Supplement informations

Stress impacts of different types of micro- and nanomaterials on vegetable crops

Weiwen Qiu[a#], Minling Gao[b#], Xue meng[b], Youming Dong[b], Liu Qinghai[c], Chen Qiu[c], Zhengguo Song[b]*

[a] The New Zealand Institute for Plant and Food Research Limited, Private Bag 3230, Hamilton 3240, New Zealand

[b] Department of Materials and Environmental Engineering, Shantou University, Shantou, 51 5063, China

[c]Institute of Agricultural Resources and Environment, Xizang Academy of Agricultural and Animal Husbandry Sciences, Lhasa Xizang 850032, China.

[#]Weiwen Qiu and Minling Gao contributed equally to this work.

*Corresponding author:

Email: forestman1218@163.com

2 Materials and methods

2.1 Dehydrogenase (DHA) activity measurement for cherry radish and lettuce

A 0.1 g fresh cherry radish or lettuce root sample was placed in a 10 mL centrifuge tube. 1 mL of triphenyltetrazolium chloride (TTC) solution and 1 mL of TTC detection buffer were added to the sample and thoroughly mixed. The mixture was incubated in the dark for 2 hours. Afterwards, 0.4 mL of TTC termination solution was added and the mixture was thoroughly stirred. The root samples were then removed and transferred to a new centrifuge tube after drying the surface water. 2 mL of methanol was added to the new tube, and the sample was soaked at 40°C for 2 hours until it turned completely white. A control blank tube was prepared

without plant samples. The absorbance at 485 nm was measured using a UV-visible spectrophotometer to calculate the activity.

2.2 Soluble sugar and soluble protein measurement of cherry radish

0.1 g of fresh cherry radish fleshy root sample was weighed, and it was ground into a slurry with 1 mL of distilled water addition. The slurry was then poured into a covered centrifuge tube, placed in a 95°C water bath, cooled, and centrifuged at 8000 g at 25°C for 10 minutes. The supernatant was transferred to a 10 mL test tube, diluted to 10 mL with distilled water, and shaken well. Into an EP tube, 200 μL of the sample (with a blank tube having no sample), 200 μL of distilled water, 100 μL of anthrone (with ethyl acetate) solution, and 1 mL of concentrated sulfuric acid were added sequentially, mixed, and the EP tube was placed in a water bath at 95°C for 10 minutes, then cooled to room temperature. The absorbance values of the blank and test tubes at 620 nm were obtained to calculate the soluble sugar content.

For soluble protein content measurement, 0.1 g of a fresh cherry radish fleshy root sample was weighed. 1 mL of distilled water was added to the sample, and the samples was ground in an ice bath. The mixture was then centrifuged at 8000 g at 4°C for 10 minutes. Into an EP tube, 20 μL of the sample (with a blank tube containing distilled water and a standard tube with a soluble protein standard), 1 mL of BCA solution and $CuSO_4$ solution mixed at a 50:1 ratio were sequentially added, mixed, and the mixture was incubated at 60°C for 30 minutes. The absorbance values of the blank, standard, and test tubes at 562 nm in a 1 mL cuvette were measured to calculate the soluble protein content.

2.3 H_2O_2 content measurement for cherry radish and lettuce leaf

0.1 g of fresh cherry radish or lettuce leaf sample was weighed, mixed with 1 mL of acetone, and ground in an ice bath. The mixture was transferred to a 2 mL centrifuge tube and diluted to 1 mL with acetone, then centrifuged at 8000 g at 4°C for 10 minutes. 1 mL of the supernatant was removed to a new centrifuge tube, to which 100 μL of titanium sulfate and 200 μL of

concentrated ammonia were added, followed by centrifugation at 4000 g at 25°C for 10 minutes. The supernatant was discarded, and the precipitate was retained. 1 mL of sulfuric acid solution was added to the precipitate. Once the precipitate dissolves, it is left to stand at room temperature for 5 minutes, then transferred into a cuvette to measure the absorbance at 415 nm and calculate the H_2O_2 content.

2.4 MDA content determination for lettuce leaf

Weigh 0.1g of tissue, add 1mL $Na_2HPO_4 \cdot 12H_2O$ and NaH_2PO_4 mixture. Homogenize the mixture in an ice bath. Then centrifuge at 8000 g, 4°C for 10 min. Collect the supernatant. Add 0.6 mL of the TCA and TBA mixture and 0.2 mL of the sample to a 2 mL centrifuge tube. Mix thoroughly and incubate in a water bath at 95°C for 30 minutes, ensuring the tube is tightly covered to prevent water loss. Cool the mixture in an ice bath, then centrifuge at 10,000 g at 25°C for 10 minutes. Transfer the supernatant to a 1 mL cuvette and measure the absorbance at 532 nm and 600 nm using a spectrophotometer. Calculate the MDA content based on these measurements.

2.5 CAT content measurement for lettuce leaf

Weigh 0.1 g of tissue and add 1 mL of $Na_2HPO_4 \cdot 12H_2O$ and NaH_2PO_4 extraction solution. Homogenize the mixture in an ice bath, then centrifuge at 8000 g at 4°C for 10 minutes and collect the supernatant. Incubate the working solution in a water bath at 25°C for 10 minutes. Preheat the spectrophotometer for at least 30 minutes, set it to 240 nm. Add 35 μL of the sample and 1 mL of the working solution ($Na_2HPO_4 \cdot 12H_2O$ and NaH_2PO_4 solution) to a 1 mL cuvette and mix. Immediately measure the initial absorbance at 240 nm and then the absorbance after 1 minute at room temperature. Calculate the CAT content.

Figures

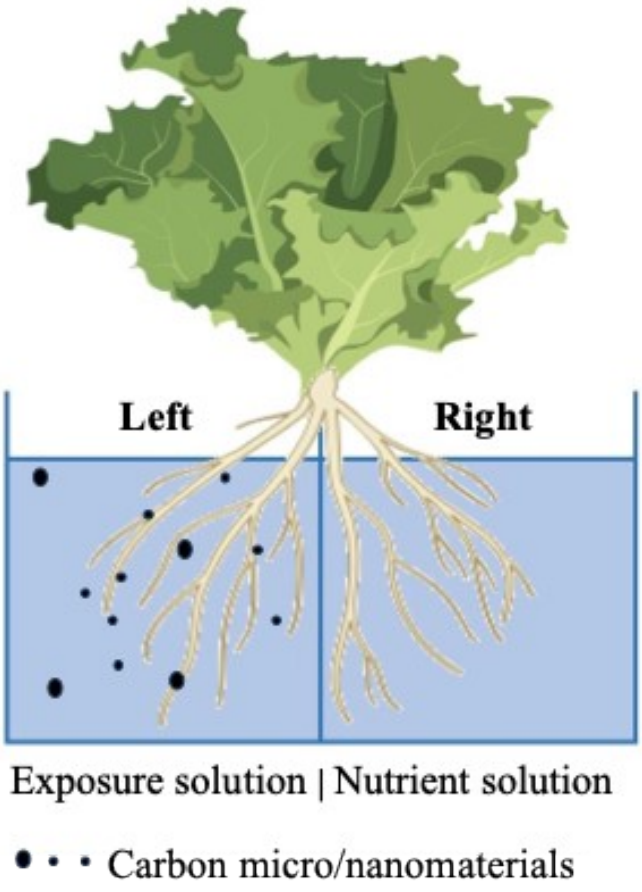

Figure S1. Divided roots experiment

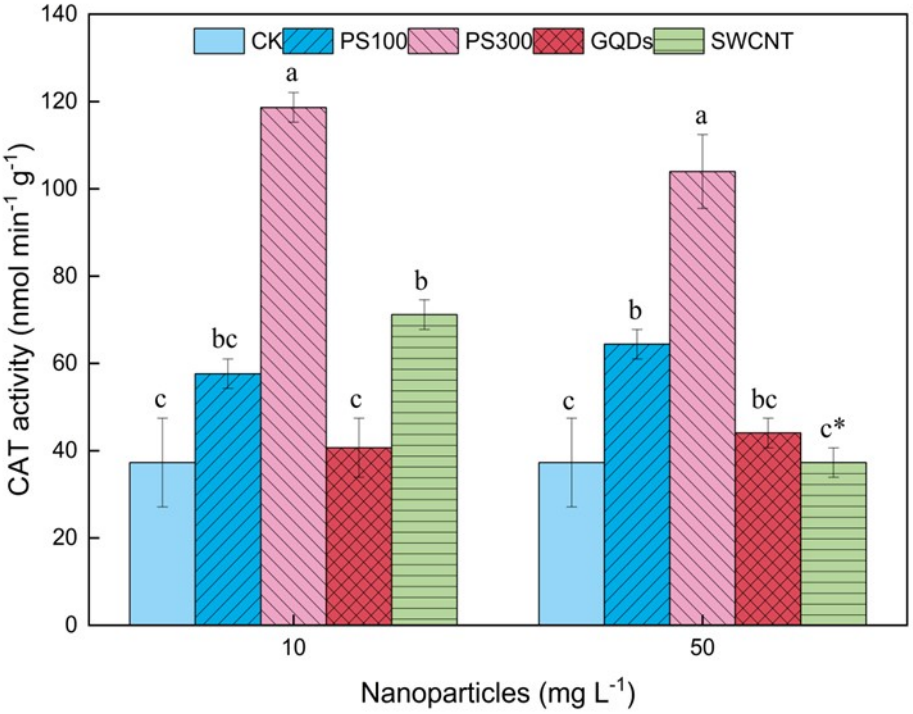

Figure S2. The effect of different nano particles on the CAT content in lettuce leaves

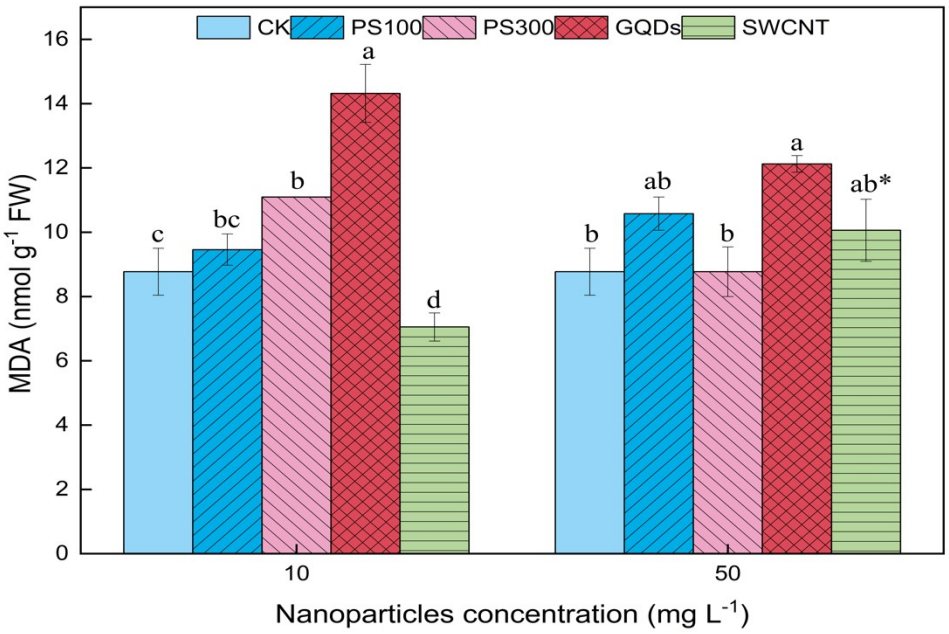

Figure S3. Effect of different nano particles on MDA content in lettuce leaves

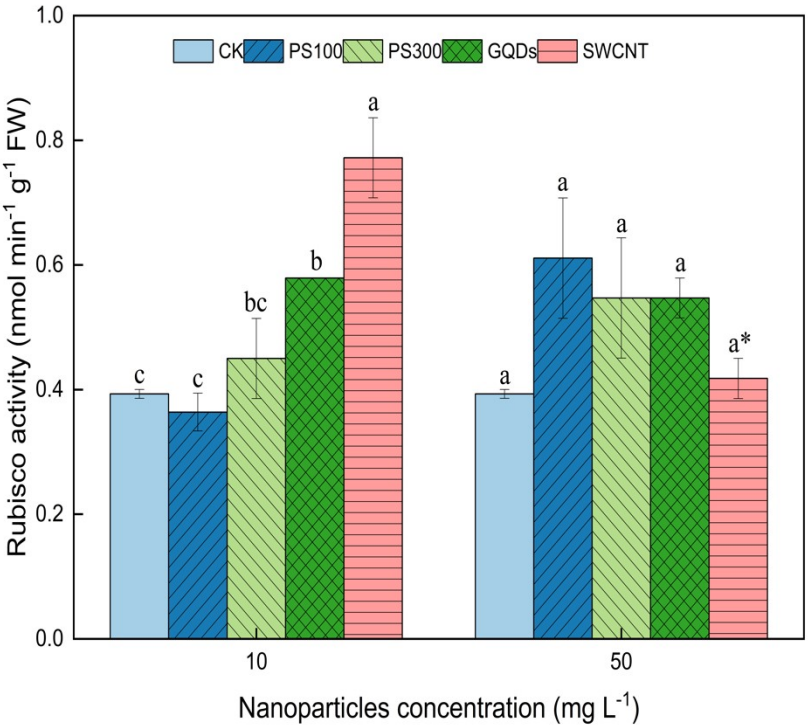

Figure S4 Effect of different nano particles on Rubisco enzyme activity in lettuce leaves

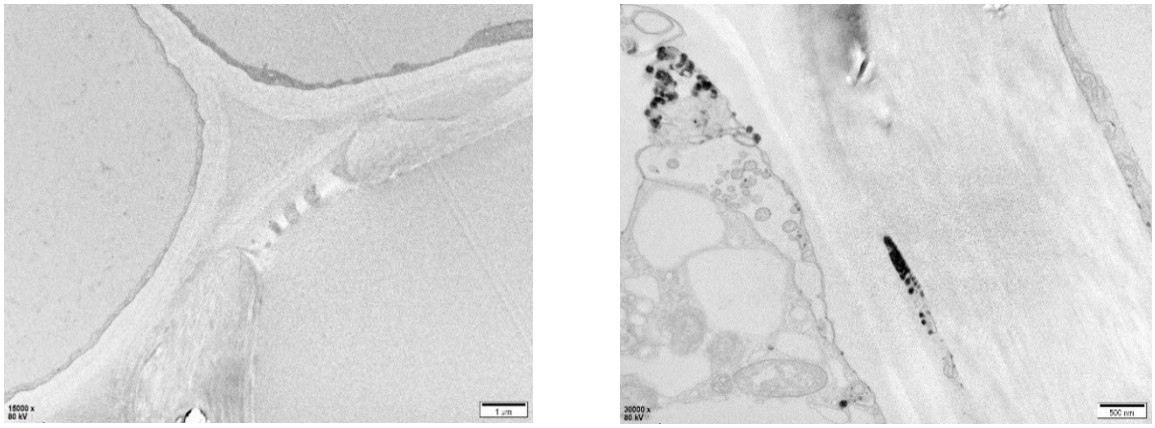

Figure S5. TEM images of cherry radish fleshy roots