# Selenium nanomaterials induce flower enlargement and improve the nutritional quality of cherry tomatoes: pot and field experiment

Bingxu Cheng<sup>abc</sup>, Chuanxi Wang<sup>abc</sup>, Le Yue<sup>abc</sup>, Feiran Chen<sup>abc</sup>, Xuesong Cao<sup>abc</sup>, Xiaofei Liu<sup>abc</sup>, Zhenyu Wang<sup>\*abc</sup>, and Baoshan Xing<sup>d</sup>

<sup>a</sup> Institute of Environmental Processes and Pollution Control, and School of Environment and Civil Engineering, Jiangnan University, Wuxi, Jiangsu, 214122, China

<sup>b</sup> Jiangsu Engineering Laboratory for Biomass Energy and Carbon Reduction Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China

<sup>c</sup> Jiangsu Key Laboratory of Anaerobic Biotechnology, Jiangnan University, Wuxi, Jiangsu, 214122, China

<sup>d</sup>Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, USA

\*Corresponding author: wang0628@jiangnan.edu.cn (Dr. Zhenyu Wang)

#### Text S1. Calculation of application amount of Se ENMs in field experiment.

The amounts of ENMs used in the field experiments is determined based on soil mass, which is calculated using the following formula:

#### $M = \rho \times A \times h$

Where M is the mass of soil,  $\rho$  is the soil density, A is the application area of Se ENMs, and h is the thickness of the soil. Once the mass of soil has been determined, the amount of Se ENMs applied can be determined.

#### Text S2. Mobile phase and instrument parameter settings of UPLC-ESI-QE.

Taproot extracts (5  $\mu$ L, full loop injection) were separated on a Thermo Scientific UPLC Vanquish equipped with an HSS T3 column (100×2.1 mm, particle size 1.8  $\mu$ m, Waters) at 35 °C with a flow rate of 0.35 ml/min and applying the following gradient: 0 min 95% A (0.1% formic acid), 5% B (99.9% Acetonitrile/0.1% formic acid); 1.5min 95% A, 5% B; 14 min 100% B; 15.5 min 100% B; 16 min 95% A, 5% B; 18 min 95% A, 5% B. Eluting compounds were detected from m/z 70 to 1050 using a Thermo Scientific UPLC Vanquish coupled to the Q Exactive Orbitrap (UPLC-ESI-QE) in positive and negative ion mode using the following instrument settings (Table S2). Data analysis was accomplished by using Compound Discoverer software 3.1.

#### Text S3. Observation of ovary wall.

Cherry tomatoes fruit were fixed in stationary liquid (formalin, acetic acid, and 70% alcohol (V: V: V) = 1:1:16) for at least 48 h. Fruit samples of 0.25 cm<sup>2</sup> were immersed in 70% ethanol overnight under vacuum. Then the samples were processed in the following order: gradient dehydrated in ethanol (85%, 95%, 100%, 2h), and hyalocleared (xylene, AR). Subsequently, the wax immersion was performed and the samples were then embedded in paraffin. 15  $\mu$ m pieces were sectioned off with a Leica RM 2235 (Germany) rotary microtome. Images of cell layers in the ovary wall were captured by a fluorescence microscope (Nikon, Eclipse Ni, Japan).

#### Text S4. The dissolution experiment of resultant Se ENMs in soil.

The 20 mL soil suspension (water and soil (V/V) = 1:1) and 2 mL (20 mg·L<sup>-1</sup>) of Se ENMs suspension were mixed. Before sampling at 0, 6, 12, 24, 48, and 72 h, 6 mg of poly-aluminum chloride were added to the precipitated Se ENMs in each tube. After

centrifugating at 8000 rpm for 20 min, the supernatant was filtered by a 0.22  $\mu$ m membrane, diluted 10 times with ultrapure water, and tested by ICP-MS.

#### Text S5. Measurement of carbohydrate.

The carbohydrate content of the cherry tomatoes plant was measured by the phenol method. Totally dried tomato leaves (0.2 g) and fruits (0.2 g) were ground. After boiling water bath with 5 mL ultrapure water for 15 min, the solutions were filtrated and diluted to 100 mL. H<sub>2</sub>SO<sub>4</sub> (5 mL) was slowly added into the mixture (2 mL, 5% phenol solution and 1 mL diluted solution). The absorbance was determined at 490 nm when these solutions cooled to room temperature. Glucose standard curve: a series of concentrations (0, 20, 40, 60, 80  $\mu$ g·mL<sup>-1</sup>) of glucose, and processed in the same way as the samples. y = 0.0035x + 0.0687 (R<sup>2</sup> = 0.9921), where x is the absorbance at 490 nm and y is the carbohydrates content ( $\mu$ g·g<sup>-1</sup>). The final content of carbohydrate is y/0.0002.

#### Text S6. Measurement of chlorophyll a and chlorophyll b.

Chlorophyll a and chlorophyll b of tomato leaves were measured and calculated as follows. Briefly, 40 mg of fresh leaves were ground into small pieces under liquid nitrogen and extracted with 80% acetone. The leaves were then water bath for 15 min at 68 °C and 4000g centrifugation for 15min. Liquid supernatant were measured at 663, 646 nm by a multifunctional microplate reader (Varioskan Lux, Thermo Scientific, Finland). The content of chlorophyll a and chlorophyll b were calculated according to the following equations:  $Ca = 12.21A_{663}-2.81A_{646}$  (chlorophyll a),  $Cb = 20.13A_{646}-5.03A_{663}$  (chlorophyll b).

#### Text S7. Observation of pollen vigor.

The observation of pollen vigor was carried out by using the fluorescein diacetate (FDA) stain method. Briefly, 2.4 mM FDA was dissolved in acetone and mixed with 0.5 M sucrose solution until the solution turned milky. Fresh pollen and FDA solution were mixed. Bright green fluorescing pollen was observed by using a fluorescence microscope (Nikon, Eclipse Ni, Japan).

## Text S8. Economic comparison between Se ENMs and SeO<sub>3</sub><sup>2-</sup> application.

The potential labor cost was not taken into account for the comparison of investment

between Se ENMs and  $\text{SeO}_3^{2-}$  application. The investment per hectare (C) of Se ENMs and  $\text{SeO}_3^{2-}$  fertilizer was calculated by the following equation:

$$C = V*W$$

where V is the price of materials; W is the mass of materials applied to cherry tomatoes; The mass of both Se ENMs and  $\text{SeO}_3^{2-}$  fertilizer applied to cherry tomatoes was based on the best performance concentration (75 µg·kg<sup>-1</sup>) of Se ENMs compared to control. Se ENMs were applied at 100g per hectare and  $\text{SeO}_3^{2-}$  applied at 200g (Se 100g) per hectare. The C value of Se ENMs and  $\text{SeO}_3^{2-}$  fertilizer were \$66.61 and \$229.7 per hectare, respectively.

The economic benefit (E) per hectare of Se ENMs and  $SeO_3^{2-}$  fertilizer were calculated by the following equation:

## E = Y\*M\*R

where Y is the yield per hectare of cherry tomatoes in China; M is the average market price of cherry tomatoes in China; R is the production efficiency increase of cherry tomatoes. The yield of cherry tomatoes was 5.42 tons per hectare in this study, the average price of cherry tomatoes is 393.7/ton. The yield increase rate of cherry tomatoes was 176.9% and 53.8% for Se ENMs and SeO<sub>3</sub><sup>2–</sup> fertilizer, respectively. The E value for Se ENMs and SeO<sub>3</sub><sup>2–</sup> fertilizer were 3774.8 and 1148.0 per hectare, respectively.

| Soil index                                     |                  |
|--|------------------|
| pH   | 7.6              |
| Redox potential (mV)                           | 310.5            |
| Electrical conductivity (mS·cm <sup>-1</sup> ) | 0.342            |
| Total nitrogen (g·kg <sup>-1</sup> )           | $22.1\pm1.02$    |
| Total phosphorus (g·kg <sup>-1</sup> )         | $0.4\pm0.05$     |
| Total potassium (g·kg <sup>-1</sup> )          | $11.98\pm0.38$   |
| Total Carbon (g·kg <sup>-1</sup> )             | $59.03 \pm 1.62$ |
| Se (mg·kg <sup>-1</sup> )                      | $0.14\pm0.03$    |
| Rapidly available phosphorus                   | $130.76\pm3.23$  |
| $(mg \cdot kg^{-1})$                           |                  |
| Rapidly available potassium                    | $29.49 \pm 1.35$ |
| $(mg \cdot kg^{-1})$                           |                  |
| Organic matter (g·kg <sup>-1</sup> )           | $21.55 \pm 1.21$ |

| Table S1. Soil index |
|----------------------|
|----------------------|

| Parameter       | Conditions                     |  |
|-----------------|--------------------------------|--|
|                 | User role: Standard            |  |
| Global Settings | Use lock mass: off             |  |
|                 | Chrom. Peak wi: 5s             |  |
| Time            | Method duration: 18 min        |  |
| General         | Runtime: 0 to 18 min           |  |
|                 | Polarity: Positive/negative    |  |
|                 | Exclusion: on                  |  |
|                 | Default charge: 1              |  |
|                 | Resolution: 70,000             |  |
| Full mass       | AGC target: 1e6                |  |
| r un mass       | Maximum IT: 100ms              |  |
|                 | Scan range: 70 to 1050 m/z     |  |
| dd-MS2/dd-SIM   | Resolution: 17500              |  |
|                 | Maximum IT: 50ms               |  |
|                 | Loop count: 7                  |  |
|                 | TopN: 8                        |  |
|                 | Isolation window: 1.5 m/z      |  |
|                 | (N) CE/Stepped nce: 20, 40, 60 |  |
|                 | AGC target: 5e4                |  |
| dd setting      | Minimum AGC: 8.00e3            |  |
|                 | Inetnsity thresh: 1.6e5        |  |
|                 | Dynamic exclus: 10.0 s         |  |

 Table S2. Q Exactive Orbitrap (UPLC-ESI-QE) instrument settings.

# Table S3. Primer sequences used for the qRT-PCR of selected genes.

| Gene name | Forward primer | Reverse primer |
|-----------|----------------|----------------|
|           |                |                |

| <b>Slcod</b> A | ATCGATACGCCGAAGCTGTT   | TGGTCCTGCAGGTGCTCGCC  |
|----------------|------------------------|-----------------------|
| SISUT1         | TTCCATAGCTGCTGGTGTTC   | TACCAGAAATGGGTCCACAA  |
| SICDKA1        | AACCCCTGAATAGAACCAAATG | GTATGTGCCGTGATTGTCTG  |
| SIWEE1         | TCTTCTTCCGGGTCACTCCT   | CAGAAGGACGACGTGTTGGA  |
| SIIAA9         | GCGCAGCCTTTGTGAAAGTT   | TGCCAAGTGCATCAGAGAGT  |
| SICTD1         | GGAGACTTGCCCTGTGATTTC  | CACCTGCTTTCACACCGGA   |
| <b>SICWINV</b> | GGCAATTAACGACGAGGCAC   | TGCCCATACTCCATGCATCC  |
| SUT1;1         | GATATTGCGATAGGGCCAGT   | GCAAGACGCTGATACTCGTG  |
| Actin          | GGAACTTGAGAAGGAGCCTAAG | CAACACCAACAGCAACAGTCT |



Fig. S1. Size distribution of Se ENMs.



Fig. S2. The content of S in cherry tomatoes root.



**Fig. S3.** (a) Flowering period images of cherry tomatoes under different treatment. (b) Intracellular CO<sub>2</sub> concentrations (Ci) of cherry tomatoes leaf; (c) Transpiration rate (E) of cherry tomatoes leaf; (d) Stomatal conductance (Gs) of cherry tomatoes leaf.



**Fig. S4.** (a) Chlorophyll a and b content of cherry tomatoes leaves. (b) Heat map of the variation for mineral elements in cherry tomatoes leaves.



Fig. S5. Pollen vigor of cherry tomatoes upon different treatments.



Fig. S6. Images of cherry tomatoes flower upon different treatments.



**Fig. S7.** Effects of Se ENMs (75  $\mu$ g·kg<sup>-1</sup>) and SeO<sub>3</sub><sup>2-</sup> (75  $\mu$ g·kg<sup>-1</sup>) on cherry tomatoes fruit. (a) Images of cell layers on the pericarp; (b) Images of cherry tomatoes upon Se ENMs and SeO<sub>3</sub><sup>2-</sup> exposure; (c) Fruit diameter of cherry tomatoes treated by Se ENMs and SeO<sub>3</sub><sup>2-</sup>.



**Fig. S8.** Carbohydrate content in different plant tissues of cherry tomatoes upon different treatments. (a) Leaf carbohydrates; (b) Fruit carbohydrates.



Fig. S9. Heat map of cherry tomatoes fruit metabolites.



Fig. S10. Pathway enrichment analysis of cherry tomatoes metabolites.



**Fig. S11.** C and N content of different cherry tomatoes tissues. (a)Total carbon; (b) Total nitrogen



Fig. S12. Ascorbic acid, glutathione and tomatidine content of cherry tomatoes fruit.



Fig. S13. Change of flavonoids in cherry tomatoes fruit.



**Fig. S14.** IAA (indole-3-acetic acid), jasmonic acid and salicylic acid content of cherry tomatoes fruit.



Fig. S15. (a)Field experiment scene and (b) cherry tomatoes fruit image.



Fig. S16. (a) PCA analysis; (b) KEGG pathway enrichment results.



Fig. S17. Changes in metabolism pathways of cherry tomatoes fruit under Se ENMs treatment.



**Fig. S18.** The relative abundance of macronutrients and micronutrients in cherry tomatoes fruit (field experiment).



Fig. S19. Carbohydrate content in cherry tomato fruits upon different treatments.