Supporting information

Quantifying the Distribution of Ceria Nanoparticles in Cucumber Plants: the Influence of Labeling

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The supporting information includes:

1. Number of pages: 5

2. Number of figures: 4

1. Preparation and Characterization of Nano-ceria

In the present work, 0.5 M solution of hexamethyleneteramine (HMT) and 0.0375M solution of $Ce(NO_3)_3 \cdot 6H_2O$ were mixed and stirred in 75°C water bath for 3 h. The particles obtained were washed three times by ultrapure water. The morphology and size distribution of the as-synthesized nano-ceria were characterized by TEM (FEI Co., Eindhoven, The Netherlands). The samples were dispersed in ultrapure water and sonicated for 15 min before dropped on a Cu grid for observation. X-ray diffraction (XRD) of nano-ceria was obtained on a Panalytical's X'Pert PRO diffractometer (Panalytical BV, the Netherlands) using monochromatized Cu Kα radiation at 30 kV and 40 mA. The XRD patterns were scanned from 5-90° 2θ range using 0.02° step intervals. The size distribution and the surface charge density (in terms of zeta potential) of as-synthesized nano-ceria (AS-ceria) and diiodofluorescein-coated nano-ceria (DIF-ceria) in ultrapure water were evaluated by dynamic light scattering (DLS) analysis (Zeta-Sizer, Malvern Instruments, UK). The hydrodynamic sizes of AS-ceria and DIF-ceria were 34.2±9.7 nm (PDI=0.218) and 39.5±13.4 nm (PDI=0.241), respectively; their zeta-potentials were 36.6±5.9 mV and 34.3±7.0 mV.

Figure S1. The XRD pattern of nano-ceria.

The specific surface area of nano-ceria was 86.85 $m^2 \cdot g^{-1}$, measured by the BET

method using a surface area analyzer (Autosorb-1 model, Quantachrome, USA). Assuming that each DIF molecule could cover 0.9 nm^2 of the particulate surface (fluorescein is a nonspherical solute with an approximate size of 0.47, 0.81, and 1.09 nm in different directions)[23], 25% of the surface of nano-ceria was covered with DIF when 25 mg nano-ceria were mixed with 50 mL 2×10^{-5} M DIF solution.

2. Effect of DIF exposure on the root elongation of cucumber

The root elongation of cucumber was determined after a 5-day incubation in DIF solution at a concentration of 2×10^{-5} M. In a parallel study, the seeds were pre-soaked in the respective DIF solution for 2 h before the 5-day germination test.

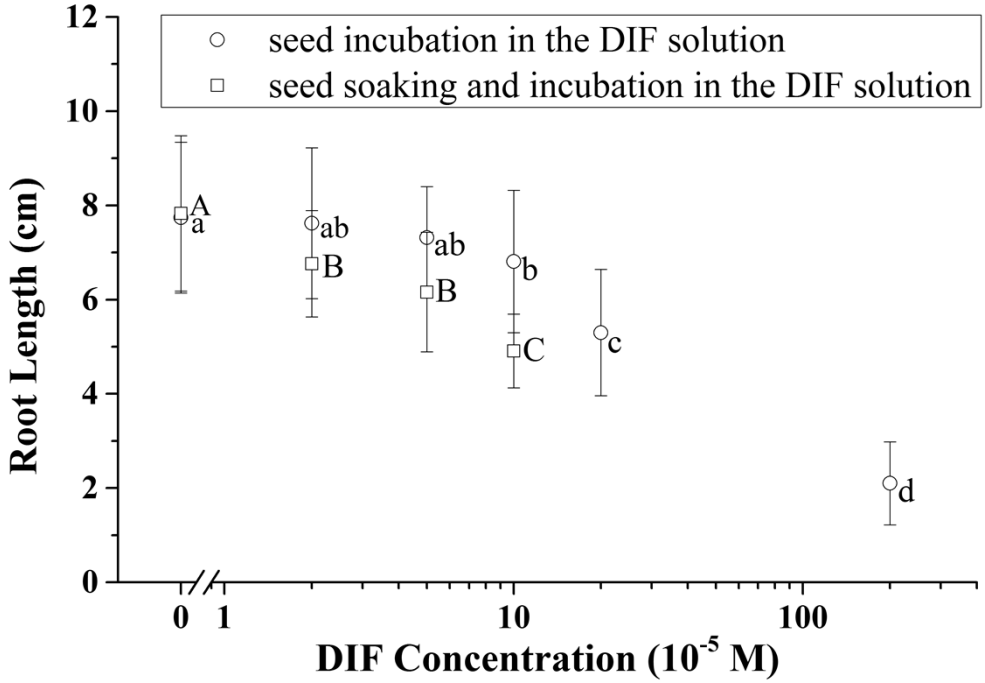

Figure S2. Cucumber root elongation after a 5-day incubation in DIF solution or a 2-hour pre-soaking followed by a 5-day incubation in DIF solution. The cucumber root length is expressed as mean±SD of 40 replicates. In the seed incubation treatment, data labeled with different lowercase letters are significantly different at $p < 0.05$; in the seed soaking and incubation treatment, data labeled with different uppercase letters are significantly different at $p < 0.05$.

3. TEM images of the cross sections of cucumber roots treated with AS-ceria and

DIF-ceria

After a 5-day germination, cucumber roots were washed with deionized water thoroughly and the root apexes were cut and fixed in 2.5% glutaraldehyde solution. Then they were dehydrated in a graded acetone series and embedded in Spurr's resin. Ultrathin sections (90 nm) were obtained using an UC6i ultramicrotome (Leica, Austria) with a diamond knife. The sections were collected on copper grids and observed on a JEM-1230 (JEOL, Japan) transmission electron microscope operating at 80 kV without staining.

As shown in figure S3, most of the AS-ceria and DIF-ceria agglomerates were found on the surface of roots though the seedlings had been thoroughly washed.

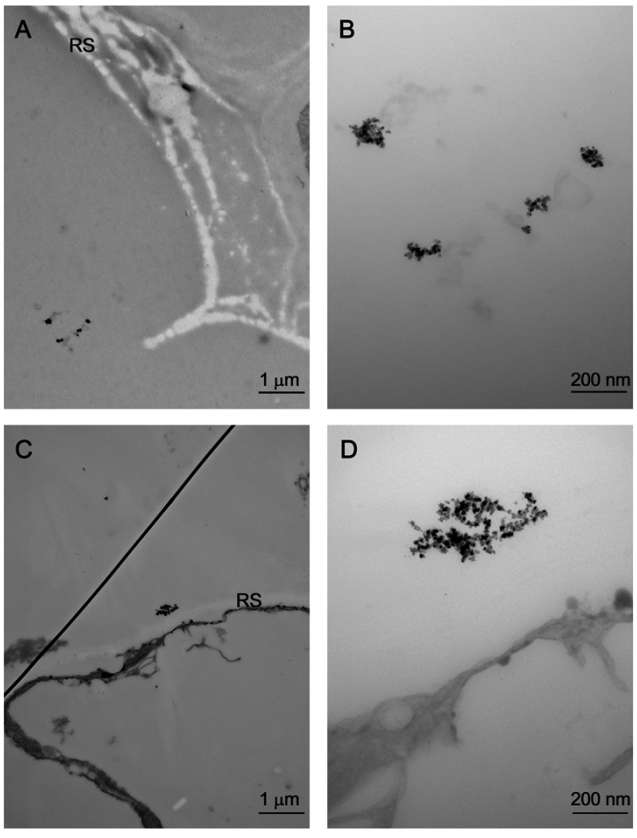

Figure S3. TEM images of the cross sections of cucumber roots treated with As-ceria (A&B) and DIF-ceria (C&D). RS: root surface.

4. The influence of DIF coverage

Our results showed that fluorescent labeling could change the behaviors and bio-effects of nano-ceria when 25% of the particulate surface was coated with DIF. We further determined the labeling-caused changes by different DIF-coverage rates. The results suggest that to minimize the labeling-caused changes, the DIF coverage rate should not be larger than 5%.

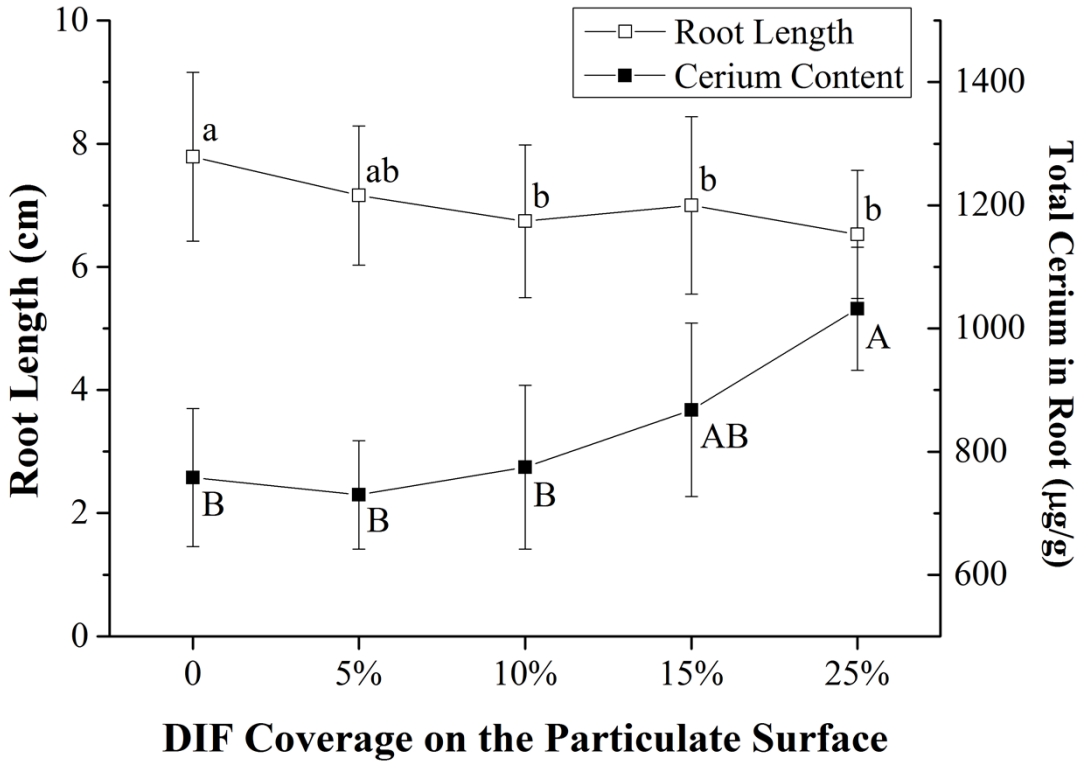

Figure 4. The changes in the root length and root cerium content caused by the different DIF coverage rates on the surface of nano-ceria. Cucumber root lengths labeled with different lowercase letters are significantly different at $p < 0.05$; As for the contents of total cerium in roots, data labeled with different uppercase letters are significantly different at $p < 0.05$.