

Anionic Cerium Oxide Nanoparticles Protect Plant Photosynthesis from Abiotic Stress by Scavenging Reactive Oxygen Species

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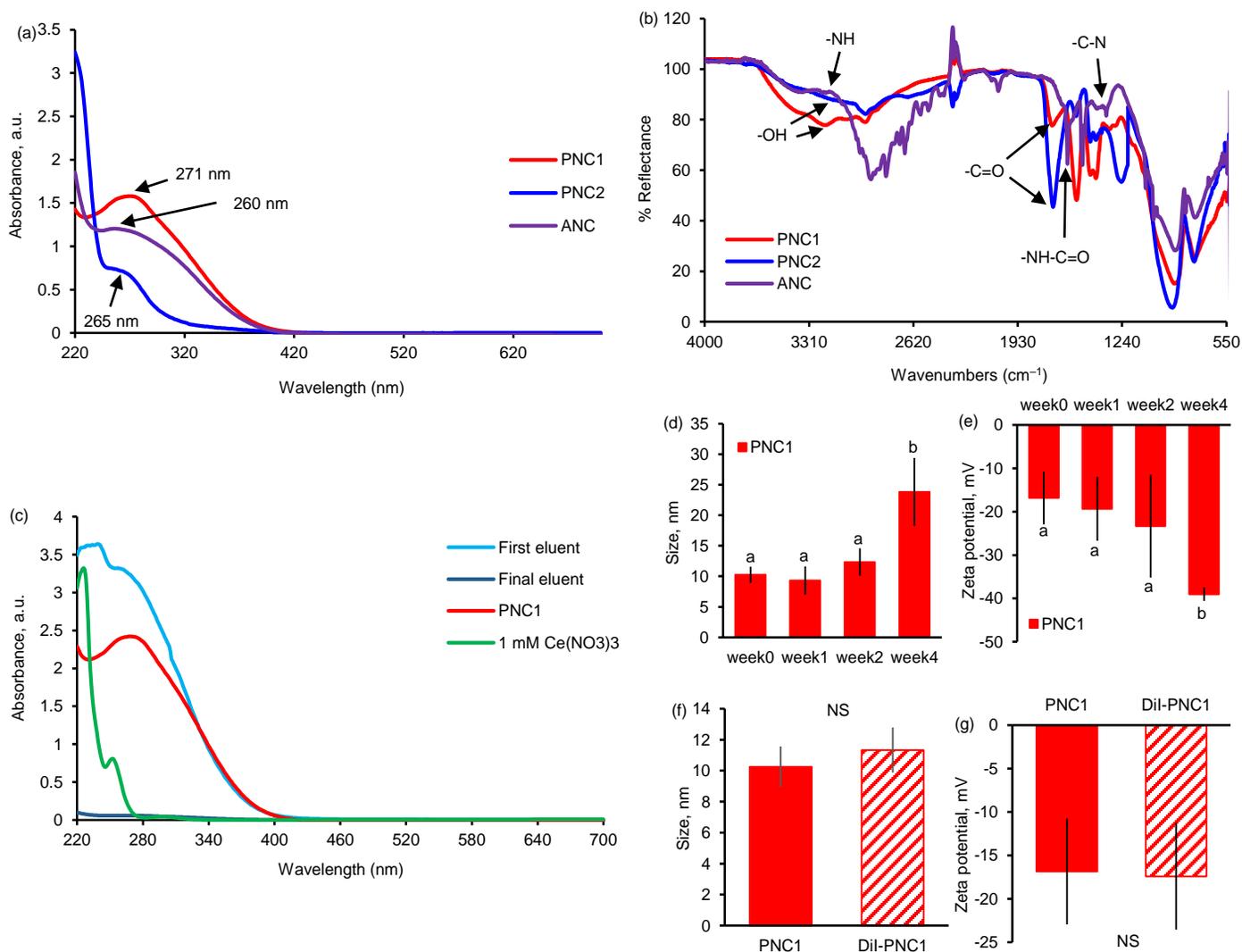


Figure S1. Nanoceria absorbance and Fourier Transform Infrared (FTIR) spectra, hydrodynamic diameter, and zeta potential. (a) Absorbance of PNC1, PNC2 and ANC measured by UV-VIS spectrophotometry show characteristic peaks of nanoceria in the UV. (b) Chemical characterization of PNC1, PNC2 and ANC by FTIR spectroscopy indicates the presence of C=O-OH bonds in PNC1, PNC2 and NH-C=O bonds in ANC. (c) No dissolved Ce(NO₃)₃ absorbance peaks were found in either purified PNC1 solution or final eluent after at least 5 cycles of purification. Temporal changes in PNC1 (d) size, measured by dynamic light scattering (DLS), and (e) zeta potential. No significant changes in size or zeta potential of PNC1 were found before the nanoparticles were two weeks old. No significant changes in (f) DLS size and (g) zeta potential were found in DiI dye labeled PNC1 relative to non-labeled PNC1. One-way ANOVA based on Duncan's multiple range test was used in d and e. Lower case letters represent significant differences at 0.05 level. Statistical comparisons in f and g were performed by independent-samples t-test (SPSS 23). NS represents no significant difference. Mean ± SD (n =3-5).

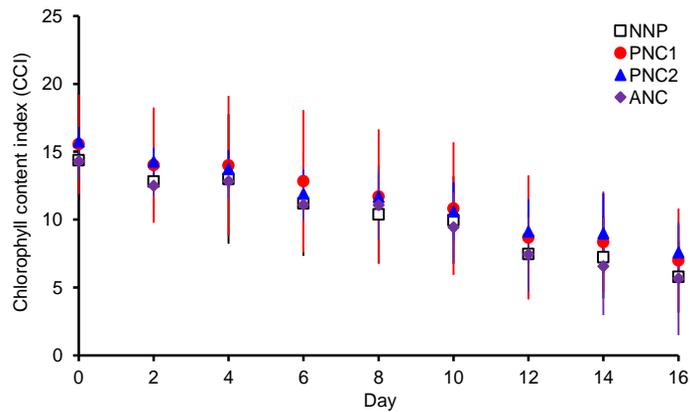


Figure S2. Temporal patterns of leaf chlorophyll content index (CCI) in *Arabidopsis* plants infiltrated with PNC1, PNC2 and ANC. PNC1, PNC2 and ANC at 450 μM did not affect leaf chlorophyll content index of *Arabidopsis* plants in normal growth chamber conditions at 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, 14/10 hr light and dark period. Statistical comparisons were performed by independent-samples t-test (SPSS 23). Mean \pm SD (n = 10).

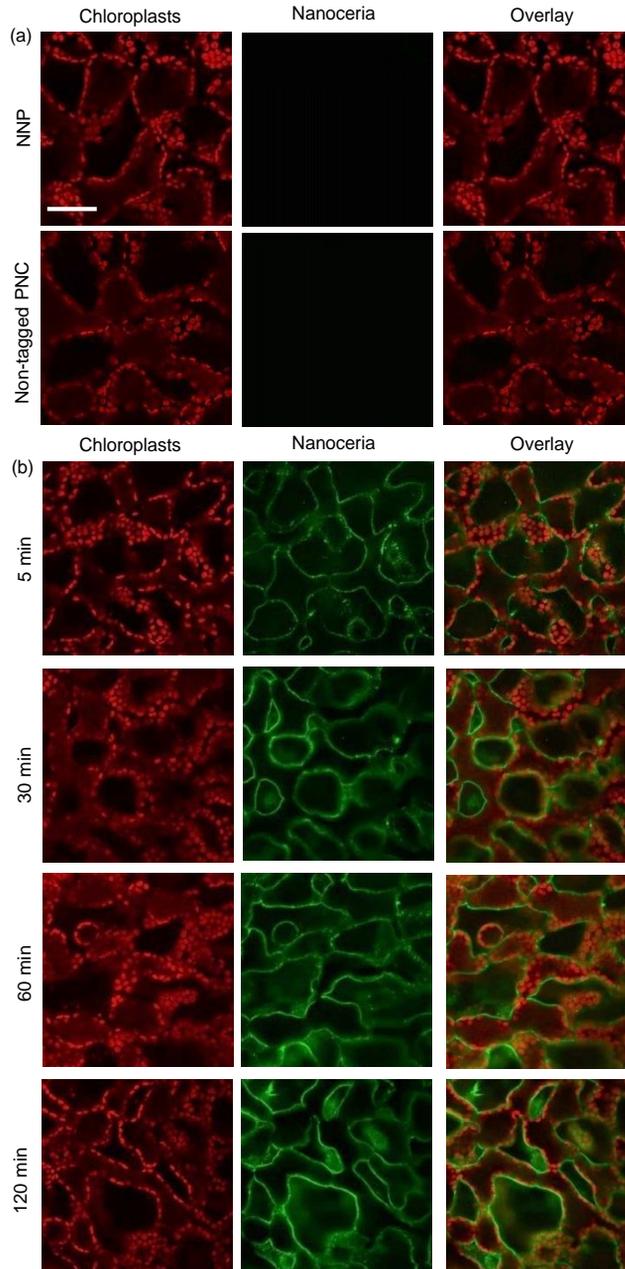


Figure S3. Temporal changes in nanoceria colocalization with chloroplasts. (a) Representative confocal images of leaf mesophyll cells in plants infiltrated with TES buffer and non-labeled nanoceria as control. (b) Representative confocal images show colocalization of chloroplast autofluorescence (red) in leaf mesophyll cells with PNC1 (green) after 5, 30, 60, and 120 min of leaf infiltration with PNC1. Scale bar 50 μm .

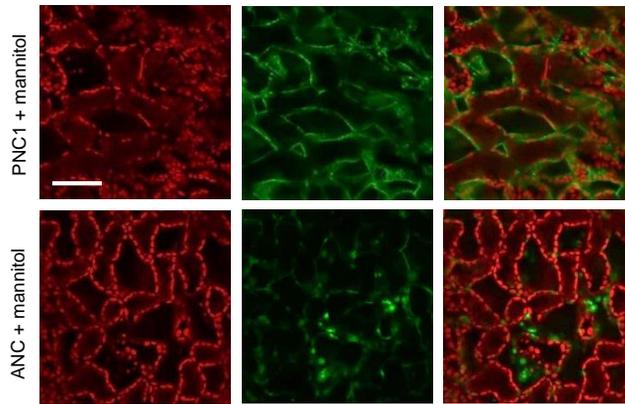


Figure S4. Osmotic stress does not affect PNC1 and ANC colocalization with chloroplasts. Representative confocal images of chloroplast autofluorescence (red) and PNC1 and ANC in leaf mesophyll cells. Colocalization of chloroplasts with nanoceria was not significantly affected by infiltration with 170 mM mannitol (isotonic to 100 mM NaCl). Scale bar 50 μ m.

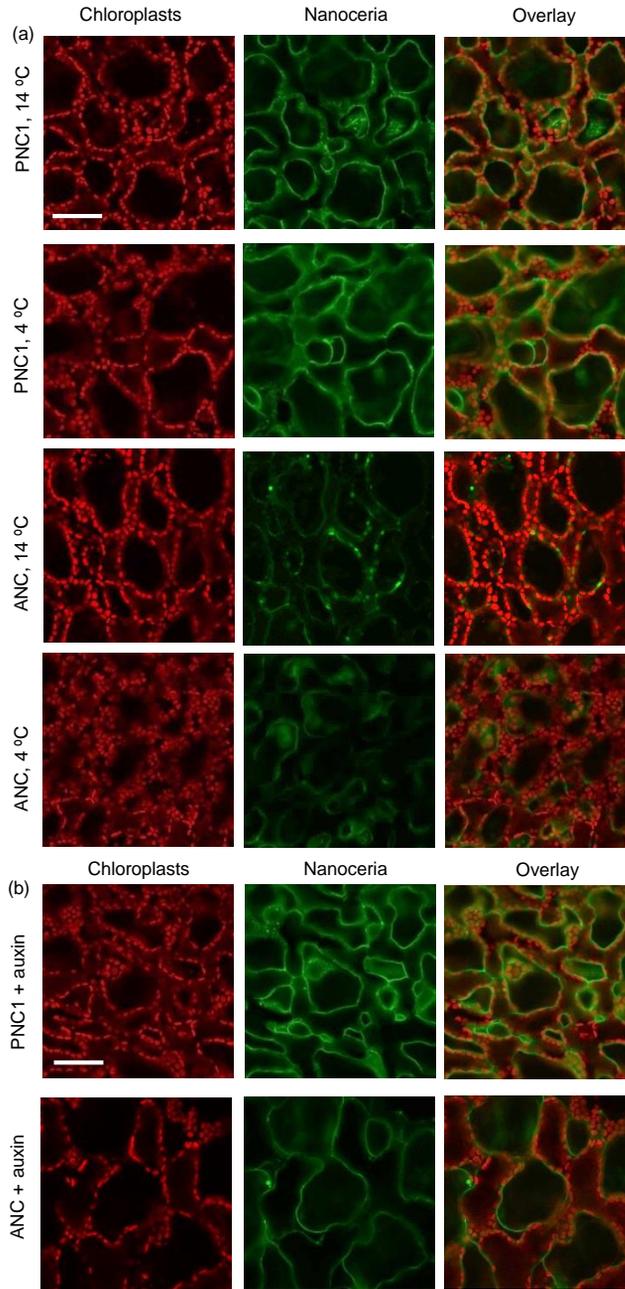


Figure S5. Temperature and auxin do not influence nanoceria colocalization with chloroplast. (a) Similar colocalization percentage of chloroplast after incubation at 14 °C and 4 °C for both PNC1 and ANC. (b) Representative confocal images of leaf mesophyll cells showing colocalization of chloroplasts with PNC1 and ANC after infiltration with auxin, an endocytosis inhibitor. Scale bar 50 μ m.

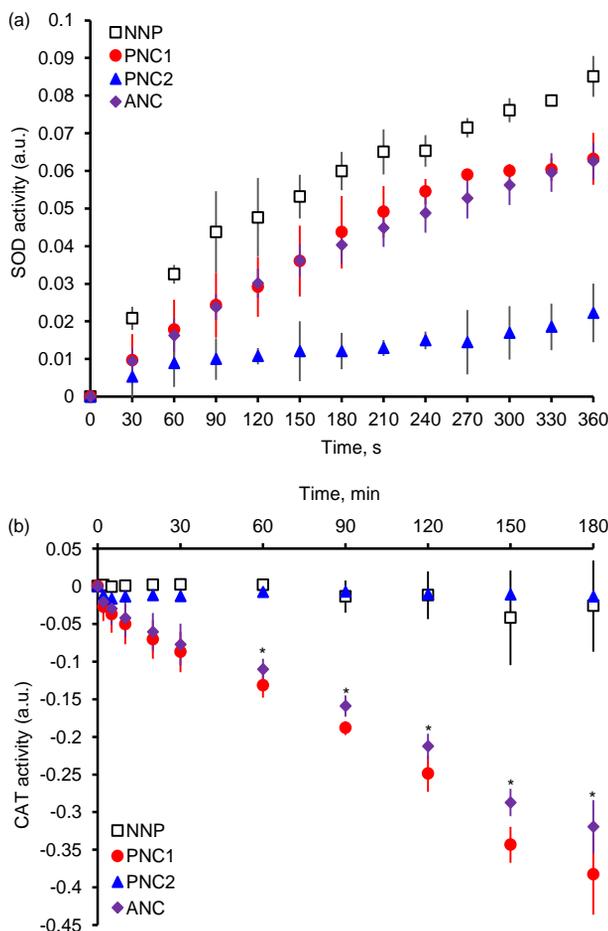


Figure S6. Superoxide dismutase (SOD) and catalase (CAT) mimetic activity of PNC1, PNC2 and ANC. (a) Superoxide anion leads to the reduction of ferricytochrome C to ferrocytochrome C (absorbance 550 nm). Competition of nanoceria with ferricytochrome C for reduction by superoxide anion was monitored by measuring absorbance at 550 nm. This assay indicates that PNC1 and ANC have a significant SOD mimetic activity. The PNC2 have a SOD mimetic activity that is stronger than PNC1 and ANC. Values are mean \pm SD ($n = 3-4$). (b) In the presence of horseradish peroxidase, Amplex Red reacts with hydrogen peroxide and is converted to resorufin (absorbance 560 nm). Absorbance of resorufin, which is indicative of hydrogen peroxide levels, was monitored at 560 nm. Both PNC1 and ANC exhibit CAT mimetic activity where PNC1 has higher CAT activity than ANC (asterisks indicate significance at $P < 0.05$, independent-samples t-test). No significant CAT mimetic activity was detected for PNC2 compared to controls without nanoparticles (NNP). Mean \pm SD ($n = 4-6$).

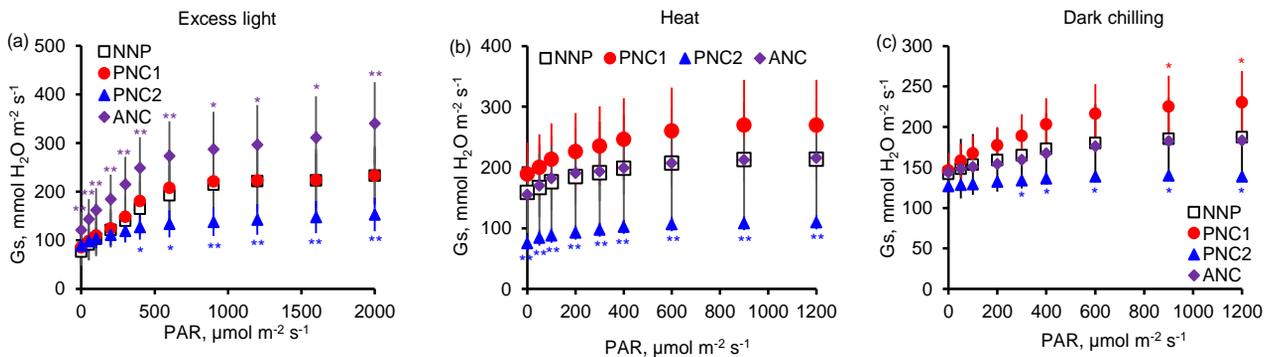


Figure S7. Stomatal conductance is not affected by PNC1 in plants under excess light and temperature stress. No significant difference of stomatal conductance (G_s) was found between plants infiltrated with PNC1 or infiltration buffer (NNP) under excess light (a), heat (b), and dark chilling (c), except for 900 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR in the dark chilling stress treatment. A significantly lower G_s was found in PNC2-Leaves relative to NNP-Leaves under excess light (a), heat (b), and dark chilling (c). A higher G_s was found in ANC-Leaves under excess light in comparison with NNP-Leaves (a). Statistical comparisons were performed by independent-samples t-test (SPSS 23, * $P < 0.05$, ** $P < 0.01$). Mean \pm SD ($n = 8-15$). Asterisks represent significant differences between leaves with nanoceria and buffer controls (NNP-Leaves).

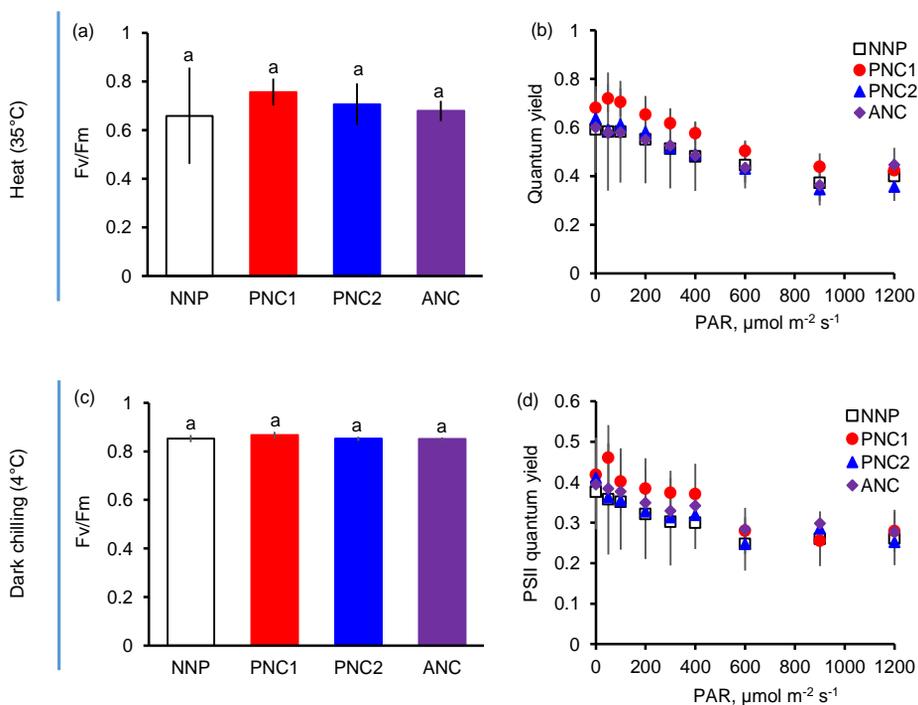


Figure S8. Nanocerium (PNC1, PNC2 and ANC) do not protect PSII from heat and dark chilling stress. No significant differences of Fv/Fm (a, c) and quantum yield of PSII (b, d) in PNC1-leaves, PNC2-Leaves and ANC-Leaves compared to leaves without nanoparticles (NNP). Statistical comparisons were performed by independent-samples t-test (SPSS 23). NS represents no significant difference. Statistical analysis in a and c were performed by a one-way ANOVA based on Duncan's multiple range test. Same lower case letters represent no significance at 0.05 level. Comparisons in b and d were performed by independent-samples t-test (SPSS 23). Mean \pm SD (n = 9-12).