Environmental Science Nano

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Journal:	*Environmental Science: Nano*
Manuscript ID	EN-ART-04-2021-000390.R1
Article Type:	Paper

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Heng Zhou[1#], Honghong Wu[2, 3#], Feng Zhang[1], Ye Su[1], Wenxue Guan[1], Yanjie Xie[1], Juan Pablo Giraldo[3*], and Wenbiao Shen[1*]

Environmental Significance

Salinity leads to significant worldwide losses in crop yield. Plant nanobiotechnology is an emergent approach that is demonstrating improvement of plant stress tolerance. Here, we elucidated the mechanisms of biocompatible poly (acrylic acid) coated cerium oxide nanoparticles (PNC) improve rice tolerance and yield under salinity stress. Nanoceria are able to enhance NO production by increasing the transcripts and modulating the dephosphorylation level of nitrate reductase (NR), and thus maintaining the reactive oxygen species (ROS) and ion homeostasis. This is also the first study demonstrating that scalable soil application of PNC increases rice grain yield without resulting in cerium accumulation in the rice grain under both the normal and salinity stress conditions.

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Heng Zhou[1#], Honghong Wu[2, 3#], Feng Zhang[1], Ye Su[1], Wenxue Guan[1], Yanjie Xie[1], Juan Pablo Giraldo[3*], Wenbiao Shen[1*]

1 College of Life Sciences, Laboratory Center of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu, China, 210095

2 MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science & Technology, Huazhong Agricultural University, Wuhan, Hubei, China, 430070

3 Department of Botany and Plant Sciences, University of California, Riverside, CA, U.S.A. 92521

[#] These authors contributed equally to this work.

[*]Corresponding author: wbshenh@njau.edu.cn (Lead contact); juanpablo.giraldo@ucr.edu

Abstract

Herein, we demonstrate and elucidate how biocompatible poly (acrylic acid) coated cerium oxide nanoparticles (PNC) improve rice tolerance and crop yield under salinity stress. The rice seedlings hydroponically supplied with PNC (1 μM, 98 μg/L) have a higher shoot length (33.3%), fresh weight (56.9%), and chlorophyll content (123.9%) compared to controls after being exposed to 100 mM NaCl for 8 days. Similarly, greenhouse experiments reveal that during the reproductive stage, PNC (10 μM, 0.98 mg/L)-exposed rice plants upon NaCl stress (7.2 dS/m; two months) exhibited increased chlorophyll content (23.9%), dry weight (50.5%), and grain weight (47.1%) compared with non-nanoparticle stressed controls. Importantly, cerium (Ce) content in harvested grain of PNC-supplemented rice plants (18.3 ± 3.1 μg/Kg dry weight) is similar to those of samples without nanoparticles, in both control and stress conditions. Molecular evidences showed that PNC enhance nitric oxide (NO) production (30.5%) by inducing transcription of *nia2* (a gene encoding nitrate reductase) and controlling the dephosphorylation of its protein, thus resulting in NO production and plant tolerance against salinity. The crucial role of NIA2-dependent NO synthesis was evaluated by manipulation of endogenous NO levels and in *nia2* mutants. The nanoparticle-mediated NO control of salinity tolerance could be explained by reducing reactive oxygen species accumulation (39.5%) and maintaining ion homeostasis. Overall, NO signaling is involved in PNC control of salt tolerance. Our results also indicated that PNC are promising nanobiotechnology tools for improving crop performance and yield in salt stressed fields without increasing Ce content in cereal grains.

Keywords: cerium oxide nanoparticles, Na^+/K^+ homeostasis, rice, nitric oxide, nitrate reductase, salinity stress

1. Introduction

Human population is projected to rise to 9.6 billion by the year 2050,[1] requiring a boost in agricultural production, which should be increased by 25%-70% to meet 2050 crop demand.[2] Between 720 and 811 million people in the world faced hunger in 2020. Meanwhile, approximately 1125 million hectares of agricultural land are impacted by salinity.[3] A yield loss of up to 1 t ha^{-1} per unit EC (dS m^{-1}) has been reported in rice.[4] Traditional breeding programs to generate robust salt tolerant crop species, are laborious and lengthy, requiring novel approaches for addressing the expected agricultural production gap.[5,6] For example, exogenously application of exnitrogen-fixing bacteria, arbuscular mycorrhizal fungi, or even ethanol are alternative approaches to improve salt tolerance in plants.[7–9]

Plant nanobiotechnology is an emergent approach to improve plant stress tolerance.[10,11] Cerium oxide nanoparticles (nanoceria) have been reported to increase salinity stress tolerance by interfacing them with canola roots (200-1000 mg/kg soil)[12] and *Arabidopsis* leaves (50 mg/L).[13] It should be noted that without the control of size, dosage and proper care about aggregation issues, nanomaterials such as nanoceria may cause phyto-toxicity.[14] For example, nanoceria with size about 200 nm caused the decrease in photosynthetic rate and CO_2 assimilation efficiency in herbaceous annual plants (*Clarkia unguiculata*).[15] Since nanoceria are potent catalytic scavengers of reactive oxygen species (ROS), they were widely used for research in living organisms from diverse taxa.[11,16-18] Nanoceria ROS scavenging abilities rely on the large number of surface oxygen vacancies that alternate between Ce^{4+} and Ce^{3+} oxidation states. The dangling Ce^{3+} bonds of the defect sites effectively scavenge ROS while lattice strains promote the regeneration of these sites via redox cycling reactions. Despite the classification of cerium as rare-earth element, it is the most abundant (20-70 ppm) of all the lanthanides in the

earth's crust with a concentration just below that of copper (50-85 ppm).[19,20] Previous results revealed that the impact of nanoceria on improvement of plant performance under salt stress were associated with ROS scavenging and regulation of K^+ retention.[12,13,21] Studies in *Arabidopsis* plants also reported that nanoceria alter the expression of genes associated with ion transport[21] and plant stress responses.[22] However, the molecular basis of how nanoceria influences crops under salinity stress is not fully understood. Meanwhile, although nanoceria were suggested as a great potential to improve crop salt tolerance in the laboratory, their impact on crop such as rice growth and yield has not been fully assessed under realistic greenhouse trials.

Nitric oxide (NO, an important gaseous signal functioning in both animal and plant kingdoms) has been widely reported to enhance the antioxidant defense system and maintaining ion homeostasis in response to plant salt stress, at both enzymatic and molecular levels.[23] Nitrate reductase (NR) and a NO-associated protein (NOA1) represent important enzymatic sources of NO production in plants.[24] The requirement of NR-mediated NO production in stomatal closure and cold acclimation has been genetically demonstrated in Arabidopsis.[25,26] In rice, there are two NR encoding genes, *NIA1* and *NIA2*, and the transcriptional level of *NIA2* is markedly higher than that of *NIA1*.[27,28]

Rice is the most important food crop of the developing world and the staple food of more than half of the world's population. Its grain yield is highly sensitive to environmental stresses.[29] Herein, the aim of this study was to elucidate the molecular mechanisms of how cerium oxide nanoparticles (PNC) confer salt tolerance in rice plants, and importantly, explore the scalable soil application of PNC in improvement of cereal grain yield under salt stress. We hypothesized that nanoceria-improved salt stress tolerance in rice plants is dependent on the modulation of nitrate reductase gene expression (at both transcriptional and post-translational levels) and its catalyzed

NO synthesis. The effects of the nanoparticles on plant phenotypic performance were quantified by measuring shoot length and fresh weight, chlorophyll content, Na^+/K^+ content, and NO production, in salt stressed plants. To determine the role of NO in nanoceria-improved salt tolerance, we manipulated endogenous NO levels by using NO donors and scavengers. A genetic analysis was also carried out to investigate the physiological role of NIA2 in nanoceria-triggered NO production and control of salinity tolerance. We further discovered the beneficial impact of nanoceria on salt tolerance and grain yield of rice plants at the reproductive stage in greenhouse experiments. Thus, this work not only elucidates the unique mechanisms underlying the vital function of NO in the bioactivity of nanomaterials in crops, but also demonstrates a promising case of nanoceria control of the stress tolerance for field applications.

2. Experimental Methods

2.1. Nanoceria synthesis

Low Ce^{3+}/Ce^{4+} ratio (about 35%) cerium oxide nanoparticles (nanoceria) coated with poly (acrylic acid) (PNC, about 10 nm) were synthesized and characterized as described in previous study.[13] Briefly, 1.08 g cerium (III) nitrate (Sigma Aldrich, 99%) and 4.5 g poly (acrylic acid) (1,800 MW, Sigma Aldrich) were weighed and dissolved separately in 2.5 mL and 5 mL molecular biology grade water in two 50 mL conical tubes (Corning, Mediatech, Inc.). These two solutions were then mixed thoroughly at 2,000 rpm for 15 min using a digital vortex mixer (Fisher Scientific). The resulted mixture was added dropwise to 15 mL ammonium hydroxide solution (Sigma Aldrich, 7.2 M) in a 50 mL glass beaker. The solution was stirred at 500 rpm (RCT basic, IKA) at ambient temperature for 24 h in a fume hood. Then, the resulted mixture was transferred to a 50 mL conical tube and centrifuged at 3,900 x g (Allegra X30, Beckman) for

1 h to remove any debris and large agglomerates. The supernatant solution was then transferred into 10 kDa filters (15 mL volume, MWCO 10K, Millipore Inc.) and centrifuged at 3,900 x g for 15 min to purify the PNC from free polymers and other reagents. The remainder of the filter was filled with molecular grade water to make a total dilution of 15 mL in each filter tube. This centrifugation step was repeated at least six times. The purified PNC solution was collected and filtered against a 20 nm pore size syringe filter (Whatman, Anotop^{TM} 25). The final filtered PNC solution was collected in a 50 mL conical tube and stored in a 4 ^{0}C refrigerator for further use.

2.2. Labeling nanoceria with DiI fluorescent dye

PNC was labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) fluorescent dye (Fisher Scientific) following our previous protocol.[13] The DiI dye does not coat the nanoparticle surface but instead is encapsulated inside the hydrophobic polymer shell of nanoceria. Briefly, 0.4 mL of 5 mM (58 mg/L) PNC solution was mixed with 3.6 mL molecular biology grade water into a 10 mL glass vial and stirred at 1,000 rpm (RCT basic, IKA). Then, 200 µL DiI dye solution (dilute 24 µL of DiI, 2.5 mg/mL into 176 µL of dimethyl sulfoxide) was added dropwise under continuous stirring (1, 000 rpm) at ambient temperature to allow the incubation for 1 min. The resulted mixture was purified from free DiI and other reagents with centrifugation at 3,900 x g (Allegra X30, Beckman) for 5 min using a 10K Amicon cell (MWCO 10K, Millipore Inc.). The rest of the filter tube was filled with molecular biology grade water to make the total dilution of 15 mL. This centrifugation step was repeated at least five times. The collected DiI-PNC solutions were filtered through a 20 nm pore size syringe filter (Whatman, Anotop^{TM} 25). The absorbance of final filtered DiI-PNC solution was measured by spectrophotometry (UV-2600, Shimadzu), and its concentration was calculated using Beer-Lambert's law as described above. DiI dye is encapsulated inside the hydrophobic polymer shell

of nanoceria and does not coat the nanoparticle surface.[30] The stability of the fluorescent dye in DiI-PNC has been reported for about 2 weeks.[13] The final DiI-PNC solution was stored in a refrigerator at 4 ºC until further use.

2.3. Plant materials and growth

Rice (*Oryza sativa* L., Dongjin) and *OsNIA2* T-DNA insertion mutant lines (*nia2*, Dongjin)[31] were provided from Prof. Yali Zhang (State Key Laboratory of Crop Genetics and Germplasm Enhancement, and Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture, Nanjing Agricultural University, China). Seeds were surface-sterilized and germinated in distilled water for 2 days at 28 °C. Germinated seeds were sowed in bottom-cut 48-well plates and placed on the top of a black opaque plastic beaker containing 550 mL half-strength Murashige and Skoog (MS) medium. Plants were grown with 16/8 h (28/25 °C) day/night regime at 150 μmol m^{-2} s^{-1} irradiation. For salt-tolerance analysis in seedlings stage, two-week-old seedlings were transferred to $^1/_2$ MS medium with or without PNC (0.00098, 0.0098, 0.098, 0.49, and 4.9 mg/L; 0.01-50 μM) in the presence or absence of 100 mM NaCl, which is a severe stress condition to rice seedlings[32] for 8 days. To investigate the role of NO in PNC mediated salt tolerance, the sodium nitroprusside (SNP; a NO-releasing compound, 10 μM)[31] and 2-phenyl4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide (PTIO; a scavenger of NO, 200 μM)[33] were supplied to manipulating endogenous NO level. The medium with or with above chemicals was replaced every two days. Each treatment contained 48 seedlings per time and was repeated for three times (48×3 seedlings).

2.4. Plant phenotype and photosynthesis measurements

After various treatments, the corresponding phenotypes, including the shoot length, fresh weight, and chlorophyll content were measured. Meanwhile, representative images were taken with a

digital camera (EOS M100, CANON). For salt-tolerance assays in reproductive stage (Fig. 6), two-week-old hydroponic seedlings were first treated with or without PNC (1 μM; 0.098 mg/L) for 8 days, and then transplanted to a soil pot (4 L) with 5 Kg soil (EC =0.42 dS/m; Sodium percentage =0.017%) in the glasshouse. Three rice seedlings were planted in a pot. The soil was taken from a field experiment site in Nanjing Agricultural University in Nanjing, Jiangsu. After grown in glasshouse for two months until the panicle development stage, rice plants were further treated with or without PNC (10 μM; 0. 98 mg/L) and exposed to salinity treatment by irrigating with NaCl to reach EC =7.2 dS/m; Sodium percentage =0.4% in soil, which is equal to 75 mM NaCl and is a severe stress condition to rice plants.[34] After two months until maturity stage, the phenotypic characteristics of rice plant, in terms of height, dry weight, yield per plant, and 1000 grain weight, were measured after seeds were harvested. In total, about 1 mg PNC nanoparticles were added into each pot. Each treatment has three replicates. Photosynthetic gas exchange parameters were measured by using LI-6400 portable photosynthesis analysis system (Li-Cor, Lincoln, NE, USA) equipped with an LED light source from 8:00 to 10:00 a.m; leaf temperature was maintained at 25 °C in the leaf chamber. The airflow rate was set at 500 μmol s^{-1} and PAR was 1200 μmol m^{-2} s^{-1}. The flag leaves from seedlings were assayed. The maximum efficiency of photosystem II (Fv/Fm) of flag leaves was measured using the MINI-PAM-II (Heinz Walz, Effeltrich, Germany) after dark adapted for 10 min.

2.5. Confocal imaging of DiI-PNC in rice plants

Two-week-old hydroponic rice seedlings were transferred to $^1/_2$ MS medium containing DiI-PNC (500 nM) or PNC (500 nM, as control) for two days, the samples were collected after washing in 20 mM HEPES buffer (pH 7.8). The imaging of PNC distribution in rice seedlings was performed using a Zeiss LSM 710 confocal microscope (Carl Zeiss, Oberkochen, Germany,

excitation at 514 nm, emission at 550–615 nm, 40 × objective). All manipulations were performed at 25 ±1 °C. Images were taken based on 20 overlapping confocal planes of 2 μm each using Z-stacks tool. Each treatment condition has 5 replicates per experiment. Colocalizations between two fluorophores were calculated by using ImageJ 'Pearson and Spearman correlation (PSC) coefficients'.[35] Results are presented as Pearson correlation coefficients, which produce r values in the range (−1 to 1), where 0 indicates no discernable correlation, while +1 and −1 indicate strong positive and negative correlations, respectively.

2.6. Ion estimation of elemental nutrient and Ce

For the ion estimation, the leaves from rice seedings and flag leaves, straw, root, and rice grains from mature rice plants were first washed twice with EDTA-Na_2 solution and rinsed briefly in de-ionized water. Afterwards, samples were oven-dried at 60 °C, then digested with HNO_3 using a Microwave Digestion System (Milestone Ethos T, Italy) for 30 min. The Na and K contents were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Perkin Elmer Optima 2100DV) from leaves of two-week-old hydroponic seedlings with or without PNC (1 μM), SNP (10 μM), and PTIO (200 μM), alone or their combinations, in the presence or absence of 100 mM NaCl for 8 days. The contents of the elemental nutrient of P, Ca, Mg, Fe, Mn, Cu, B, Ni, and K from flag leaves, straw, and rice grain of mature rice plants after administration of PNC or NNP (no nanoparticle control) in the presence or absence of NaCl were also determined using ICP-OES, while Ce content from flag leaves, straw, rice grains, and root from mature rice plants and soil samples were determined by ICP-mass spectrometry (MS), and calculated according to the dry weight of samples. Three individuals (3 replicates for each sample) in total were used for these experiments.

2.7. Measurement of NO content

NO production was determined by using Griess reagent.[36] 0.5 gram of leaves from two-week-old hydroponic seedlings with or without PNC in the presence or absence of 100 mM NaCl for 24 h were grinded 50 mM cool acetic acid buffer (pH 3.6, containing 4% zinc diacetate) and supernatant were collected for determination. The supernatant was incubated with 0.1 g of charcoal first. After vortex and filtration, the filtrate was leached and collected. Importantly, for avoiding the interference caused by concentrated nitrate and nitrite contents in plants, identical samples were preincubated in 200 μM cPTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt, the scavenger of NO) for 30 min, and were used as the blank. After the addition of Griess reagent for 30 min, absorbance was recorded at 540 nm, and NO content was determined by comparison to a standard curve of $NaNO_2$.

2.8. Confocal imaging of NO fluorescent dye intensity *in vivo*

Endogenous NO levels were monitored by confocal microscopy using a specific NO fluorescent probe 4-amino-5-methyl-amino-2',7'-di-fluorofluorescein diacetate (DAF-FM DA).[37] Leaves from two-week-old hydroponic seedlings with or without PNC in the presence or absence of 100 mM NaCl for 24 h were incubated with 10 μM DAF-FM DA for 20 min before washing in 20 mM HEPES buffer (pH 7.8) three times for 5min each, and then imaged using a Zeiss LSM 710 confocal microscope (Carl Zeiss, Oberkochen, Germany, excitation at 488 nm, emission at 500–530 nm for NO analysis). All manipulations were performed at 25 ± 1 °C. Images were taken based on 10 overlapping confocal planes of 2 μm each through Z-stacks. Each treatment condition had 5 replicates per experiment. Data are presented as the means of fluorescence intensity relative to control condition (0 h).

2.9. Determination of nitrate reductase activity

Maximum nitrate reductase activity (NRAmax) and active nitrate reductase (NRAact) were both measured in leaves of two-week-old hydroponic seedlings with or without PNC in the presence or absence of 100 mM NaCl for 24 h according to the method described previously.[28] Briefly, the leaf samples or protoplasts were harvested and grinded in the extraction buffer containing 25 mM potassium phosphate buffer (pH 8.8), and 10 mM cysteine. The protein extracted in the presence of excess Mg^{2+}, is considered to be the NRAact *in situ* in leaf tissues, while NRAmax is measure in the presence and preincubation of EDTA for 30 min. The reaction mixture contained 0.4 mL of the extracted aliquots, 1.2 mL of a 0.1 mM potassium phosphate buffer (pH 7.5), 0.1 mM KNO_3, and 0.4 mL of 0.25 mM nicotinamide adenine dinucleotide (NADH). NRA was expressed as μmol $NO_2^-g^{-1}$ FW h^{-1}.

2.10. Protoplast preparation and transiently expression of OsNIA2

Full-length cDNA fragment was amplified with the *Kpn*I and *BamH*I sites for cloning of *OsNIA2* gene, and then cloned between the CaMV35S promoter and Flag tag of the *1300221-Flag* vector. Protoplast isolation and transfection with *1300221-OsNIA2-Flag* were based on the protocol for rice protoplasts.[38] Rice protoplasts (1 ml, usually 5×10^5 cells ml^{-1}) from 10-day-old *nia2* mutant seedlings were transfected with 100 μg of fusion constructs *1300221-OsNIA2-Flag* using a PEG-calcium-mediated method. Then the transfected protoplasts were incubated in the incubation solution overnight in the dark at 25 °C.

2.11. Phosphorylation level detection of OsNIA2

Protoplasts of *nia2* leaves with transiently overexpression of *NIA2* were embedded with or without 1 μM PNC in the presence or absence 50 mM NaCl for 1 h. After that, the protoplasts were harvested and protein were extracted by 100 mM Tris-HCl buffer (pH 7.0) containing 50 mM $MgCl_2$, 1% SDS, 1 mM DTT, and 0.1% Triton X100 with vortexing on ice. The total

protein was further denatured in sodium dodecyl sulphate sample buffer containing 5% β-mercaptoethanol (β-ME) at 95 °C for 10 min, and transferred to a polyvinylidene fluoride membrane (Roche) and hybridized with the phos-tag biotin antibody (APExBIO) according to the manufactures protocol. Related phosphorylation level was calculated according to its Flag-labeled protein level.

2.12. Histochemical staining (DAB and NBT)

The detection of H_2O_2 and O_2^- was performed by DAB (3,3'-diaminobenzidine) and NBT (nitro blue tetrazolium) staining as described previously.[39] For DAB staining, rice seedlings leaves were immersed in DAB solution (1% w/v, pH 3.8), vacuum infiltrated, and then incubated in darkness overnight. For NBT staining, seedlings leaves were immersed in NBT solution (0.1% w/v in 50 mM sodium phosphate buffer, pH 7.5) and incubated in darkness overnight. After staining, seedlings were transferred to distilled water and then incubated at 95 °C for 10 min in a solution containing acetic acid: glycerol: ethanol (1:1:3, v/v/v). Tissues were then photographed (model Stemi 2000-C; Carl Zeiss, Germany).

2.13. H_2O_2 content

The content of H_2O_2 was measured based on the peroxide-mediated oxidation of Fe^{2+}, followed by the reaction of Fe^{3+} with xylenol orange.[40] An aliquot of supernatant (500 μL) was added to 500 μL of assay reagent (500 μM ammonium ferrous sulfate, 50 mmol/L H_2SO_4, 200 μM xylenol orange, and 200 mmol/L sorbitol). Absorbance of the Fe^{3+}-xylenol orange complex (A560) was detected after 45 min of incubation. The specificity for H_2O_2 was tested by eliminating H_2O_2 in the reaction mixture with catalase (CAT). Standard curves were obtained by adding variable amounts of H_2O_2. Data were normalized using an extinction coefficient of 2.2×10^5 M^{-1} cm^{-1} and expressed as nmol H_2O_2 per gram of fresh weight (FW).

2.14. Determination of thiobarbituric acidreactive substances (TBARS) content

Lipid peroxidation of rice seedlings was estimated by measuring TBARS contents. 0.2g leaves samples from two-week-old hydroponic seedlings with or without PNC in the presence or absence of 100 mM NaCl for 6 days were grinded with 1.5 ml 5% trichloroacetic acid (TCA), then 1.5 mL 0.5% 2-thiobarbituric acid (TBA) was added in 5% TCA. After the incubation in 95 °C water bath for 30 min, the supernatant was collected by centrifugation at 12,000g for 20 min, then determined by measuring absorbance at 450, 532, and 600 nm. The level of lipid peroxides, together with oxidatively modified proteins, was quantified in terms of TBARS amount using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight.

2.15. Antioxidant enzyme activity

Rice seedling leaves (0.2 g) were homogenized in 100 mM Tris-HCl buffer (pH 7.0) containing 1 mM DTT and 1 mM *β*-mercaptoethanol. The homogenates were centrifuged at 12000 *g* for 15 min at 4 °C and supernatant was collected for estimation of antioxidant enzymes activity. Total superoxide dismutase (SOD) activity was measured according to its ability to reduce nitroblue tetrazolium (NBT) by the O_2^- generated by the riboflavin system under illumination.[41] One unit of SOD was defined as the amount of crude enzyme extract required to inhibit the reduction rate of NBT by 50%. Catalase (CAT) activity was measured by the absorbance decrease at A240 nm due to the H_2O_2 decomposition.[42] Ascorbate peroxidase (APX) activity was determined by monitoring the rate of H_2O_2-dependent oxidation of ascorbate in A290 (extinction coefficient 2.8 mM^{-1} cm^{-1}).[43] Supernatant fractions were assayed for maximal extractable activities of glutathione peroxidase (GPX) on the basis of NADPH consumption (extinction coefficient of 6.22 mM^{-1} cm^{-1}) at 340 nm for 3 min.[44]

2.16. Estimation of starch and seed storage proteins content

Fully filled grains were used for measuring grain quality traits. Iodine stained starch in cut endosperms of rice grain was performed by using *Lugol's_iodine* reagent.[45] De-hulled rice grains were ground into flour and stored at -20 °C for related traits determination. Starch contents were measured by calculating generation of glucose in the insoluble fractions of ethanol-water extracts. The intensity of color formed by anthrone-glucose was determined spectrophotometrically at 620 nm according to the previous method.[46] For amylose content, finely powered rice was extracted in alkali followed by boiling, and then incubated with acetic acid and iodine reagent for color reaction. The amount of amylose was calculated according to the standard curve of amylose.[47] Contents of seed storage protein including albumin, globulin, prolamin, and glutelin were determined. In briefly, 100 mg rice flour was first extracted by distilled water for albumin, and the pellet was extracted by 1 M NaCl for globulin, and the pellet was extracted by 70% ethanol for prolamin, and 0.05 M NaOH for glutelin extraction. The protein content of each extraction was quantified by Coomassie brilliant blue G-250 dye.[48]

2.17. qPCR analysis

Total RNA was isolated from the leaves of rice seedlings after different treatments at the indicated time points using the Trizol reagent (Invitrogen, Gaithersburg, MD, USA) according to the manufacturer's instructions. Real-time quantitative reverse-transcription PCR were performed using a Mastercycler ep® realplex real-time PCR system (Eppendorf, Hamburg, Germany) in a reaction mixture of 20 μL of SYBR® Premix Ex Taq™ (TaKaRa Bio, China) according to the manufacturer's instructions. Primers for the corresponding genes are listed in Supplementary Table S1. Relative expression levels of corresponding genes are presented as values relative to corresponding control samples at the indicated times or under the indicated conditions, after normalization to *OsActin1* (Os03g50890) and *OsActin2* (Os10g36650)

transcript levels. Experiments were repeated in three individuals (each biological replicate was measured for three times).

2.18. Statistical analysis

All data were analyzed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Comparisons were performed by independent samples *t*-test (two tailed) or one-way ANOVA based on Duncan's multiple range test (two tailed). *, **, and *** represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Different lower-case letters indicate significance at $P < 0.05$.

3. Results and discussion

3.1. Characterization of anionic nanoceria and its translocation in rice plants

Compared to the metal ion-based nanomaterials (e.g. Ag, Zn, Ti, and Ce), the widely used carbon-based nanotubes are more easily bioaccumulated in the environment posing safety concerns.[49] Among them, nanoceria is a unique antioxidant material able to self-regeneration of its properties after interaction with oxidants.[50] There is a clear distinction between nanoceria and other nanomaterials which are unable to reversible oxidation-reduction reactions. It means that even low concentration of nanoceria was able to provide long-time protection for cell against oxidative stress.[51]

In this study, cerium oxide nanoparticles coated with poly (acrylic acid) (PNC) have a core size of 5.6 ± 0.2 nm (Fig. 1a) and a hydrodynamic diameter of 9.5 ± 0.9 nm (Fig. 1b). The nanoparticles are negatively charged with a zeta potential of -21.6 ± 4.3 mV (Fig. 1c). Fourier transform infrared spectroscopy (FTIR) analysis confirmed the presence of –OH and –C=O groups on the surface of PNC, thus indicating the presence of -COOH groups on the nanoparticle surface (Fig. S1a). PNC have low Ce^{3+}/Ce^{4+} ratios of 31.1 ± 0.5% that promote scavenging of

ROS in plants,[13] including superoxide anion radicals and hydrogen peroxide (Fig. 1d). To assess the translocation and distribution of PNC in rice plants, PNC was labeled with a fluorescent dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI-PNC) (Fig. S1b) and imaged by confocal fluorescence microscopy. The DiI dye is encapsulated inside the hydrophobic polymer shell of nanoceria. PNC and DiI-PNC have similar hydrodynamic diameter and zeta potential (Fig. 1b and 1c). Confocal imaging of rice seedlings indicated that DiI-PNC hydroponically applied to seedlings is detected in both rice roots and leaf mesophyll cells, after two days of exposure (Fig. 1e and 1f). The colocalization rates between the DiI and chlorophyll autofluorescence channels (r_s) indicate 52.5% colocalization of PNC with chloroplasts. PNC were also present in primary and lateral roots, as well as root junctions (Fig. 1f). Together, these results indicate that anionic PNC smaller than 10 nm are able to translocate from root to shoots of rice seedlings, which is in according with the results shown in previous literature.[52]

3.2. Nanoceria enhance plant tolerance against salt stress

The optimal concentration of PNC applied to rice seedling roots was determined by salinity experiment in hydroponics supplemented with 0.00098, 0.0098, 0.098, 0.49, and 4.9 mg/L (0.01-50 μM) nanoceria (Fig. S2). After the exposure to 100 mM NaCl for 8 days, seedling growth was inhibited either with PNC or NNP (in particularly) compared to the non-stressed controls. For example, NaCl caused large reductions in chlorophyll content (-68.7%), shoot length (-39.2%), and root length (-29.4%) in seedlings in the absence of nanoparticles ($P < 0.05$). However, plants co-treated with nanoparticles at concentrations higher than 0.1 μM could differentially improve their performance under salt stress alone. PNC at 1 μM (0.098 mg/L) was selected further since this can significantly alleviate the inhibitory impacts of salinity, relative to non-stressed controls on shoot length (-23.3 ± 1.7% vs. -42.5 ± 3.2%), fresh weight (-24.8 ± 0.7% vs. -52.1 ± 1.3%),

and chlorophyll content (-35.6 ± 1.6% vs. -71.2 ± 4.1%) ($P < 0.05$; Fig. 2a-d). It should be mentioned that the above salt concentration medium did not alter the hydrodynamic size of PNC (Fig. S3).

It is well-known that the maintenance of ion homeostasis is crucial for plant survival upon salinity stress.[5] Compared with plants under non-saline conditions, the exposure of rice seedlings to NaCl caused a significant Na^+ increase from about 0.047 ± 0.006 to 0.892 ± 0.008 mmol/g DW, and a K^+ decrease from about 1.031 ± 0.076 to 0.634 ± 0.031 mmol/g DW, and thus dramatically increased Na^+/K^+ ratio from 0.049 ± 0.003 to 1.418 ± 0.007 ($P < 0.001$) (Fig. 2e-g). In contrast, PNC treatment markedly reduced the overaccumulation of Na^+ (0.622 ± 0.057mmol/g DW) and the loss of K^+ (0.873 ± 0.043 mmol/g DW), thus maintaining a lower Na^+/K^+ ratio (0.717 ± 0.042) than plants under salinity stress alone ($P < 0.001$). Similarly, previous studies showed that 10 nm anionic nanoceria increased Arabidopsis salt tolerance by modulating the activities of non-selective cation channels to enhance mesophyll K^+ retention ability.[21] Also, 10 nm anionic nanoceria improved shoot Na^+ exclusion ability to maintain cytosolic Na^+/K^+ ratio to improve cotton salt tolerance.[53] It suggests that the ability to maintain Na^+/K^+ ratio might be a common mechanism underlying nanoceria improve plant salt tolerance.

Nanoceria have been reported to be potent ROS scavengers in barley,[18] canola,[12] carrot,[54] sorghum,[55], Arabidopsis[13,21], and cotton[53,56]. Here, histochemical analyses for H_2O_2 (DAB staining) and O_2^- (NBT staining) in rice leaves indicated that salinity stress induces significant accumulation of these ROS (Fig. S4a), while the hydroponically applied PNC mitigated the ROS accumulation in rice leaves compared with non-nanoparticle stressed controls. These results were confirmed by the reduction in spectrometric determination of H_2O_2 content (35.9%) and lipid peroxidation (45.4%) in rice leaves (Fig. S4b and c). The PNC also enhanced the activities of

representative antioxidant enzymes, including superoxide dismutase (SOD; 20.7%), catalase (CAT; 12.5%), ascorbate peroxidase (APX; 18.5%), and glutathione peroxidase (GPX; 23.9%), in rice seedling leaves under salt stress (Fig. S5). Thus, we propose that the reestablishment of redox homeostasis alleviates salinity stress symptoms in plants when treated with PNC.

3.3. NO mediates nanoceria-improved salinity tolerance

NO is a key signaling molecule involved in the regulation of plant adaption against stress, including salt tolerance,[23] and plant growth and development.[31] Thus, basal level of NO is present in plants under normal conditions. Under stress conditions, increased NO production is a common response in plants.[23] To investigate the role of NO in PNC-controlled plant salt tolerance, we monitored endogenous NO levels in leaves of rice seedling by laser scanning confocal microscopy (LSCM). NaCl stress rapidly triggered the production of NO during 48 h of treatment, with a fast induction as early as 3 h (up to 30.1%; Fig. 3a), followed by a peak at 24 h (59.1%). NaCl-induced NO production was magnified in PNC-treated rice seedlings as observed by confocal fluorescence images (Fig. 3b) and quantified by Greiss reagent assays (Fig. 3c), after 24 h of treatment. For example, the latter results showed that PNC-treated rice seedlings under salt stress exhibited the highest NO content (39.83 ± 3.55 nmol/g FW), compared to NaCl treated alone (30.51 ± 3.25 nmol/g FW) and non-stressed controls (12.14 ± 1.47 nmol/g FW) ($P < 0.05$).

There are many pathways for NO generation in plants. Besides the enzyme complexes xanthine oxidoreductase (XOR) located in mitochondrion, nitrate reductase (NR) in cytosol, amidoxime reducing component (ARC), and plasma membrane-bound NR could catalyze the nitrate reduction to NO or nitrite and NO. NO-associated protein and NOS-like protein catalyze unknown NO production, and non-enzymatic NO production have also been reported in plants.[23,57] Among all the pathways for NO production, NR has been shown to play a main role.[58]

During this pathway, NR is responsible for the reduction of absorbed NO_3^- to nitrite (NO_2^-) and further reduction to NO. RT-qPCR results indicated that the relative gene expression of *NOA1* (1.72-fold change), *NIA2* (3.91-fold change), XOR (2.11-fold change), ARC2 (2.65-fold change), and ARC2-like (1.92-fold change) were up-regulated by NaCl, but this induction was not observed in *NIA1* transcripts ($P < 0.05$) (Fig. 3d and S6). Upon salinity stress, the addition of PNC promoted the up-regulation of *NOA1* (1.94-fold change), *NIA2* (4.94-fold change), and *ARC2* (2.96-fold change) ($P < 0.05$). No such significant differences in *NIA1*, *XOR*, and *ARC2-like* were found in both control and NaCl conditions. These results clearly indicate that *NIA2* might be mainly responsible for the PNC-induced NO production in response to NaCl stress.

Further results revealed that the increased NO production was associated with higher nitrate reductase activity. NaCl-induced maximum- and activated-nitrate reductase activities (NRAmax and NRAact) were intensified by PNC treatments (Fig. 3e and f). Interestingly, NRAact in rice seedling leaves challenged with PNC alone was increased by 49.1% compared with the non-PNC control under non saline conditions, whereas no such difference was observed in NRAmax. It indicated that PNC may regulate nitrate reductase at post-translational level. In fact, post-translational modification e.g. phosphorylation, plays an important role on the regulation of NR activity.[58] It has been reported that the phosphorylated Ser-motif of NR could be recognized by a 14-3-3 dimer, and in the presence of divalent cations then convert NR into a completely inactive complex.[59] To test this idea, we cloned the *OsNIA2* gene and transiently overexpressed *OsNIA2* into protoplasts of *nia2* mutant rice seedlings,[31] and this approach was used to evaluate the effects of PNC on the phosphorylation modification status of NIA2 protein. As expected, western blotting results showed that NIA2 protein from PNC-embedded protoplast exhibited lower phosphorylation levels relative to controls with no nanoparticles (NNP) under normal and

salinity stress condition (Fig. 3g). However, salinity stress has no significant effect on phosphorylation status of NIA2. These results on the phosphorylation level of NIA2 were confirmed by the changes in their activity (Fig. 3h), indicating that PNC could trigger the dephosphorylation of NIA2 and thus promote NO production. Interestingly, the enzyme-mimicking activities, including phosphatase activity of nanoceria, are well recognized. Previous studies showed that nanoceria could accelerate the dephosphorylation of simple organophosphates, energetically rich biomolecules such as adenosine triphosphate (ATP), and 3′,5′-cyclic adenosine monophosphate (cAMP).[60,61] Importantly, the subcellular localization of PNC is mainly in chloroplasts (Fig. 1) and cytosol[13], which is consistent with the location of NR (cytosol). Together, these results indicated that PNC may directly dephosphorylate NIA2 protein.

The role of endogenous NO generation in PNC-enhanced salinity stress tolerance was assessed using a NO donor (sodium nitroprusside)[31] and scavenger (2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide; PTIO)[33]. As expected, the addition of SNP (a positive control) and PTIO (a negative control) alleviated or aggravated the toxic effects of NaCl stress (Fig. S7a), in terms of changes in shoot length (Fig. S7c), fresh weight (Fig. S7d), and chlorophyll content (Fig. S7e), and all of those were accompanied by increases or decreases in endogenous NO content (Fig. S7b). These pharmacological results clearly illustrate the key role of endogenous NO in rice tolerance against salinity stress.[23] Further experiments revealed that PNC-stimulated NO production and -alleviated salinity stress were sensitive to the removal of endogenous NO with PTIO. Together, these results indicate that NO production is a downstream mediator governing PNC-improved salt tolerance in rice.

3.4. Nanoceria do not improve the performance of *nia2* mutants under salinity stress

Our results point out that NIA2-dependent pathway may responsible for PNC control of NO production in response to salinity stress (Fig. 3). In fact, the abundance of *NIA2* transcripts is considerably higher than that of *NIA1* in both *Arabidopsis*[60] and rice.[27,28] Consistent with these findings, it was observed a pronounced increase in *NIA2* transcripts, compared to that of *NIA1*, in stressed rice plants when incubated with PNC (Fig. 3d). To further elucidated the function of NIA2 on PNC-improved rice salt tolerance, the *nia2* rice mutants with a severe reduction in NO production[31] was used. The results showed that *nia2* plant exhibited severe growth inhibition under salinity stress (Fig. 4a), as indicated by the reductions ($P < 0.05$) in shoot length (51.4%; Fig. 4b) and chlorophyll content (76.7%; Fig. 4c). Interestingly, interfacing *nia2* mutants with PNC did not improve growth performance of rice seedlings under salinity. In contrast, the application of NO donor significantly rescued the reduction ($P < 0.05$) in shoot length (52.7%) and chlorophyll content (66.9%) in *nia2* rice caused by salinity. Similarly, significantly lower Na^+/K^+ ratios were observed in salt stressed *nia2* rice seedlings treated with NO donor in the presence (1.31 ± 0. 10) or absence (1.23 ± 0.11) of PNC, but not in PNC alone (1.85 ± 0.13; $P < 0.05$) (Fig. 4d). As expected, NO content was increased in *nia2* rice seedlings exposed to salt stress or SNP, regardless of the addition of PNC (Fig. 4e and S8). We also observed that the addition of PNC did not influence endogenous NO production in stressed *nia2* plants (24.20 ± 2.24 vs. 22.50 ± 1.86 nmol/g FW). These results highlight the key role of NIA2 in PNC-mediated salt stress tolerance.

Consistently, the evaluation of TBARS (83.7 ± 5.7 vs. 89.8 ± 7.2) and H_2O_2 content (21.8 ± 3.9 vs. 22.4 ± 3.2) showed that PNC could not mitigate ROS accumulation under salt stress in *nia2* mutant (Fig. S9). These genetic findings point out that NIA2-dependent NO production is required for PNC improved ROS homeostasis and rice salt tolerance and also indicate that

nanoceria improvement in NO production is coordinated with the re-establishment of ROS homeostasis, thus enhancing salt tolerance in rice.

3.5. NIA2 is required for nanoceria-mediated gene expression encoding antioxidant enzymes, NADPH oxidase, and ion transporters and channels

The molecular mechanisms underlying PNC impact on transcription levels of genes encoding antioxidant enzymes, Rboh family (NADPH oxidases), and ion transporters and channels, were investigated under the short-term (1 day) and long-term (7 days) salt stress exposure. After 1-day treatment, the expression of genes encoding antioxidant enzymes, including *SODA* (*superoxide dismutase A*), *SODC* (*superoxide dismutase C*), *CATA* (*catalase A*), *cAPX2* (*cytosolic ascorbate peroxidase 2*), and *GPX1* (*glutathione peroxidase 1*) were up-regulated by PNC in NaCl-stressed wild-type and *nia2* mutant, relative to NaCl alone (Fig. 5). Similarly, the transcripts of gene encoding NADPH oxidases, including 9 members of Rboh family (*RbohA* to *RbohI*) were down-regulated by PNC in NaCl-stressed wild-type, and *nia2* mutant (except *RbohB* and *RbohG*), relative to NaCl alone. However, above induction or inhibition of related genes by PNC was only observed in the wild-type plants, but not in *nia2* mutant, after 7 days of salt treatment.

Reestablishing ion homeostasis is one of the main strategies that plants employ to survive and grow under salinity stress.[62] Salt tolerant rice varieties have been reported to have lower cytosolic Na^+/K^+ ratio than the sensitive ones.[63] To maintain the homeostasis of Na^+/K^+ ratio, plants reduce the accumulation of cytosolic Na^+ through activating Na^+ extrusion or vacuolar Na^+ sequestration, and prevented leakage of cytosolic K^+ mainly through KOR (outward-rectifying K^+ channel) and NSCC (non-selective cation channel).[64] KOR and NSCCs are known to be activated by ROS,[64] which can be effectively scavenged by nanoceria. In NaCl-stressed wild-type plants, the transcriptional levels of genes encoding plasma membrane ion transporters

for Na^+ and K^+, including Na^+/H^+ antiporter (*SOS1*, salt overly sensitive 1), sodium hydrogen exchanger (*NHX1*), and high-affinity potassium transporters (*HKT1;1*, *HAK5*, and *HAK21*), were enhanced by PNC, in both short- and long-term treatments (Fig. 5); whereas, the expression of *KOR1* (*outward-rectifying K^+ channels 1*) showed the opposite tendency. Tonoplast and plasma membrane H^+- ATPase are crucial for building the H^+ electrochemical gradient across the tonoplast that provides the driving force for potassium retention and uptake through voltage-gated channels and the exchange of Na^+/H^+ via NHX.[65] This is an important physiological mechanism for maintaining low Na^+ and high K^+ in the cytoplasm under salinity stress.[62] Similarly, the transcriptional levels of *VHA-A* (*tonoplast H^+-ATPase A subunit*) and *SA2* (*plasma membrane H^+-ATPase 2*) genes were significantly upregulated, whereas the relative expression of *VP1* (*tonoplast H^+- pyrophosphatase 1*) and *VP3* (*tonoplast H^+- pyrophosphatase 3*) genes was downregulated by PNC treatment, relative to NNP controls after both short and long term of NaCl treatments. The majority of these gene expression responses were differentially impaired or abolished in *nia2* mutant plants. Combined with the phenotypic analysis, our genetic and molecular evidence suggest that the molecular mechanism underlying PNC control of plant tolerance against salinity stress is mainly mediated by NR-catalyzed NO synthesis, via the reestablishment of redox homeostasis (especially in the late stage of stress conditions) and ion homeostasis. Our results also hint a useful route to design novel nanoparticles, particularly for regulating Na^+/K^+ homeostasis, in crops sensitive to salt stress.

3.6. Nanoceria enhance grain yield of rice plants under salinity stress without affecting grain quality and resulting in cerium accumulation

Salinity stress not only severely inhibits plant growth, but also reduces crop yield at reproductive stages. Therefore, the sustained growth and yield under salt stress are main criterions for

engineering salt-tolerant crop plants.[6,63] Previous results revealed that salinity tolerance is enhanced in *Arabidopsis* and canola by interfacing nanoceria with leaves or roots.[12,21] Here, our greenhouse experiments further demonstrated that the phenotypic performance of rice plants cultured in saline condition is significantly improved by supplement of PNC (10 μM; 0.98 mg/L) (Fig. 6a), as determined by changes in chlorophyll content, photosynthetic rate, plant height, maximum efficiency of photosystem II, and dry weight (Fig. 6b-f). The grain length, but not width and thickness in saline-free control conditions, was increased by PNC, while grain length and thickness, but not width were increased by PNC under salinity stress (Fig. 7a-e).

Remarkably, higher yield per plant (7.3%) and 1000 grain weight (6.8%) were found in PNC-treated rice plants than NNP controls (Fig. 6g and 7f). In PNC-treated rice plants, the changes in yield per plant were more pronounced in salt stress conditions, showing a 47.1% increase, compared to NNP controls. To assess the potential environmental safety issues caused by the bioaccumulation of nanoceria, we detected the content of cerium (main component of nanoceria) by using ICP-mass spectrometry in harvested rice grains. Interestingly, the results showed that cerium content in harvested rice grain, flag leaves, root tissues, and cultured soil of PNC treated plants was very similar to rice plants grown without PNC under both normal and salinity stress conditions, in contrast with the accumulation of cerium in straw (Fig. 7g and S10). These indicate that applying PNC (0.98 mg/L) to soil does not result in detectable exposure of nanoparticles to rice grain, would not impact food for human consumption. Besides, the elemental nutrient contents of P, Ca, Mg, Fe, Mn, Cu, B, Ni, and K, were also evaluated. The results showed that contents of P, Ca, and Mg are higher in flag leaves of PNC-treated rice plants than those of controls under normal condition. Salinity stress can cause nutrient element loss in flag leaves, including P, Cu, and K. The PNC treatment significantly reduced this loss

(Supplementary dataset). Importantly, cerium is found in soils in hydrous phosphates and oxide form.[66] In our experimental condition, the cerium content in soil is about 320 mg/Kg soil, which is far beyond the cerium content from PNC addition (1 mg/5 Kg soil). Hence, we can conclude that the beneficial role of PNC is not from addition of cerium element. However, studies showed that much higher concentration of MWCNT (10–80 mg/L), SiO_2 (1.5–7.5 g/L), or TiO_2 (50–750 mg/kg) were used for improving plant growth or yield.[67-70] In some cases, these nanoparticles could accumulate in grain. For example, hydroponic supplemented MWCNT (50 mg/L) were found that moved into the grain of all tested species after long term exposure.[71] At the high CO_2 concentration, application of $nTiO_2$ at 200 mg/kg increased accumulation of titanium in rice grain by 8.6%.[68] Nanoceria has a low dissolution rate and tend to accumulate in the soil if not leached out.[72] More importantly, recent research reported that nanoceria exhibited negligible mobility in the soil-water interface or immobile in the soil solid phase, as well as non-toxicity to nanomaterials sensitive ammonium oxidizing bacteria.[73] Hence, the environmental risk of the nanoceria appears to be lower than other pollutants.

Nanoceria are stable nanoparticles in biological systems or environmental systems and remain unaltered after uptake by plant roots.[74-76] In our study, we noticed that the stability of PNC is not significantly affected by the presence of NaCl (100 mM), indicated by no significant differences of hydrodynamic size between PNC and PNC+NaCl treatments (Fig. S3). However, becasue root rhizosphere is a complex system, more efforts should be conducted to investigate the behavior of PNC and NaCl in the rhizosphere of salt stressed plants. Meanwhile, PNC was applied at far less concentration (1 mg/5 Kg soil) than that found in soil (about 320 mg/Kg soil). However, its impact on soil microbiome and fate in the environment should be further elucidated.

Moreover, our results showed that PNC led to a slight decrease in starch content, but with no changes in amylose content compared with NNP controls in the both normal and salinity stress conditions (Fig. S11a-d). Meanwhile, no obvious changes in seed proteins, including albumin, globulin, prolamin and glutelin, were found in rice grains from plants in the presence or absence of PNC, regardless of the addition of NaCl (Fig. S11e-h). Thus, the application of nanoceria offer a promising tool to improve cereal grain yield, especially in saline soil, also avoiding nanoparticle exposure to grains for human consumption.

Agriculture has a notoriously thin profit margin, thus the cost of the proposed nanobiotechnology approach was assessed. According to our results, the cost of the synthesis of PNC in laboratory condition is about $390 per hectare. This cost can be largely reduced after scaling up production at industry levels. Moreover, instead of soil application, foliar spray[13] or seed priming[56] with nanoparticles could be other more efficient delivery approaches. The current cost of PNC for cotton seed nanopriming is less than $30 per hectare and that for a foliar spray is ~$100 per hectare.[77]

Overall, PNC represent a promising nano-enabled agrochemical for improving rice yield in lands affected by salinity. Rice is the most important staple food in the world, especially in Asia, and its grain yield is highly sensitive to environmental stresses.[63] Thus, this nanobiotechnology approach provides a useful pathway for enhancing food production, a major challenge of sustainable agriculture in the 21st century.

4. Conclusion

In summary, we elucidate a key mechanism of PNC improvement of rice salt tolerance. PNC induce the transcription of *nia2* and dephosphorylation of its protein, thus resulting in NIA2-

dependent nitric oxide (NO) production (Fig. 8). The PNC-promoted NO production could affect the relative expression levels of antioxidant enzymes, NADPH oxidases, H^+ pump, Na^+- and K^+-related channels and transporters, thus maintaining the ROS and Na^+/K^+ homeostasis. Finally, the reestablishment of ion homeostasis and thereafter salinity tolerance in rice plants were observed. Furthermore, we found that nanoceria applied in 10 μM (0.98 mg/L; reproductive stage seedlings cultivated in soil), significantly improved the phenotypic performance and grain yield of rice plants at reproductive stage under normal and salinity stress condition. Most importantly, the increase in crop yield is achieved without cerium accumulation and changes in amylose and proteins content in the rice grain under both normal and salinity stress condition. Collectively, above results clearly indicated that nanoceria could use as a promising tool to improve cereal grain yield, especially in saline soil, also avoiding nanoparticle exposure to products for human consumption.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

H.Z. and H.W. contributed equally to this work. This work was supported by the Funding from Center of Hydrogen Science, Shanghai Jiao Tong University, China to W.S., the USDA National Institute for Food and Agriculture under Grant No. 2019-67013-29104 to J.P.G, and the NSFC fund (No. 31901464, 32071971) and project 2662020ZKPY001 supported by the Fundamental Research Funds for the Central Universities to H.W.

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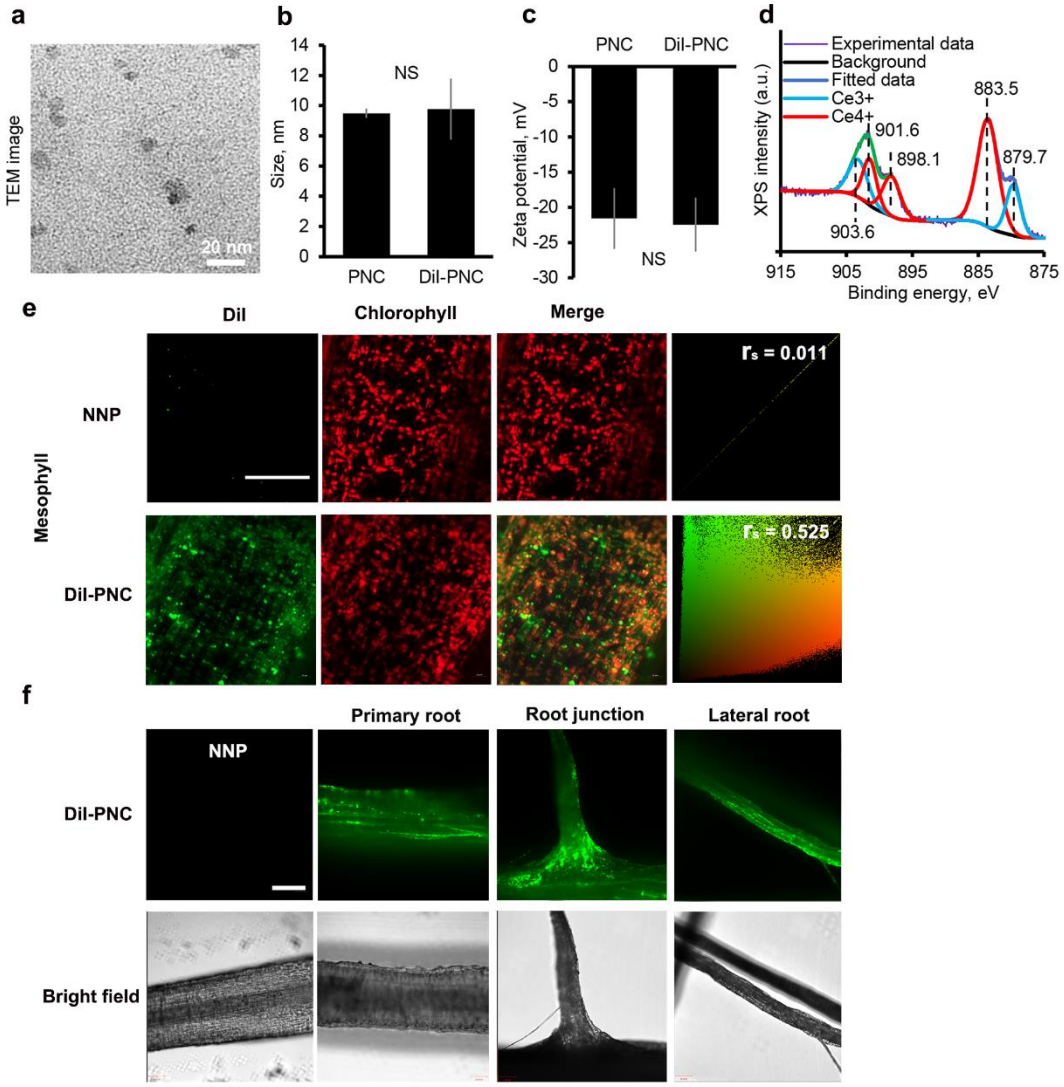

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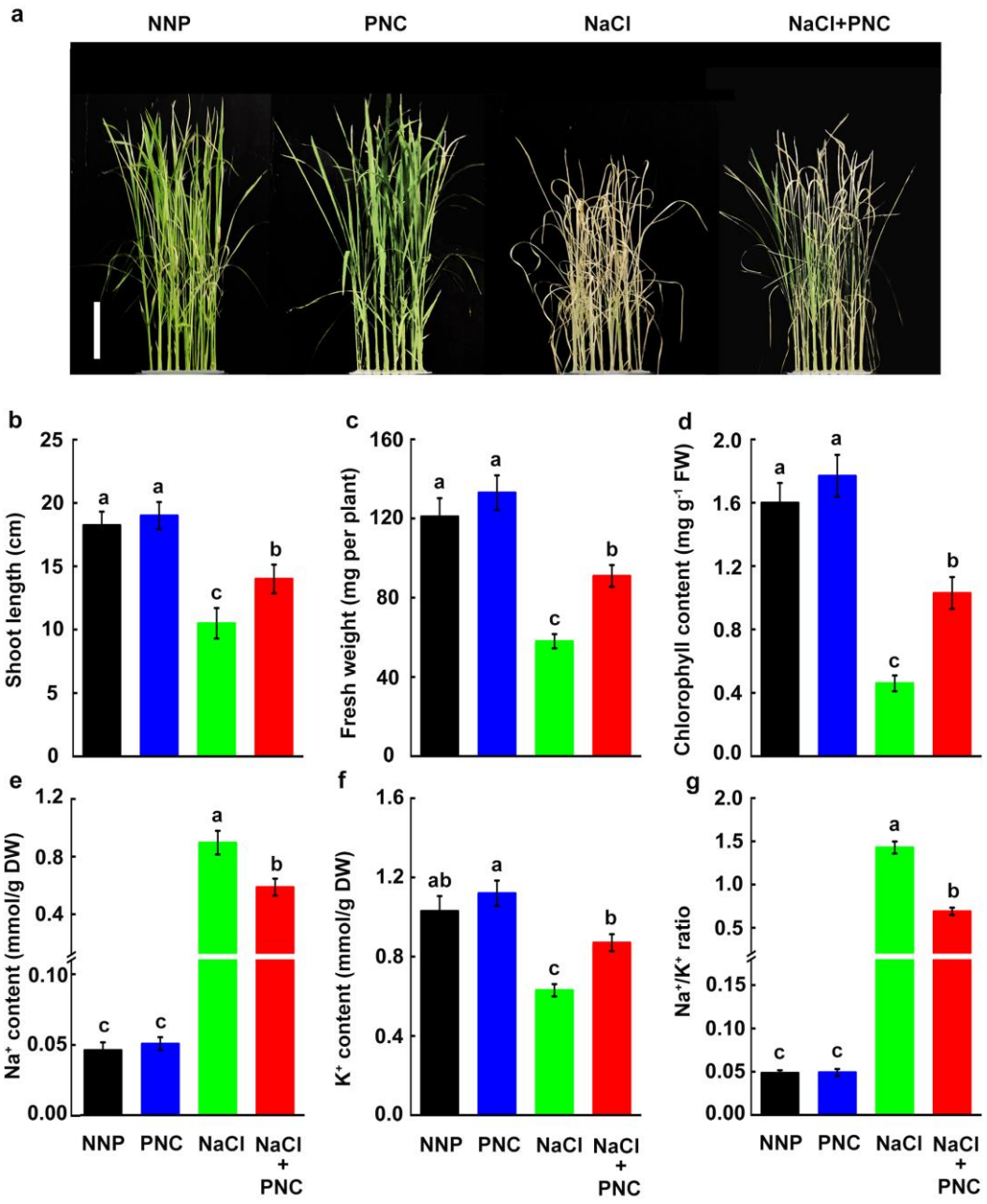

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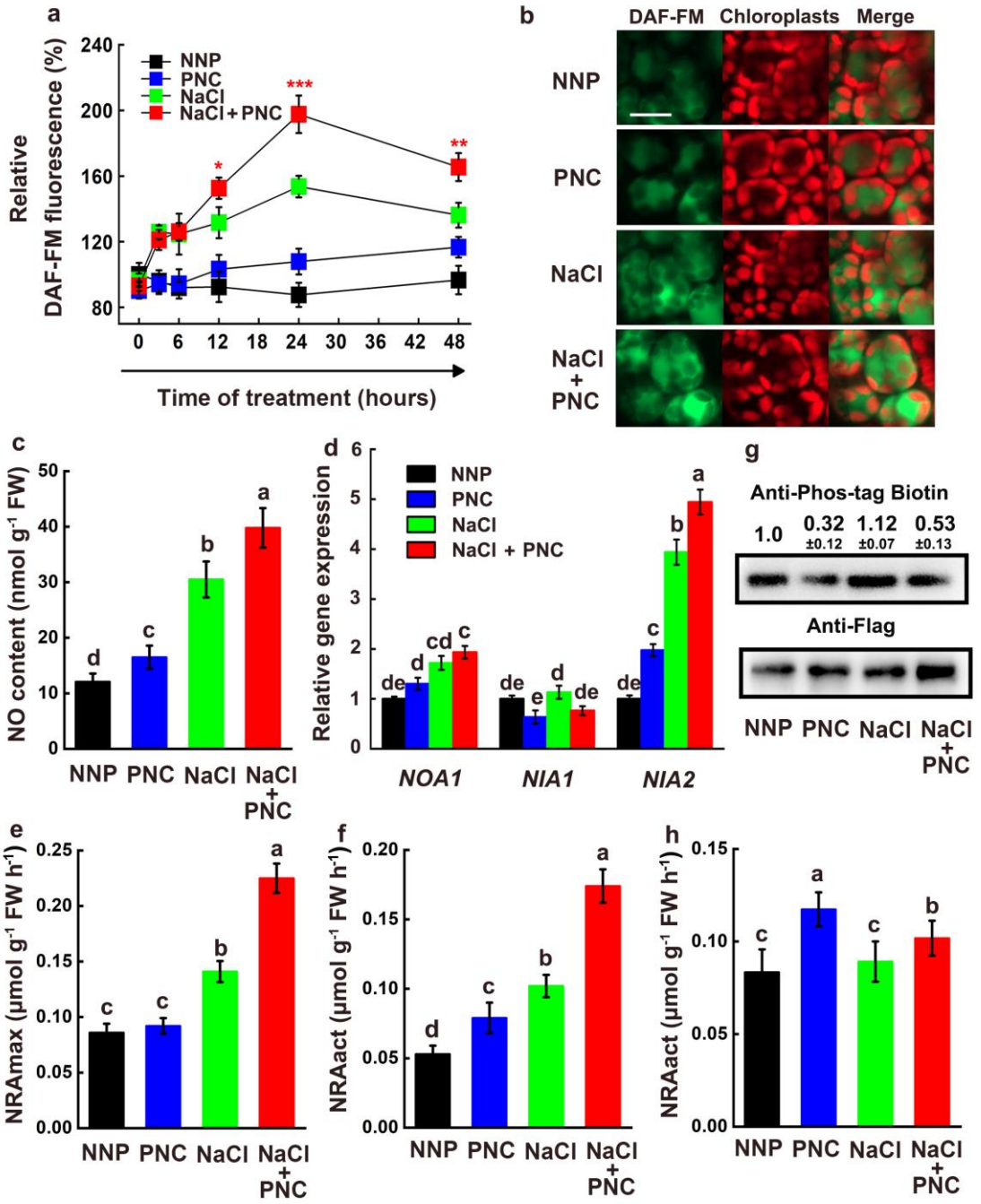

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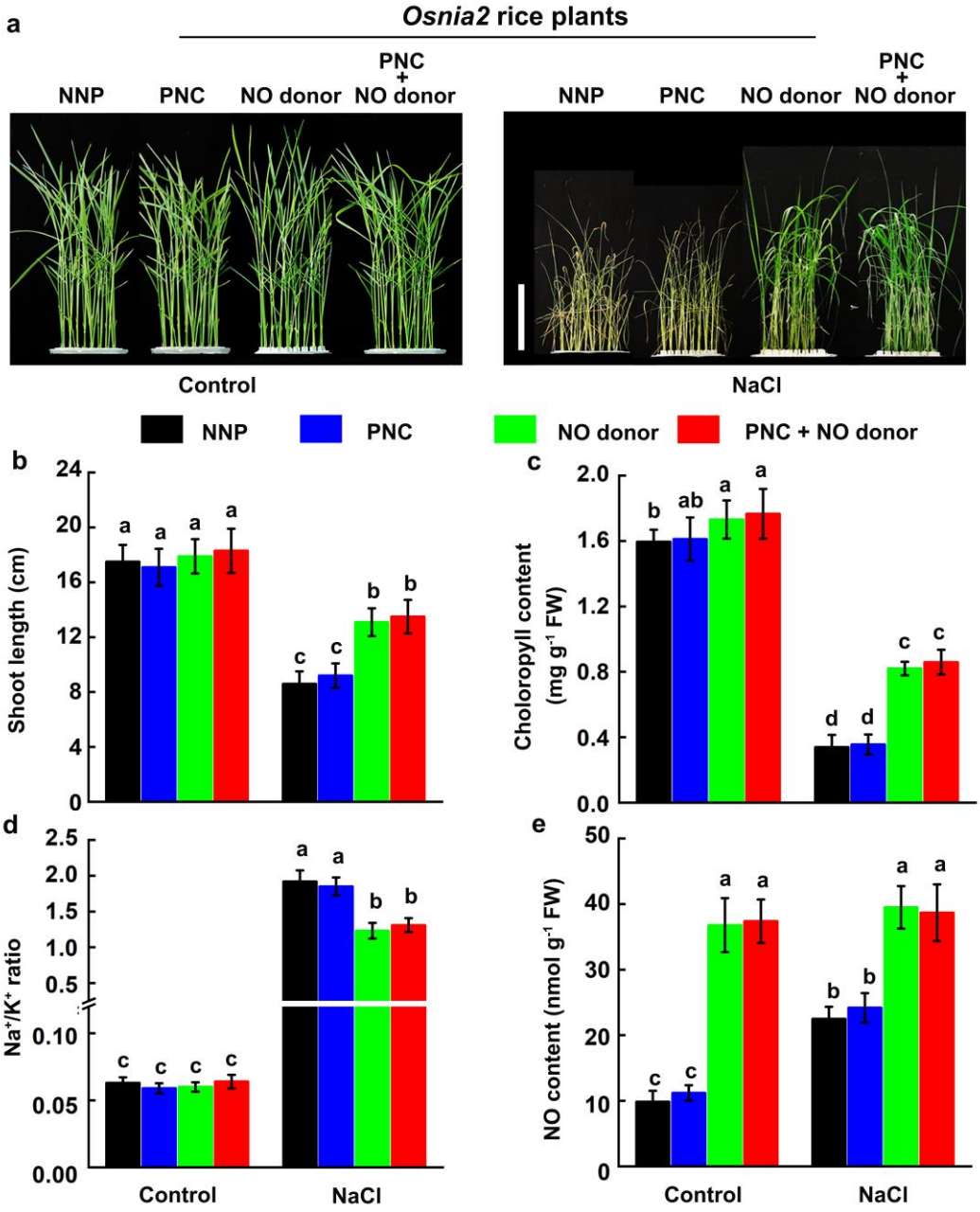

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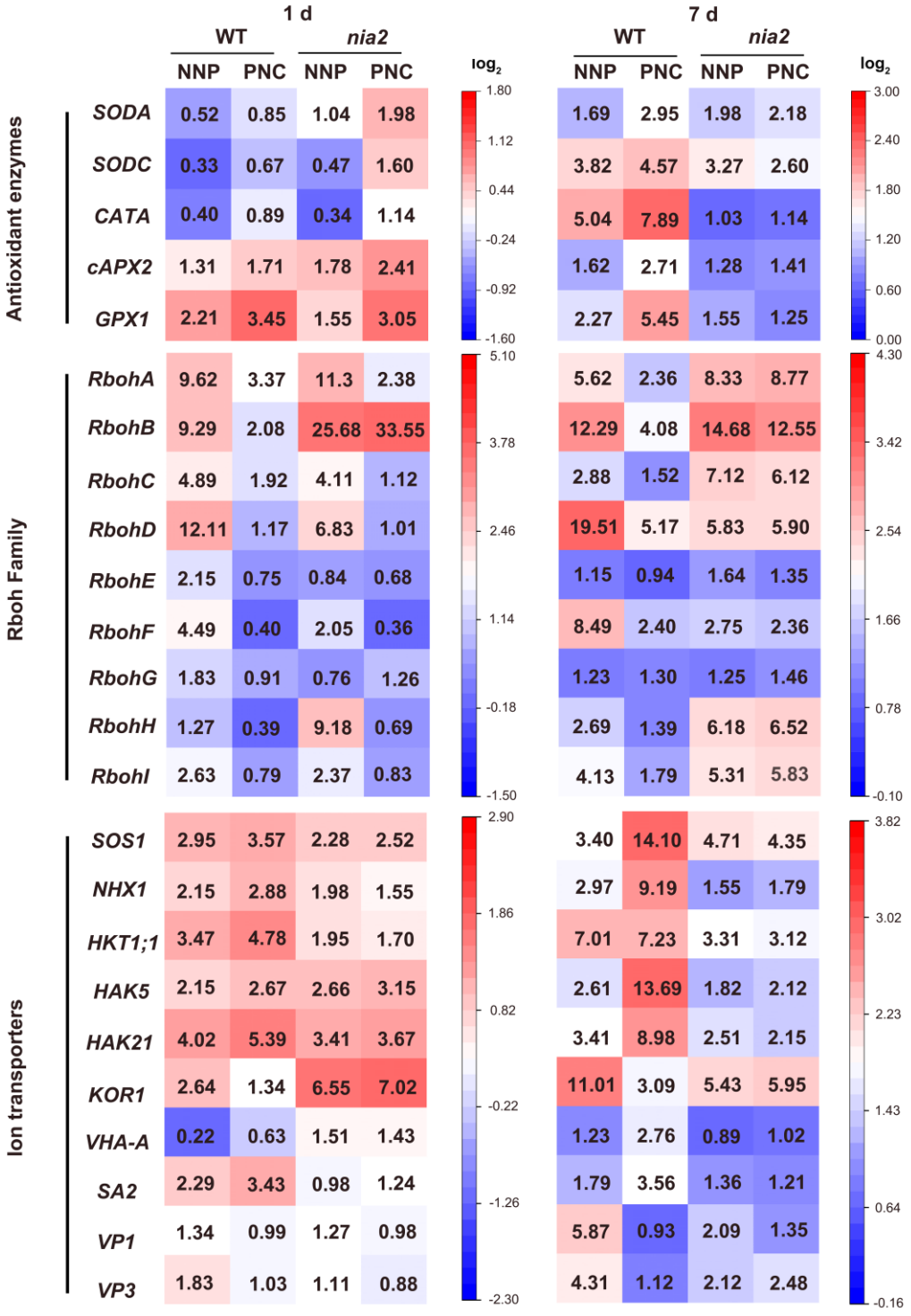

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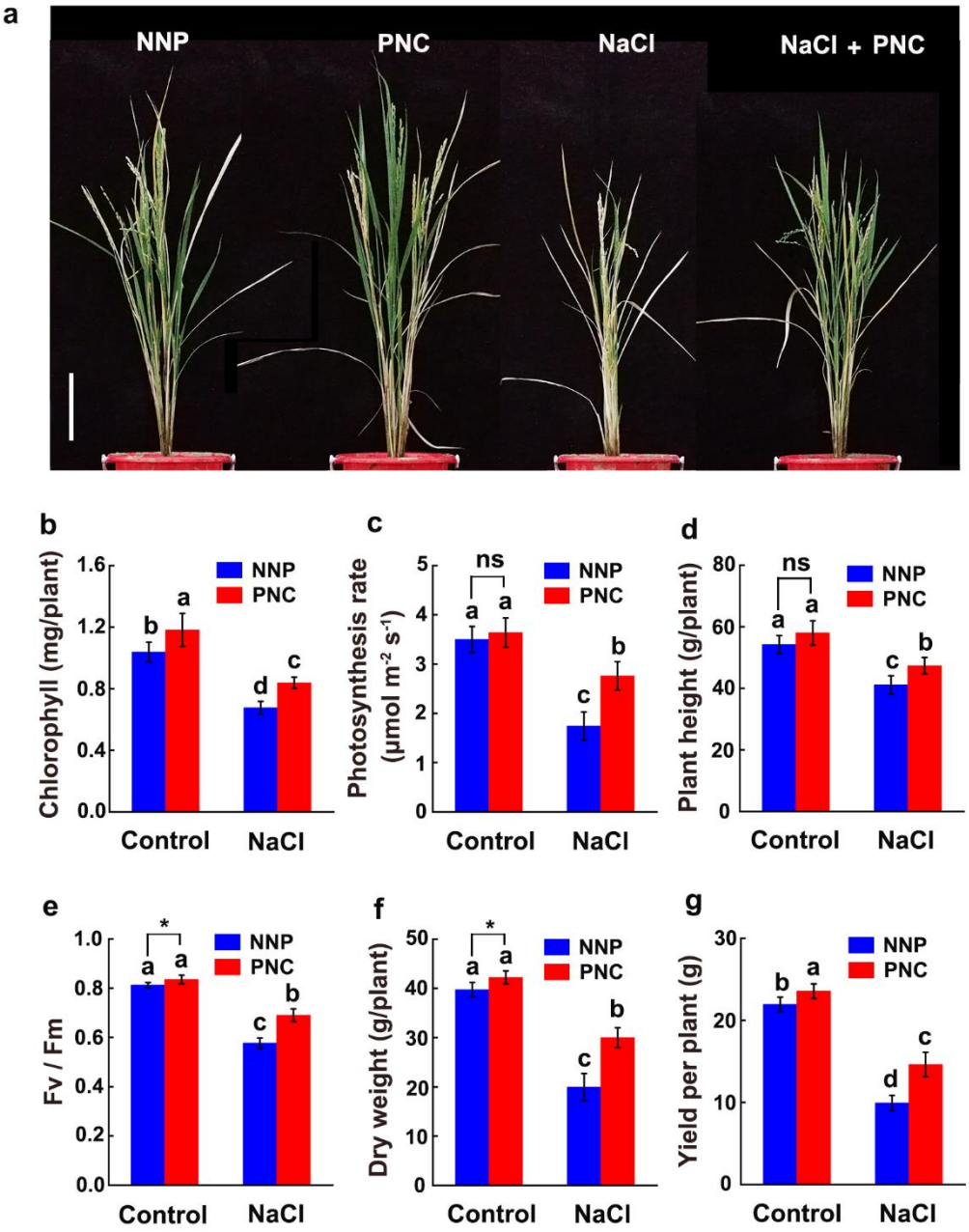

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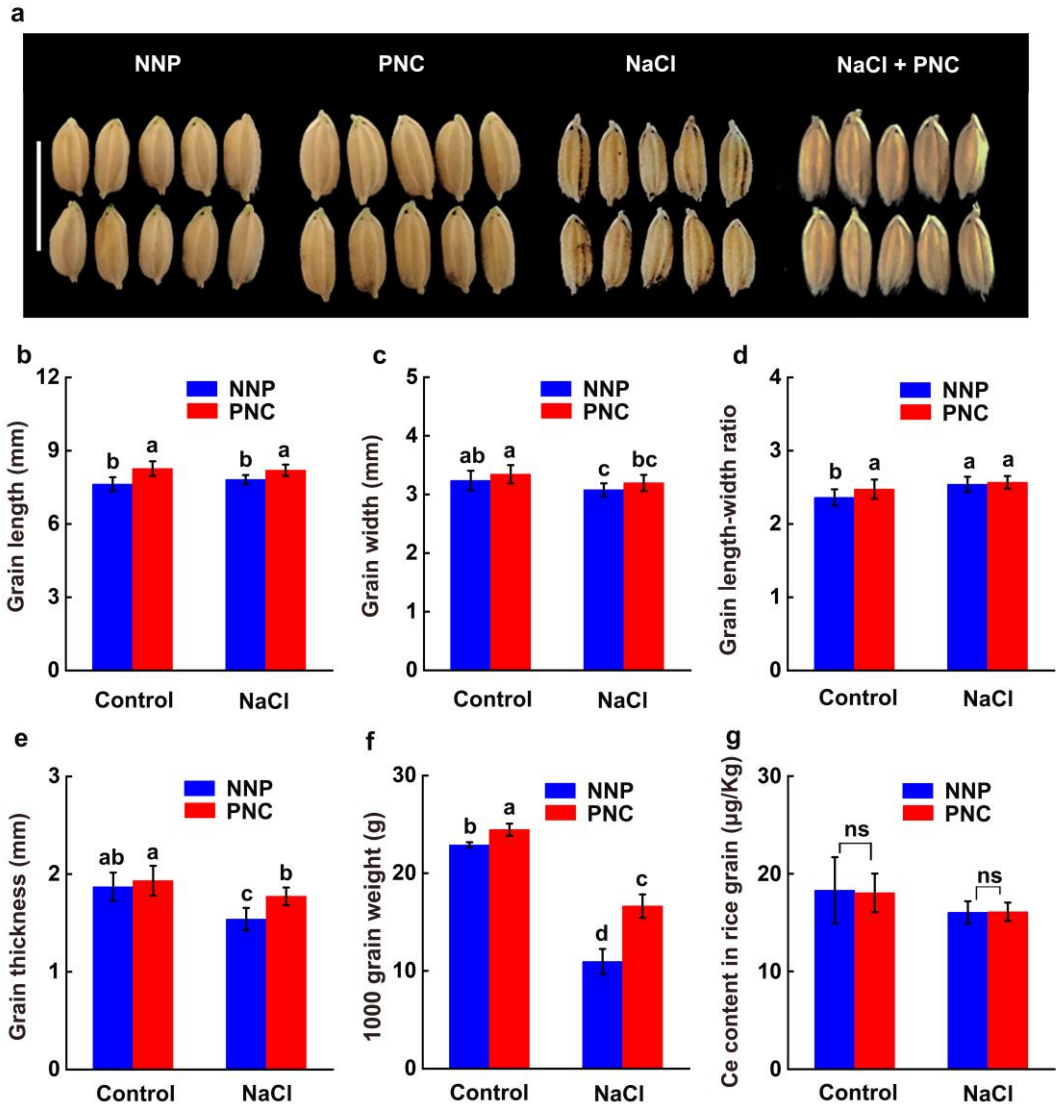

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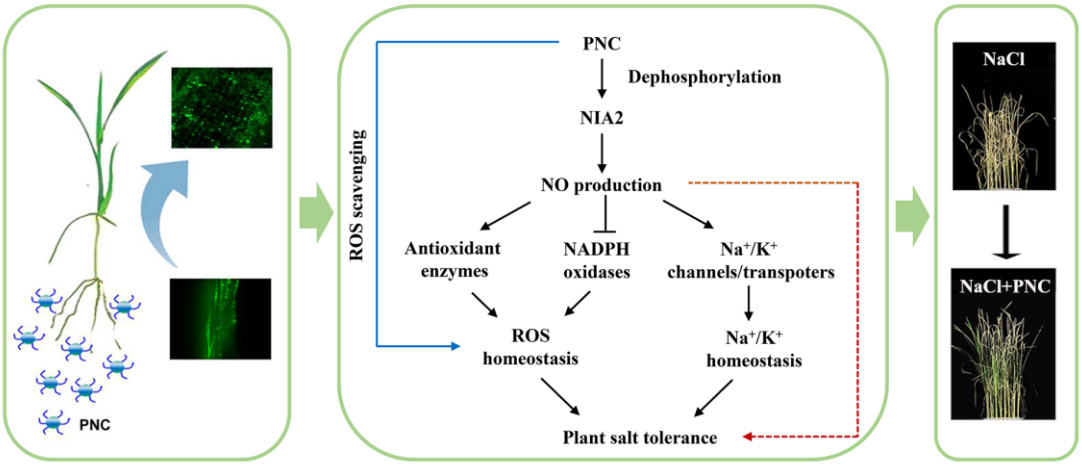

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