SUPPORTING INFORMATION

Minimal transgenerational effect of ZnO nanomaterials on the physiology and nutrient profile of *Phaseolus vulgaris*

Illya A. Medina-Velo[1,4], Nubia Zuverza-Mena[2], Carlos Tamez[3], Yuqing Ye[1], Jose A. Hernandez-Viezcas[1,4], Jason C. White[2], Jose R. Peralta-Videa[1,3,4] and Jorge L. Gardea-Torresdey[1,3,4,*]

[1]Department of Chemistry, The University of Texas at El Paso; 500 West University Ave., El Paso, TX 79968, United States.

[2]Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, 123 Huntington St., New Haven, CT 06504, United States

[3]Environmental Science and Engineering Ph.D. Program, The University of Texas at El Paso; 500 West University Ave., El Paso, TX 79968, United States.

[4]University of California Center for Environmental Implications of Nanotechnology (UC CEIN), The University of Texas at El Paso; 500 West University Ave., El Paso, TX 79968, United States.

Corresponding author: jgardea@utep.edu (J. Gardea); Phone: 915-747-5359; Fax: 915-747-5748.

Materials and methods

Antioxidant enzymatic activity in young seeds

One immature (45 days after planting) pod from each plant/replicate was collected and one S2 seed was isolated from each pod. The seeds from each replicate were mixed and two sub-samples of ~1 g were ground in liquid nitrogen and extracted with 10 mL of 0.1 M phosphate buffer (pH 7.8) using a mortar and pestle. The homogenate was centrifuged at 16 000 × g at 4 °C for 15 min (Sorvall Legend X1R, Thermo Scientific, Waltham, MA).[1] The supernatant was distributed into five Eppendorf tubes, frozen in liquid nitrogen, and stored at -80 °C until analysis.

The enzyme analyses were performed by measuring absorbance in a quartz cuvette using a UV/Vis Spectrometer (single-beam mode, Perkin-Elmer Lambda 14, Uberlingen, Germany).

The APX (EC 1.11.1.11) activity was determined according to Bailly et al. (2001)[1] and Nakano and Asada (1981)[2] by the decrease in the absorbance of H_2O_2 at 290 nm during a 2 min interval. The reaction mix contained 50 µL of sample, 285 µL of 0.5 mM ascorbic acid, and 665 µL of 0.4 mM H_2O_2. The CAT (EC 1.11.1.6) activity was determined as reported by Bailly (1996).[3] Sixty seven µL of extract were mixed with 933 µL of 3.125 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0). Changes in absorbance were recorded at 240 nm during a 3 min interval. The APX and CAT activities were expressed as nmol H_2O_2 decomposed (g fresh seed)$^{-1}$ min^{-1}. The SOD (EC 1.15.11) [3–7] assay contained 450 µL of 500 µM nitroblue tetrazolium (NBT), 500 µL of 78 mM L-methionine, 200 µL of 1.5 mM EDTA, 300 µL of 0.02 mM riboflavin, 1500 µL of 100 mM potassium phosphate buffer (pH 7.8) and 50 µL of enzyme extract. The SOD activity was estimated by measuring inhibition of the photochemical reduction of NBT by the enzyme extract. The reaction mixture was placed in a glass test tube and illuminated with a fluorescent

light bulb in a closed box during 15 min, and then absorbance (A_1) was measured at 560 nm. Non-illuminated tubes with the reaction mix without seed served as blanks, and absorbance after illumination was recorded (A_0). One unit of SOD is defined as the enzymatic activity that causes 50% inhibition of the assay reaction; the % inhibition of NBT reduction by SOD was calculated by $\frac{A_0-A_1}{A_0}$.

References cited:

(1) Bailly, C.; Audigier, C.; Ladonne, F.; Wagner, M. H.; Coste, F.; Corbineau, F.; Come, D. Changes in Oligosaccharide Content and Antioxidant Enzyme Activities in Developing Bean Seeds as Related to Acquisition of Drying Tolerance and Seed Quality. *J. Exp. Bot.* **2001**, *52* (357), 701–708.

(2) Nakano, Y.; Asada, K. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol* **1981**, *22* (5), 867–880.

(3) Bailly, C.; Benamar, A.; Corbineau, F.; Côme, D. Changes in Malondialdehyde Content and in Superoxide Dismutase, Catalase and Glutathione Reductase Activities in Sunflower Seeds as Related to Deterioration during Accelerated Aging. *Physiol. Plant.* **1996**, *97* (1), 104–110.

(4) Beyer, W. F.; Fridovich, I. Assaying for Superoxide Dismutase Activity: Some Large Consequences of Minor Changes in Conditions. *Anal. Biochem.* **1987**, *161*, 559–566.

(5) Rico, C. M.; Morales, M. I.; McCreary, R.; Castillo-Michel, H.; Barrios, A. C.; Hong, J.; Tafoya, A.; Lee, W.-Y.; Varela-Ramirez, A.; Peralta-Videa, J. R.; et al. Cerium Oxide Nanoparticles Modify the Antioxidative Stress Enzyme Activities and Macromolecule Composition in Rice Seedlings. *Environ. Sci. Technol.* **2013**, *47*, 14110–14118.

(6) Giannopolitis, C. N.; Ries, S. K. Superoxide Dismutases. *Plant Physiol* **1977**, *59*, 309–314.

(7) Zhang, C.; Bruins, M. E.; Yang, Z.-Q.; Liu, S.-T.; Rao, P.-F. A New Formula to Calculate Activity of Superoxide Dismutase in Indirect Assays. *Anal. Biochem.* **2016**, *503*, 65–67.