Supporting Information for

Molecular Basis of Cerium Oxide Nanoparticle Enhancement of Rice Salt Tolerance and Yield

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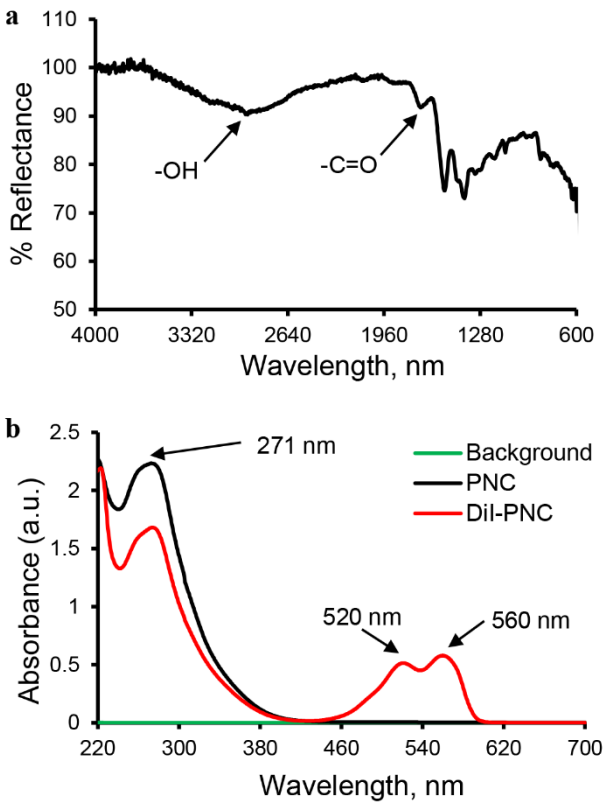

Fig. S1 Characterization of nanoceria surface chemistry and absorption. (a) FTIR spectra confirmed the presence of –OH and –C=O groups on the surface of PNC, indicating the presence of -COOH groups on the nanoparticle surface. (b) A peak of absorbance at 271 nm was observed for PNC. Another two peaks (520 and 560 nm) indicate the presence of DiI in DiI-PNC nanoparticles.

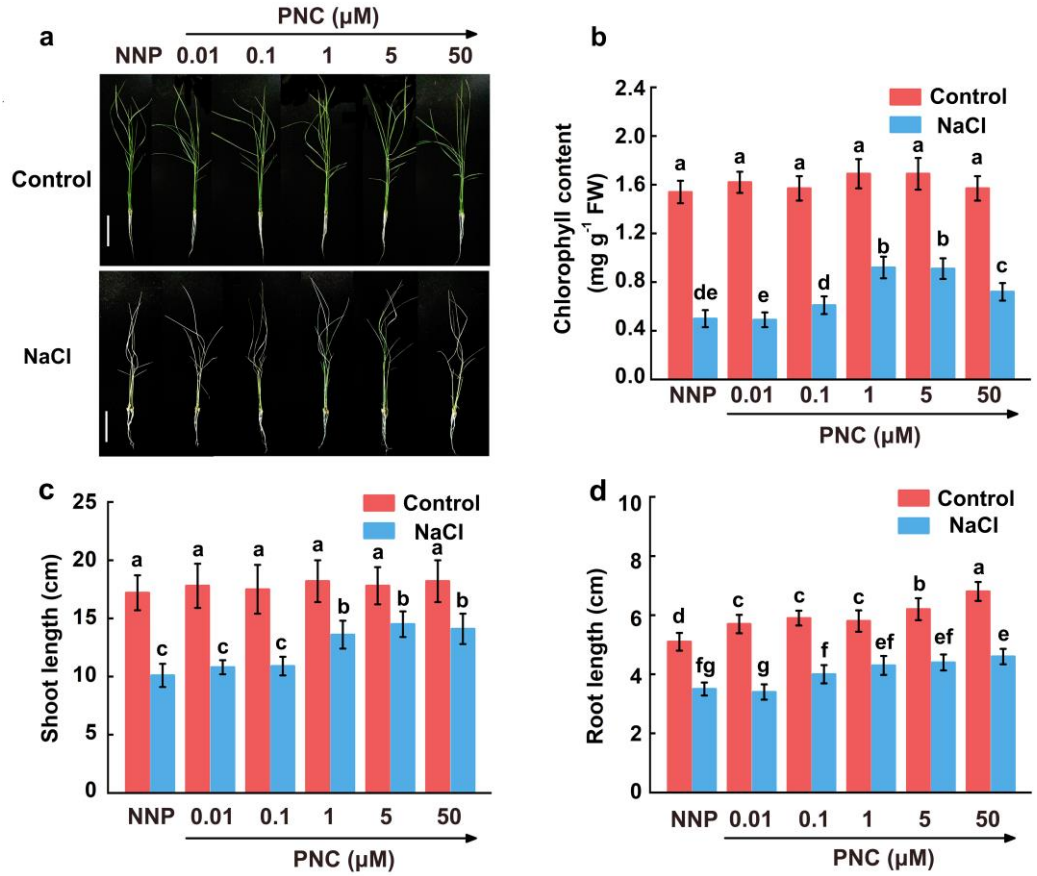

Fig. S2 Nanoceria alleviate growth inhibition in rice under salt stress. (a-d) Concentration dependent effect of nanoceria on morphology (a), chlorophyll content (b), shoot length (c), and root length (d) of rice seedlings exposed to 100 mM NaCl for 8 days. Seedlings without nanoparticles (NNP) were regarded as control. Scale bar = 3 cm. Data are means ± SD of three independent experiments. Lower case letters indicate significant differences at $P < 0.05$ (Duncan's multiple range tests).

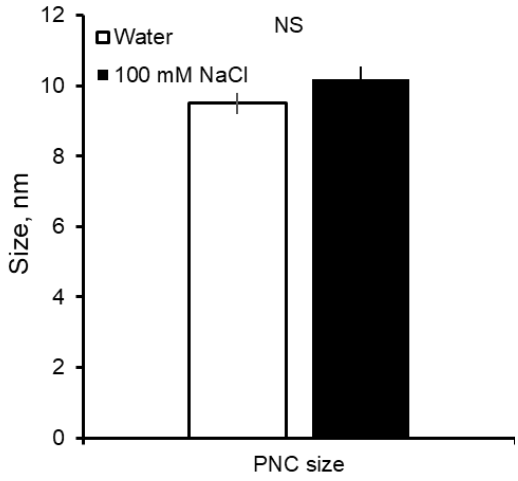

Fig. S3 The size of PNC in the medium. No difference of hydrodynamic size was found between PNC (1 μM) with or without NaCl (100 mM). NS, no significant difference. A statistical comparison was performed by independent samples *t*-test (two tailed).

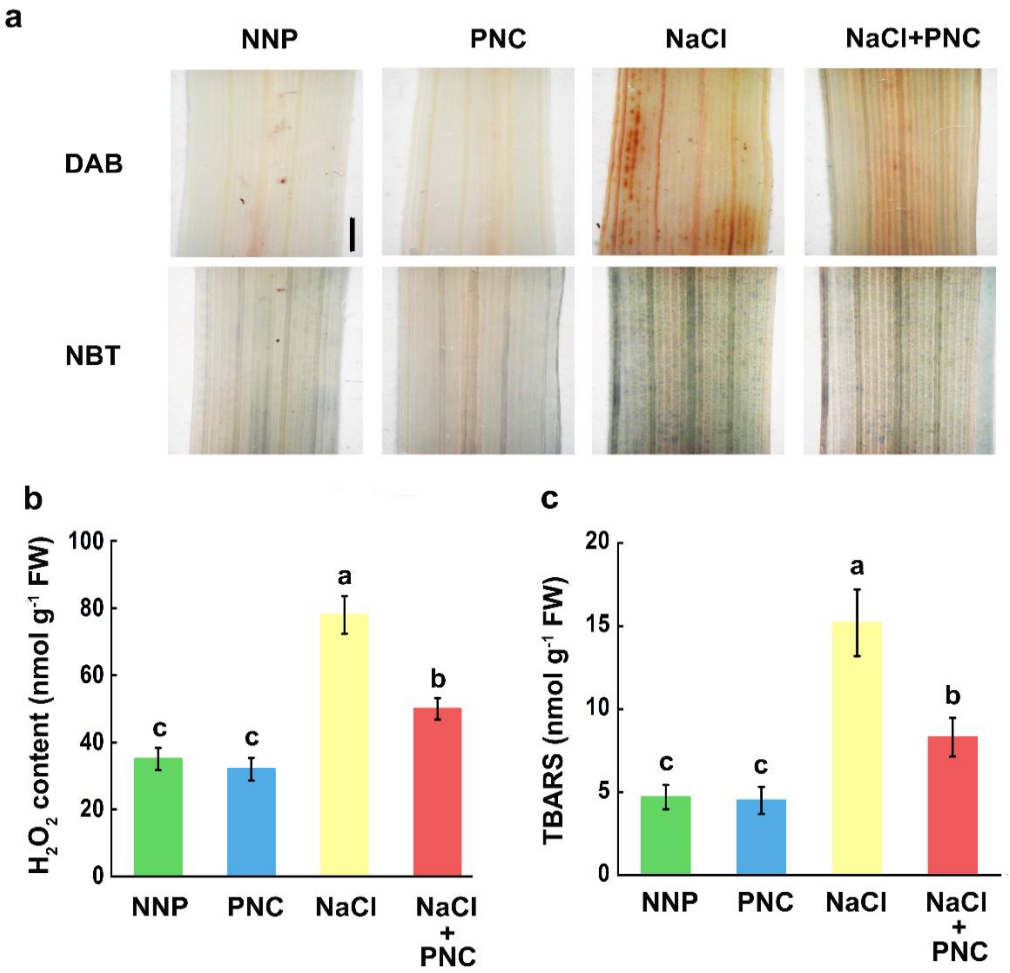

Fig. S4 ROS scavenging by nanoceria in plants exposed to salt stress. (a) In the presence of 100 mM NaCl for 8 days, rice seedling leaves interfaced with PNC were stained with DAB and NBT to assess levels of H_2O_2 and O_2^-, respectively. Seedlings without nanoparticles (NNP) were regarded as control. Scale bar = 1 cm. (b) Leaf H_2O_2 content was determined by spectrophotometric method. (c) Lipid peroxidation analyzed by thiobarbituric acid reactive substance (TBARS) test further confirmed the role of PNC as ROS scavengers in plants (c). Data are means ± SD of three independent experiments. Lower case letters indicate significant differences at $P < 0.05$ (Duncan's multiple range tests).

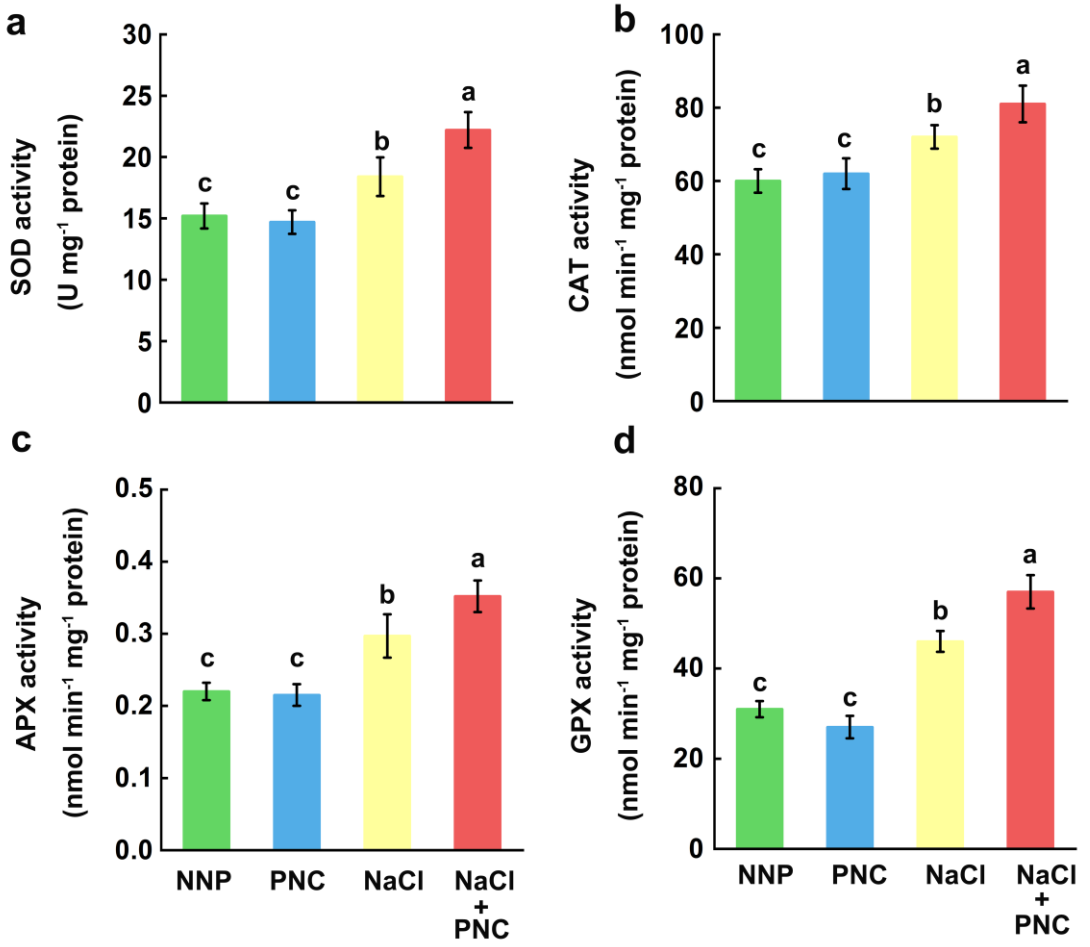

Fig. S5 Enhancement of antioxidant enzyme activity by nanoceria in rice plants under salt stress. (a-d) Activities of leaf antioxidant enzymes including superoxide dismutase (SOD, a), catalase (CAT, b), ascorbate peroxidase (APX, c), and glutathione peroxidase (GPX, d) were analyzed after the addition of NaCl or PNC, alone or combination for 6 days. Seedlings without nanoparticles (NNP) were regarded as control. Data are means ± SD of three independent experiments. Lower case letters indicate significant differences at $P < 0.05$ (Duncan's multiple range tests).

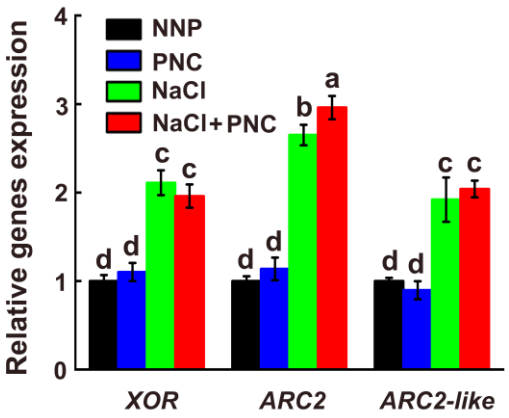

Fig. S6. The relative transcript levels of NO production-related genes including *XOR*, *ARC2*, and *ARC2-like* in rice seedling leaves (24 h after treatment). Transcript levels were measured by RT-qPCR and normalized by *OsActin1* and *OsActin2*. Different lower-case letters indicate significant differences at $P < 0.05$ (one-way ANOVA, Duncan's multiple range tests).

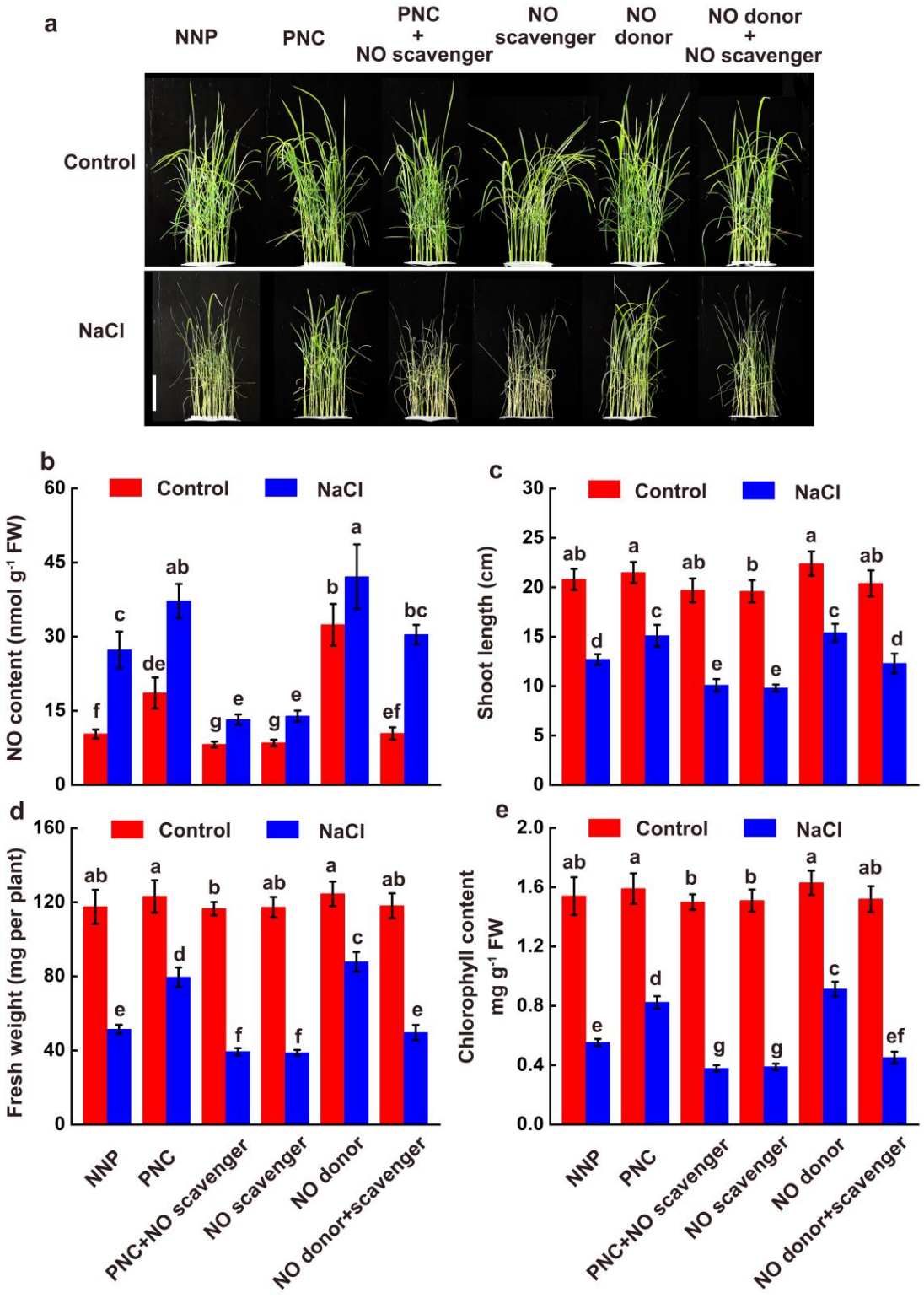

Fig. S7 Nanoceria enhancement of salt tolerance in seedlings is sensitive to endogenous NO levels. (a) Images of two-week-old rice seedlings treated with PNC (1 μM), NO donor (SNP, 10 μM), and NO scavenger (PTIO, 200 μM) and

combinations of these chemicals, in the presence or absence of salt stress (100 mM NaCl) for 8 d. PNC-enhanced endogenous NO content determined by Griess reagent (24 h; b) and performance (8 d) in shoot length (c), fresh weight (d), and chlorophyll content (e) of rice seedlings under salinity stress were sensitive to the scavenger of NO. Scale bar = 5 cm. Data are means ± SD of three independent experiments. Different lower case letters indicate significant differences at $P < 0.05$ (one way ANOVA, Duncan's multiple range tests).

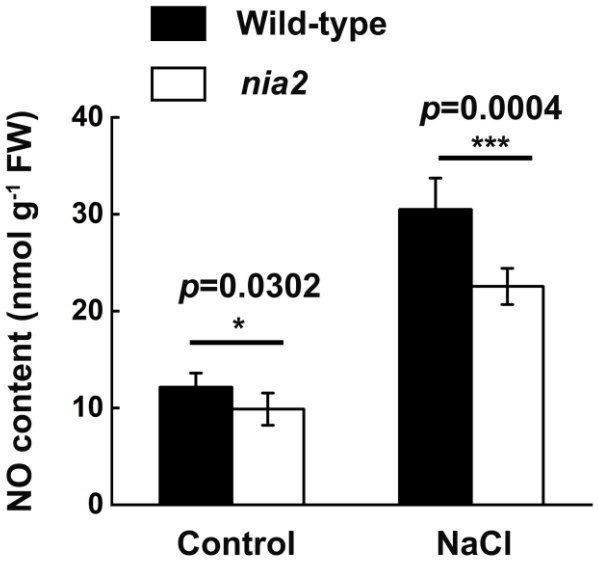

Fig. S8 NO contents in WT and *nia2* mutant. Comparison of endogenous NO contents of rice seedling leaves (24 h after treatment) of wild-type and *nia2* mutant in the presence and absence of NaCl as shown in Figure 4c and Figure 5e. Statistical comparisons were performed by independent samples t-test (two tailed) between leaves with PNC and NNP treatments (*P < 0.05, ***P < 0.001).

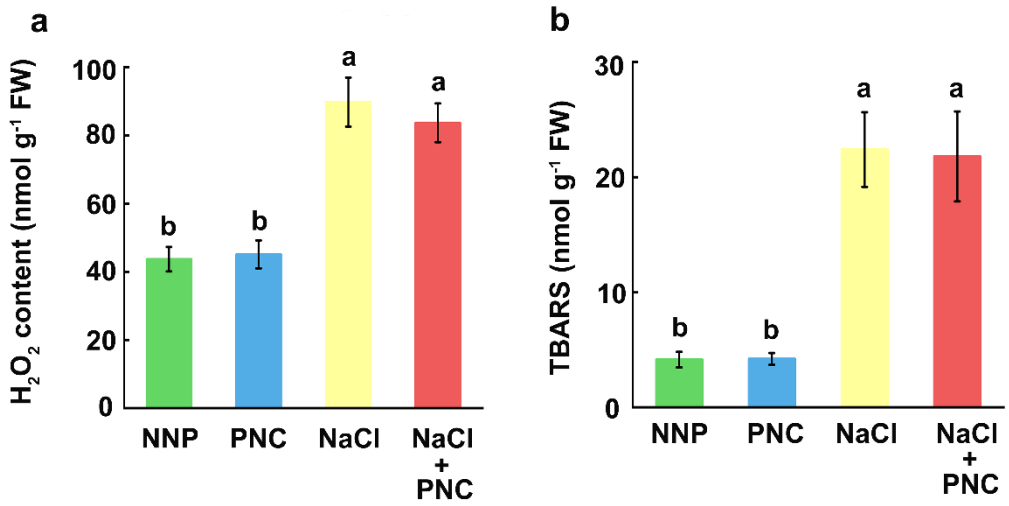

Fig. S9 PNC failed to mitigate ROS overaccumulation in *nia2* mutant rice leaves under salt stress. (a) H_2O_2 content and (b) lipid peroxidation was determined in two-week-old *nia2* mutant embedded with or without PNC in the presence or absence of 100 mM NaCl for 8 days. Data are means ± SD of three independent experiments. Lower case letters indicate significant differences at $P < 0.05$ (Duncan's multiple range tests).

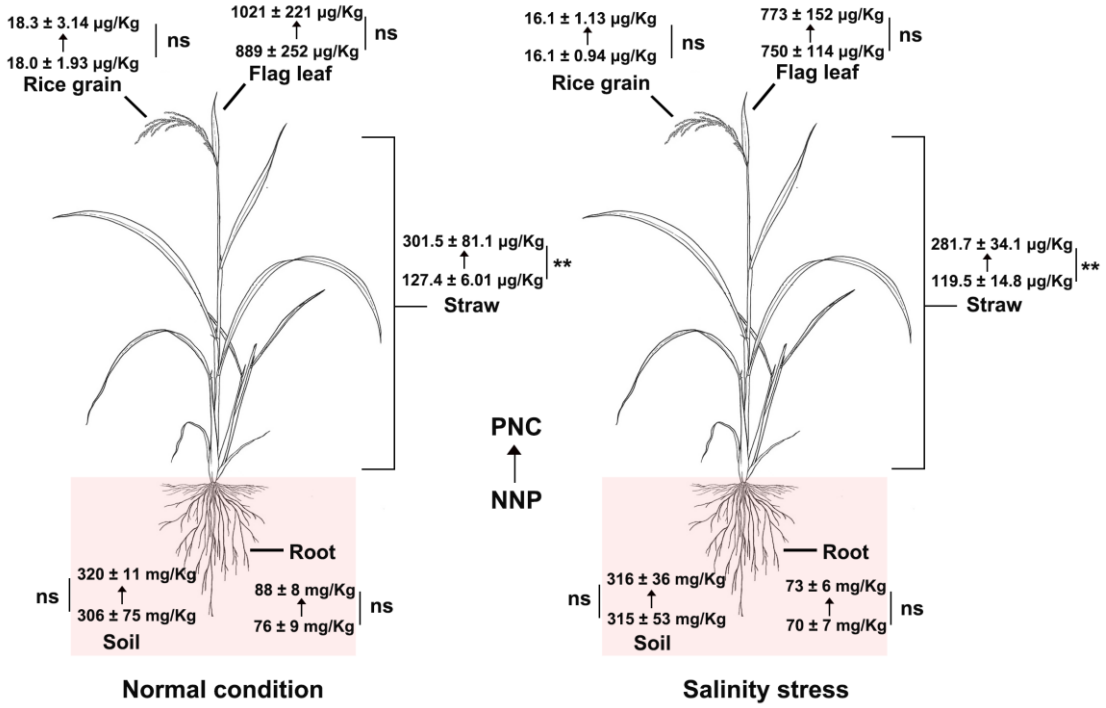

Fig. S10 Cerium contents in soil and different parts of rice plants with or without PNC administration in the presence or absence of NaCl treatment. Data are means ± SD of three independent experiments. Statistical comparisons were performed by independent samples t-test (two tailed) between samples with PNC and NNP (**P < 0.01). NS, no significant difference.

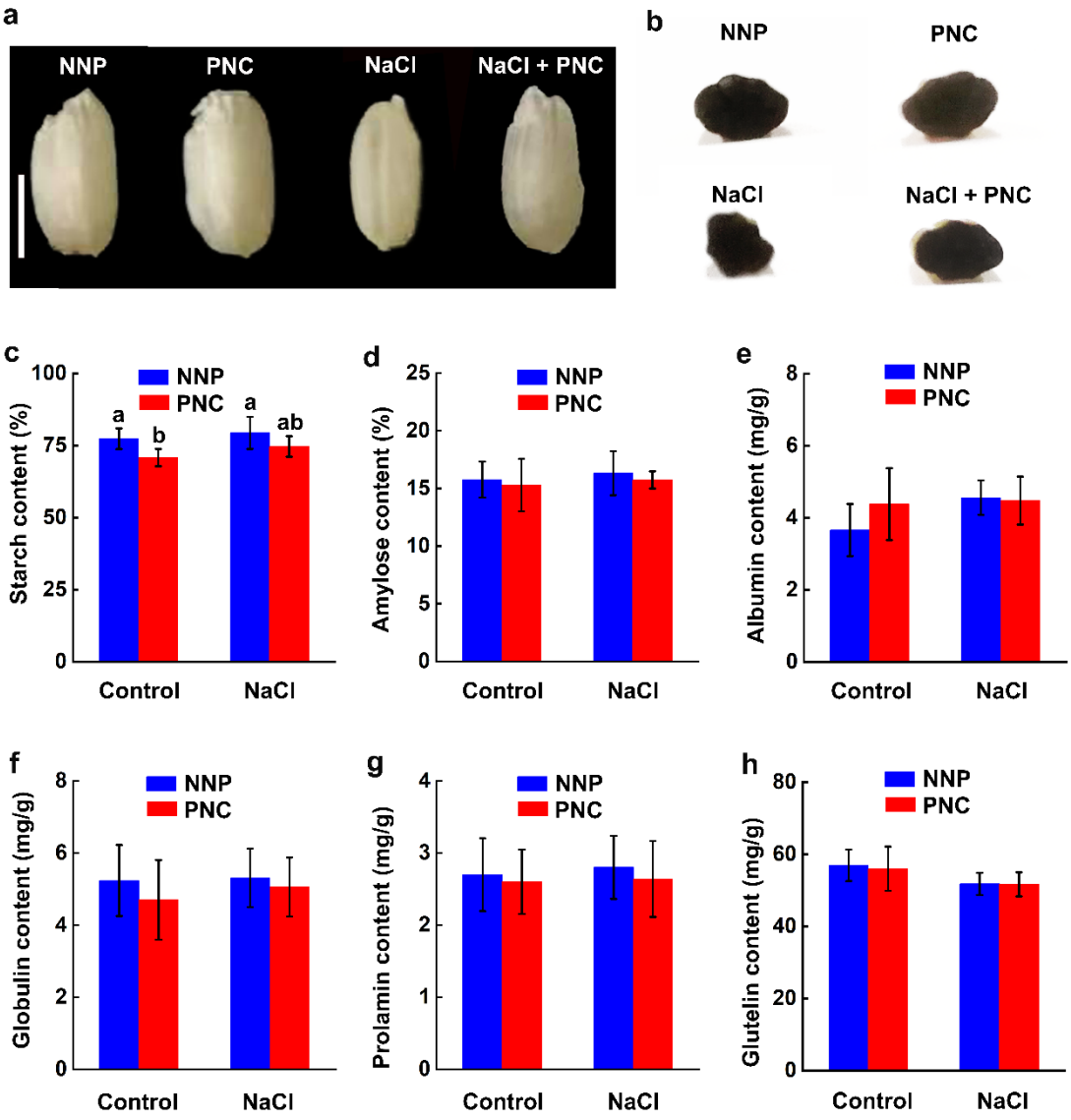

Fig. S11 Effect of nanoceria on rice grain quality. (a) De-hulled rice grain from rice plants interfaced with PNC or no nanoparticles (NNP) under the normal and salt stress conditions. (b) Endosperms of rice grains from NNP treatments exhibit deeper iodine staining (starch indicator) than those of PNC treated plants. (c) Total starch and (d) amylose contents in rice grains. (e-h) Contents of rice seed storage proteins, including albumin, globulin, prolamin, and glutelin. Scale bar = 3 mm. Data are means ± SD of three independent experiments. Lower case letters indicate significant differences at $P < 0.05$ (Duncan's multiple range tests).

Table S1 Primers used in this study.

Target genes	Primer sequences
OsActin1	5'-CAACACCCCTGCTATGTACG-3'
	5'-CATCACCAGAGTCCAACACAA-3'
OsActin2	5'-ACAGGTATTGTGTTGGACTCTGG-3'
	5'-AGTAACCACGCTCCGTCAGG-3'
OsNOA1	5'-TGCTTCTGTGGTTGGGAC-3'
	5'-TCTAAGGGCACGGTGTTT-3'
OsNIA1	5'-CCAATTCTTTCATCGTGTTCT-3'
	5'-CATGCAGCATTTCGTTTCT-3'
OsNIA2	5'-ACTGGTGCTGGTGCTTCTGG-3'
	5'-CGGCTGGGTGTTGAGGGACT-3'
OsSODA	5'-ATCTGGATGGGTGTGGCTAGCTTT-3'
	5'-AGTACGCATGCTCCCAGACATCAA-3'
OsSODC	5'-AATGGTGAAGGCTGTTGTTG-3'
	5'-TTTCCGGCAGGATTGTAGTG-3'
OsCATA	5'-CAACCGCAACGTCGACAACTTCTT-3'
	5'-TTCACCGGCAGCATCAGGTAGTTT-3'
OscAPX2	5'-CATTGCCCGTGGTACTCT-3'
	5'-TTTCATACCAACACATCT-3'
OsGPX1	5'-CAAAGGTGGGCTTTTCGGTG-3'
	5'-TTGCAAGTGGTAGTGCAGGA-3'

OsRbohA	5'-GCTGGACTGTTCACCTGGAA-3'
	5'-TGTTACGGCATACTGGCAGG-3'
OsRbohB	5'-TGCTCTTTGTCCATGGAACGTG-3'
	5'-ACAGCGAGGTACATCCATGTCG-3'
OsRbohC	5'-ATGGCCATATTACGGAGGCTG-3'
	5'-GCCAAGCTGTTCGGGATTCAA-3'
OsRbohD	5'-TGAAAAGCCATGCTGCAACC-3'
	5'-CCATACCACGGAGTAGCGTC-3'
OsRbohE	5'-TGGTCTTGGAATTGGTGCTACTCC-3'
	5'-ACCATGTATGCTTTCCACCTCTTC-3'
OsRbohF	5'-TTGAATGGTTCGTCTGCCCT-3'
	5'-CCCCCTGGTCTGGATATGGA-3'
OsRbohG	5'-CCGAACAAACGGAGACTGGA-3'
	5'-ACAAGAAGCAGCACGTCGTA-3'
OsRbohH	5'-ATTCGTCTTCCACAAGGAGAAT-3'
	5'-CTTAATCATCACTCCCCCTACC-3'
OsRbohI	5'-ACTACGTCAGCGTCCACATCAG-3'
	5'-GCAGACCCTTGAGAAGACGTTC-3'
OsSOS1	5'-ATCAGGTGGAGGCTAGAGCA-3'
	5'-TGACGCACTCCTTTGCAGAT-3'
OsNHX1	5'-TGGGAGTTTGCCAGTGACAG-3'
	5'-GTTCGACAAGAACGACAGCG-3'

OsHKT1;1	5'-TTCACCACTCTTGCGGCTATG -3'
	5'-TGTTTGTAGCCAGTCTCCCCAG-3'
OsHAK5	5'-GTCGGCCTGAGCAAACATTC-3'
	5'-TTGCAGCGTCTTCTCCGATT-3'
OsHAK21	5'-GGTGGGACATTTGCACTCTACTC-3'
	5'-ATGGCTTCCCGCTACTGCT-3'-3'
OsKOR1	5'-TTGTCGTCTTTTGCGGCTTG-3'
	5'-GCTGCCGTATTCGCTACTCT-3'
OsVHA-A	5'-GGTGTTTCAGTCCCTGCTCTTG-3'
	5'-CCCATAGAACCAGGAGGAAGG-3'
OsSA2	5'-TCATCTTCGTAACTCGGTCTCG -3'
	5'-CTTCAGGGTGGTCAGCTCTCTC -3'
OsVP1	5'-GTGGGCGGGATTGGTGATAG-3'
	5'-CGATCACCGACTTGTACCCC-3'
OsVP3	5'-TCGCGCTTGGTATCTACGTC-3'
	5'-TCCAATAGCAGCGGTTGTGT-3'
OsXOR	5'-TGCCATAAAGGATGCGATATCT-3'
	5'-ACTATACACTAAGCTTGGGACG-3'
OsARC2	5'-TCTGGGAGTGGTCTGGTTCT-3'
	5'-TGGGAAGTCGTCAGCAAACA-3'
OsARC2-like	5'-CGTCTCATTGGCATCGGTCT-3'
	5'-TCCGTGCATTCTGCTTGACT-3'

Supplementary Experimental Methods

1. Nanoceria characterization

The concentration of the final filtered PNC solution was calculated using Beer-Lambert's law (A = εCL). A is the absorbance value at 271 nm peak of the PNC sample measured by the UV-VIS spectrophotometer (UV-2600, Shimadzu). ε is the absorption molar coefficient (3 cm^{-1} mM^{-1}) of nanoceria without polymer coating functionalization.[1] L is the optical pathway length (1 cm length of cuvette). C is the molar concentration of the measured sample. Sample concentration in mg/L was calculated by using the molecular weight of cerium (III) oxide and cerium (IV) oxide weighted by the Ce^{3+}/Ce^{4+} ratios for PNC (31%).

The zeta potential of the final filtered PNC solution was measured by Malvern Zetasizer (Nano ZS) (resolution < 0.1 mV). The size of PNC was measured by Sizer (Nano S) (resolution < 0.1 nm, detection limit: 0.3 nm). Chemical characterization of PNC by Fourier transformed infrared spectroscopy (FTIR) (Nicolet 6700 FTIR, Thermo Electron Corp.) was performed to confirm the presence of –COOH group in PNC (Fig. S1a). Samples were dried and measured as PNC powder and the background was collected and subtracted accordingly. Transmission electron microscope (TEM) images of nanoceria were collected using a Tecnai12 TEM at UC Riverside. TEM samples were mounted on pure C grids, 200 mesh Cu (01840, Ted Pella Inc). The ratio of the Ce^{3+} and Ce^{4+} of PNC was characterized by X-ray photoelectron spectroscopy (XPS) using a Kratos AXIS ULTRADLD XPS system equipped with an Al Kα monochromated X-ray source and a 165-mm mean radius

electron energy hemispherical analyzer. During the acquisition, vacuum pressure was kept below 3×10^{-9} torr. Dried PNC powder was mounted on a carbon tape for XPS analysis. Using CasaXPS software (CasaXPS version 2.3.18, Casa Software Ltd), XPS spectra of PNC were deconvoluted and analyzed. The peaks in XPS spectra of PNC as +3 or +4 states of cerium were identified according to the NIST XPS database.[2]

Reference

1 N. Ould-moussa, M. Safi, M. A. Guedeau-Boudeville, D. Montero, H. Conjeaud and J. F. Berret, *In vitro* toxicity of nanoceria: effect of coating and stability in biofluids, *Nanotoxicology,* 2014, **8**, 799-811.

2 C. Korsvik, S. Patil, S. Seal and W. T. Self, Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles, *Chem. Commun.,* 2007, **14**, 1056-1058.