCGTGATTAGAGCCATTCC
	Reverse	CAATAATATAATAAGCATTTTGCATAGAGAGAG
PIP1;2	Forward	CATTATTTACAACCACGACGAAGCA
	Reverse	GGATTGAAGAAGCATCATGGATTTAGA
PIP1;3	Forward	CAAATCATCATCAGAGCCATTCCAT
	Reverse	AGAAGAGAACACACGCATATTAAGAAAGA
PIN1	Forward	TCACCTACAGGGTCTAAAAATG
	Reverse	TCCAAACAAACATGTGTAAATC
PIN7	Forward	TCAGAAACTTATCGTCCTCGTC
	Reverse	GGCAGAGTGGACAGAGAAAACA
YUC2	Forward	GCAAGGCAAAAGACTTCACGATCC
	Reverse	GCGACCGCTAAGCCCG
YUC4	Forward	GGGTTGTACACCGTCGGG
	Reverse	ACTCGCCATTGCTCGGC
YUC6	Forward	ACAGAGAAGATGAACGGCGG
	Reverse	CGCCGCAGCTAATCCTGAG
CsSUT4	Forward	GGTTTCTGGGGTTCTGAGCA
	Reverse	CTTAGCAATAGTTTTCCCACAGGTT
CsHT2	Forward	CCGAATCTTCTTTGATGTCCGC
	Reverse	TTTCCCCTGCTACAATCGCCTG
CsHT3	Forward	GCGTCGGCTTCGCAAATCAG
	Reverse	ATTAGGGGTGCCACGGATTCG
CsHT4	Forward	CGGCGGCGTCTTTGTATTC
	Reverse	GCCTTTTGATAGACTCTTTCCTTG
CsSWEET1	Forward	AACTGCCTTATTTGCCTATGGTATG
	Reverse	GCGAACATTGATACGAGAGAAGC
CsSWEET2	Forward	AACTGCCTTATTTGCCTATGGTATG
	Reverse	GCGAACATTGATACGAGAGAAGC
CsSWEET5a	Forward	ACGCTATTCTCAAGTTCGATCCTAA
	Reverse	CATTATCTGTCATTTGCACCTCTGG
CsSWEET7b	Forward	ACGCCACCTTTTACAAATCAACTC
	Reverse	CCGTCGTGTCAGAAATAGGGG
CsSWEET12c	Forward	ATAGCCATAGCAAAGGAAATGAAGC
	Reverse	TGACTTCCAAGGTTTTGTGGTTATG
CsSWEET17a	Forward	GTTTTCCTGATTTACGCACCTTC
	Reverse	CAATACGCTTTTCCCCTTCC
CsSWEET17c	Forward	ACTGTCAATGGCGTTGGTGTT
	Reverse	CCTTCTTTTGTTTGGGAGCG
GMP1	Forward	ATGATCTTGCACCAGATAGAAGC
	Reverse	AGCTGTTCCAAGTGGTTCTGTC
GMP2	Forward	GCAGAGCTAACTTACGGCGTG
	Reverse	TGAACACTTCAAAGACATCCCTG
GMP3	Forward	ATGGAAGACAGTCCGTCACATATC
	Reverse	CCGCATAGTGAAGTAGTTCGTTTG
GLDH	Forward	CTCTTGGGTCGGAATTATCATG
	Reverse	GCCTTGTTGTATTCGTCAACAG
MIOX1	Forward	TGTTGTAGGAGATACACACCCTG
	Reverse	CTCTTTGGCCACCAAATACATATAG
MIOX2	Forward	CGTCCATCACAAGTACTTCAAAG
	Reverse	ACGATAAATAAGCCGGCATC

Table S3 Differentially expressed metabolites of cucumber leaves in CK and upon CeO_2 NMs

Metabolites	CK	NMs
Sucrose	44861893.0	122595108.9
Fructose	41041325.4	211633351.5
Mannose	187760144.5	491627641.2
Malic acid	1308514172.0	1707071355.0
Citric acid	437158803.9	601262011.6
cis Aconitic	224799513.5	353303396.2

Table S4 Differentially expressed metabolites of cucumber soil in CK and upon CeO_2 NMs

	Metabolites	CK	NMs
Without plants	Phenylalanine	9297222.0	16282941.5
	Tyrosine	817655.3	1417391.1
	Hesperetin	58603.7	61277.9
	Leucine	1680275.5	3862101.2
	Valine	23680566.2	43694962.5
	Tryptophan	4140608.4	5397665.3
	Indole	15776054.4	19353380.0
	Eleostearic acid	832939.7	1114759.1
	Kaempferol	63310.8	71884.2
	Glutamine	1061219.0	1717273.5
	Citrulline	18160368.6	22363332.3
	Malic acid	2447380.9	2772874.9
	Mannose	4528575.0	7279570.4
	Trehalose	6829417.7	7243418.4
	Pyruvic acid	5504414.9	10311574.1
	Salicylic acid	12871778.0	16178998.2
	Sucrose	6513952.8	7717325.2
	Gluconic acid	381021.8	235159.8
	Ferulic acid	16366865.1	12832443.9
	Mannose-6-phosphate	1358064.9	1010587.4
	Glutathione	635839.9	557871.7
	Arginine	2765879.8	2312203.3
	Citraconic acid	1100150.8	757475.7
Rhizosphere	Phenylalanine	18248943.0	72083392.1
	Tyrosine	2543448.2	16662713.3
	Leucine	40109829.7	181412837.0
	Valine	34230954.4	75771241.6
	Tryptophan	14048262.4	54538672.3
	Indole	16061509.6	28039627.6
	Sucrose	10658908.9	20882879.5
	Hesperetin	61088.7	65282.0
	Eleostearic acid	986436.2	1844934.9
	Pyroglutamic acid	4577335.8	4847866.2
	Indole-3-acrylic acid	19033059.0	59762698.8
	Glutamic acid	5889488.2	45873344.4
	Coumaric acid	2206059.5	2616040.1
	Histidine	11338588.2	14213176.3
	Proline	336789.1	218983.1
	Ferulic acid	2602197.9	2328897.3
	Citrulline	25073868.1	16992143.8
	Glutathione	583404.0	499096.0

Table S5 Differentially expressed metabolites of cucumber flower in CK and upon CeO_2 NMs

Metabolites	**CK**	**NMs**
Tryptophan	11237932349.0	13446138837.0
Indole pyruvate	39738928.2	46616100.3
Serine	10546831392.0	16255619869.0
Tyrosine	89162951.8	582902805.4
Phenylalanine	1076839816.0	1549640162.0
Pyruvate	18251668508.0	26194850912.0
Alanine	212589024.2	291205275.9
Leucine	9403789976.0	12610733470.0
Valine	2660045048.0	3581833990.0
Threonine	159156843.7	233438010.1
Aspartic acid	81271227.7	109949106.4
Asparagine	357231992.8	873546946.8
Malic acid	18158195681.0	25942348391.0
Fumaric acid	843821032.6	1318024667.0
Citric acid	801840903.0	933087315.1
Glutamine	1252557818.0	1817246847.0
Citrulline	5237113115.0	6015491998.0
Proline	3325001224.0	8318785030.0
Arginine	334899114.9	578373136.8
Apigenin	14032378.8	17545129.3
Hesperetin	87838324.4	128126725.9
Quercetin	16490119.0	19861960.2
Rutin	4999470.5	7457221.3

References

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