Effect of Selenium on the growth and Cadmium accumulation of tomato seedlings in low temperature and low light

Yufeng Zhong¹, Tianqi Xia¹, Sihan Peng¹, Yaoqing Ma¹ and Zhongqun He¹∗

¹College of Horticulture, Sichuan agriculture university, Chengdu, Sichuan, China
*Corresponding author’s e-mail: hzqun328@sicau.edu.cn

Abstract: In this study, we used two tomato cultivars ‘Medium Vegetable 4’ and ‘Middle Mix 9’ as materials, applying Na₂SeO₃ to the soil to study the effect of external Selenium (Se) on the growth and Cadmium (Cd) accumulation under low temperature (18/12 ℃, day/night) and low light (100 μmol·m⁻²·s⁻¹). Results show that Se can significantly reduce the Cd accumulation in root, leaf and stem and promote the development of tomato under Cd stress. Under low temperature, Se can enhance cold tolerance through increasing the chlorophyll content, POD activity, soluble sugar and soluble protein content and decreasing the MAD content of two cultivars. Our study provides direction for tomato cultivation under low temperature and low light.

1. Introduction

Temperature and light as the most important environmental factors control plant growth and development. Climate changes and simple facilities limit the production of vegetables [1]. Numerous studies have shown that low temperature and low light stress can significantly inhibit physiological metabolism of vegetable crops, such as decreased photosynthesis, antioxidant enzyme activity, chlorophyll degradation. Meanwhile, due to the heavy application of pesticides and fertilizers in facility production, heavy metal pollution became a problem especially Cadmium (Cd) pollution. Cd is a harmful heavy metal element that has toxic effects on crop production and human health [2]. Due to its strong toxicity, easy movement and large pollution area, Cd is considered as the primary heavy metal pollutant in soil [3]. Cd as a non-essential element in plants, not only affects the yield and quality of vegetables, but also can enter the human body through the food chain [4].

Selenium (Se) is an essential trace element for the synthesis of proteins and enzymes in humans and animals, and has been widely studied for its antioxidant and anticancer effects [5]. Appropriate Se is conducive to promote plant growth, development and metabolism, plant mineral elements absorption and transport, and crop yield and quality. Recent studies have found that Se can enhance the resilience of plants by scavenging excess free radicals and relieve stress damage caused by low temperature, drought, heavy metal toxicity and diseases [6-7]. Exogenous Se can make the concentration of heavy metals in crops meet the quality standard, and increase the Se concentration in the edible part of crops, so as to meet the human demand for Se.

2. Materials and Methods

2.1 Materials

The tomato cultivars were "Medium Vegetable 4" (low temperature and weak light resistance, MV4) and "Middle Mix 9" (low temperature and weak light sensitivity, MM9), both purchased from Medium
Vegetable Seed Technology (Beijing) Co., LTD. The test reagents Na₂SeO₃ and CdCl₂·2.5H₂O were all analytically pure.

2.2 Treatment
This experiment was conducted in the laboratory and greenhouses of Horticulture College, Chengdu Campus, Sichuan Agricultural University from November 2017 to July 2018. After air drying, the tested soil was passed through a 300-mesh sieve and put into a bucket of 30 cm×40 cm (diameter × height) with 20 kg of soil in each barrel. According to the 10 mg·kg⁻¹ Cd concentration designed in the experiment, CdCl₂·2.5H₂O were added to the tested soil. After 30 days, all the contaminated soil was mixed together and thoroughly mixed. Then, the contaminated soil was put into a 21 cm×25 cm (diameter × height) flowerpot, and each pot was put into 3 kg.

After seed soaking disinfection on wet filter paper, two cultivars of tomato seeds were sprouted in 25 °C constant temperature incubator for 6 h, then sowing in hole plate seedling with soil, nutrition composition of peat soil: matrix: perlite = 2:1:1 (volume ratio) of 32 cavity plate seedling. The artificial climate chamber is set as follows: the photoperiod is 12h day and night, the day-night temperature is 25°C and 18°C, and the light intensity is 200 mol·m⁻²·s⁻¹. When the four true leaves of tomato were spread (about 30d), half of the seedlings were watered with sodium selenite (Na₂SeO₃) solution containing 10 mol·L⁻¹ at 9:00 am, 30 mL once a day, three times in total. After 3d, transplant all seedlings into a prepared pot with Cd contaminated soil, one in each pot. 1/3 seedlings under normal temperature conditions of light climate box (25/18℃, day/night, light for 200 μmol·m⁻²·s⁻¹), a third seedling under low temperature conditions climate box (18/12℃, day/night, light for 200 μmol·m⁻²·s⁻¹), in addition a third seedlings under weak light conditions climate box (18 ℃, day/night, light for 100 μmol m⁻²·s⁻¹), Se treated and untreated seedlings were included in each condition. In this experiment, there were two cultivars, and each cultivar had six treatments. In other words, Se untreated in normal light and temperature (T1); Normal light temperature Se treatment (T2); Se untreated at low temperature (T3); Low temperature and Se treatment (T4); Low light and Se untreated (T5); Low light and Se treatment (T6). Six pots for each treatment were randomly placed, and the positions were changed every day. After 30 days of treatment, samples were taken for indicator determination.

2.3 Detection method

2.3.1 Growth index and biomass
Remove the entire plant from the pot, rinse the soil with running water, and dry it after repeated rinsing with deionized water. The plant height and root length were measured with millimeter scale and the stem diameter was measured with vernier caliper. Then the plant was divided into root, stem and leaf. The plant was dried in an oven at 105°C for 15 min and dried to constant weight at 70°C. The biomass of each part was measured by an electronic balance.

2.3.2 Physiological index (leaf)
The chlorophyll content was soaked with 80% acetone. The activity of superoxide dismutase (SOD) was determined by nitrogen blue tetrazole (NBT) method. Peroxidase (POD) activity was determined by guaiacol method. The catalase (CAT) activity was determined by ultraviolet absorption. The content of malondialdehyde (MDA) was determined by thiobarbituric acid method. The soluble protein content was determined by Coomassie bright blue method. Soluble sugar content was determined by anthranone colorimetric method.

2.3.3 Detection of Cadmium content (whole plant)
Digestion of HNO₃-HCLO₄ was determined by atomic absorption spectrophotometer.
2.3.4 Data analysis

The experimental data were analyzed by SPSS 20.0 for ANOVA, and analyzed by Duncan's multiple comparison method for significance at $p < 0.05$ level.

3. Results

3.1 Comparison of growth index and biomass under different conditions

As shown in Table 1, the growth conditions of two cultivars under Se treatment were significantly better than Cd treatment. Compared to normal light and temperature, the height, root and stem weight of ‘MV4’ plants increased by 22.15%, 10.71% and 31.03% under Se condition, respectively. At low temperature, the plant height, root weight and stem diameter of ‘MV4’ increased by 11.47%, 50.0% and 30.36% under Se condition, respectively. Under low light conditions, the height of ‘MV4’ plant increased by 16.73% under Se condition. Under normal light and temperature, the plant height and root weight of ‘MM9’ increased by 8.3% and 13.4% under Se condition, respectively. Under low light treatment, the root weight, stem weight and leaf weight of ‘MM9’ plant increased by 41.2%, 25.9% and 35.6% under Se condition respectively. There was no significant difference between leaf dry weight and stem diameter in the two cultivars.

Table 1. Effects of Se on growth indicators of ‘MV4’ and ‘MM9’

| Cultivars | Treatment | Plant height (cm) | Root length (cm) | Thick stems (cm) | Root weight (g) | Stem heavy (g) | Leaf weight (g) |
|-----------|-----------|------------------|-----------------|-----------------|----------------|----------------|----------------|
| MV4       | T1        | 23.93±0.39 b     | 21.77±0.38 a    | 0.57±0.04 ab    | 0.28±0.01 b    | 0.58±0.01 b   | 0.66±0.02 ab   |
|           | T2        | 29.23±0.35 a     | 20.50±0.30 ab   | 0.67±0.09 a     | 0.31±0.01 a    | 0.76±0.03 a   | 0.92±0.01 a    |
|           | T3        | 8.37±0.21 f      | 19.73±0.25 c    | 0.39±0.04 d     | 0.20±0.02 c    | 0.15±0.00 d   | 0.20±0.01 c    |
|           | T4        | 9.33±0.25 e      | 19.77±0.25 c    | 0.56±0.02 ab    | 0.30±0.01 a    | 0.20±0.01 d   | 0.47±0.03 bc   |
|           | T5        | 17.93±0.35 d     | 18.33±0.31 d    | 0.46±0.03 ed    | 0.20±0.00 c    | 0.31±0.01 cd  | 0.64±0.02 ab   |
|           | T6        | 20.93±0.25 c     | 19.83±0.50 bc   | 0.47±0.03 c     | 0.20±0.00 c    | 0.32±0.01 c   | 0.71±0.02 ab   |
| MM9       | T1        | 33.13±0.31 b     | 29.57±0.31 a    | 0.53±0.06 a     | 0.29±0.01 b    | 0.78±0.03 a   | 0.83±0.01 a    |
|           | T2        | 35.87±0.31 a     | 27.90±0.27 b    | 0.47±0.048 ab   | 0.33±0.02 a    | 0.75±0.03 a   | 0.93±0.02 a    |
|           | T3        | 11.13±0.40 f     | 13.97±0.21 d    | 0.41±0.04 c     | 0.20±0.01 d    | 0.20±0.01 d   | 0.34±0.03 d    |
|           | T4        | 12.90±0.44 e     | 18.00±0.20 c    | 0.47±0.03 ab    | 0.28±0.008 b   | 0.28±0.01 c   | 0.43±0.02 c    |
|           | T5        | 26.70±0.20 c     | 15.40±0.26 c    | 0.45±0.05 ab    | 0.17±0.01 e    | 0.27±0.01 c   | 0.45±0.02 c    |
|           | T6        | 24.90±0.47 d     | 16.30±1.59 c    | 0.39±0.03 c     | 0.24±0.01 c    | 0.34±0.01 b   | 0.61±0.02 b    |

Note: Different lowercase letters in the postmarks of the same column indicate significant differences ($P < 0.05$), the same as in the following table.

3.2 Comparison of chlorophyll content under different conditions

Compared to normal light and temperature of ‘MV4’ plants, the content of chlorophyll a, b and total chlorophyll increased by 15.2%, 48.6% and 25.0% under Se condition, respectively (Table 2). At low temperature of ‘MV4’ plants, the content of chlorophyll a, b and total chlorophyll increased by 34.5%, 17.0% and 29.0% under Se condition, respectively. There was no significant difference between the ‘MM9’ groups. It is worth mentioning that the chlorophyll content of both cultivars under low light was significantly higher than that normal light.

Table 2. Effects of Se on chlorophyll content of ‘MV4’ and ‘MM9’

| Cultivars | Treatment | Chlorophyll a (mg g⁻¹) | Chlorophyll b (mg g⁻¹) | The total chlorophyll (mg g⁻¹) |
|-----------|-----------|------------------------|------------------------|------------------------------|
| MV4       | T1        | 0.79±0.06 e            | 0.37±0.06 d            | 1.16±0.12 e                  |
|           | T2        | 0.91±0.03 d            | 0.55±0.02 ab           | 1.45±0.05 c                  |
|           | T3        | 0.84±0.03 e            | 0.47±0.05 c            | 1.31±0.08 d                  |
|           | T4        | 1.13±0.10 c            | 0.55±0.09 ab           | 1.69±0.19 b                  |
|           | T5        | 1.25±0.09 b            | 0.60±0.04 a            | 1.86±0.13 ab                  |
3.3 Comparison of Cd content under different conditions
Compared to normal light and temperature, Cd content of ‘MV4’ plant in roots, stems, and leaves decreased by 6.5%, 17.1%, and 7.6% under Se condition, respectively. At low temperature, the Cd content in root, stem and leaf decreased by 16.2%, 18.8% and 27.5%, respectively. Under low light conditions, Cd content in roots, stems, and leaves decreased by 11.2%, 15.8% and 15.4%, respectively.

Table 3. Effects of Se on Cd content of ‘MV4’ and ‘MM9’

| Cultivar | Treatment | Root (μg g⁻¹) | Stem (μg g⁻¹) | Leaf (μg g⁻¹) |
|----------|-----------|---------------|---------------|---------------|
| MV4      | T1        | 46.10±0.05 b  | 17.48±0.04 d  | 26.56±0.06 b  |
|          | T2        | 43.12±0.03 c  | 14.49±0.01 e  | 24.55±0.07 d  |
|          | T3        | 37.04±0.09 e  | 32.05±0.07 b  | 30.98±0.04 a  |
|          | T4        | 31.04±0.09 f  | 26.14±0.09 c  | 22.47±0.04 e  |
|          | T5        | 47.17±0.05 a  | 38.97±0.04 a  | 26.10±0.14 c  |
|          | T6        | 41.90±0.04 d  | 32.95±0.07 b  | 22.17±0.04 e  |
| MM9      | T1        | 23.07±0.05 f  | 17.28±0.03 d  | 28.55±0.07 a  |
|          | T2        | 22.11±0.13 e  | 13.00±0.02 e  | 21.00±0.01 c  |
|          | T3        | 35.97±0.05 b  | 29.98±0.03 b  | 20.00±0.02 d  |
|          | T4        | 31.91±0.12 c  | 21.93±0.10 c  | 17.55±0.08 e  |
|          | T5        | 39.89±0.15 a  | 35.86±0.23 a  | 28.19±0.42 a  |
|          | T6        | 28.50±0.04 d  | 29.75±0.07 b  | 24.46±0.05 b  |

Under normal light and temperature, the Cd content of ‘MM9’ in root, stem and leaf decreased by 4.3%, 25.2% and 26.4% under Se condition respectively. At low temperature, Cd content in roots, stems and leaves decreased by 11.1%, 26.7% and 12.3%, respectively. Under low light conditions, Cd content in roots, stems and leaves decreased by 28.6%, 17.0% and 13.2%, respectively. The cadmium content in tomato seedlings under low light was significantly higher than that under low temperature and normal light and temperature.

3.4 Physiological index of resistance
As shown in Table 4, there were no significant difference in T1 vs T2, T3 vs T4 and T4 vs T5 of two cultivars in MDA content. In low temperature, Se can enhance POD activities of two cultivars. But Se would significant reduce SOD activities of ‘MV4’ plants, but no significant difference were observed in T3 vs T4 and T4 vs T5 of ‘MM9’ cultivar. CAT activities were only promoted by Se under normal light and temperature of ‘MV4’ plants.

Table 4. Effects of Se on MDA and antioxidant enzyme activity of ‘MV4’ and ‘MM9’

| Cultivar | Treatment | MDA activities (n molg⁻¹) | POD activities (U·g⁻¹FW) | SOD activities (U·g⁻¹FW) | CAT activities (U·g⁻¹FW) |
|----------|-----------|--------------------------|--------------------------|--------------------------|--------------------------|
| MV4      | T1        | 1.40±0.03 a              | 0.63±0.05 a              | 2.03±0.08 a              |                          |
|          | T2        | 0.88±0.05 c              | 0.47±0.03 b              | 1.25±0.08 c              |                          |
|          | T3        | 0.73±0.08 c              | 0.34±0.02 c              | 1.07±0.10 c              |                          |
|          | T4        | 0.76±0.02 cd             | 0.20±0.07 d              | 0.97±0.09 d              |                          |
|          | T5        | 0.61±0.05 d              | 0.31±0.05 c              | 0.94±0.10 cd             |                          |
|          | T6        | 1.29±0.08 a              | 0.61±0.03 a              | 1.90±0.11 a              |                          |

| MM9      | T1        | 0.30±0.03 f              | 0.25±0.03 d              | 1.10±0.08 c              |                          |
|          | T2        | 0.20±0.03 d              | 0.15±0.03 e              | 0.90±0.05 d              |                          |
|          | T3        | 0.10±0.03 e              | 0.05±0.03 f              | 0.70±0.05 e              |                          |
|          | T4        | 0.05±0.03 f              | 0.03±0.03 e              | 0.50±0.05 e              |                          |
|          | T5        | 0.03±0.03 e              | 0.02±0.03 d              | 0.30±0.05 e              |                          |
|          | T6        | 0.02±0.03 d              | 0.01±0.03 e              | 0.20±0.05 e              |                          |
3.5 Different treatments of soluble protein and soluble sugar
As shown in Table 5, there were significant differences in T1 vs T2, T3 vs T4 and T4 vs T5 of two cultivars in Soluble protein. Se can significantly increase the Soluble sugar under low temperature of two cultivars.

Table 5. Comparison of soluble protein and soluble sugar content of ‘MV4’ and ‘MM9’

|       | Soluble protein (mg·g⁻¹) | Soluble sugar (%) |       | Soluble protein (mg·g⁻¹) | Soluble sugar (%) |
|-------|--------------------------|-------------------|-------|--------------------------|-------------------|
| **MV4** |                          |                   | **MM9** |                          |                   |
| T1    | 13.83±0.64 b             | 0.17±0.04 c       | T1    | 8.75±0.47 d             | 6.88±0.43 e       |
| T2    | 14.00±1.34 b             | 0.19±0.07 c       | T2    | 10.08±0.19 c            | 7.52±0.98 de      |
| T3    | 68.28±0.60 a             | 0.66±0.09 b       | T3    | 14.22±0.52 b            | 15.75±0.58 b      |
| T4    | 69.42±2.79 a             | 0.84±0.03 a       | T4    | 20.74±0.68 a            | 20.72±2.21 a      |
| T5    | 17.29±0.09 b             | 0.10±0.03 d       | T5    | 7.57±0.30 f             | 9.13±0.77 d       |
| T6    | 14.47±0.12 b             | 0.08±0.03 d       | T6    | 8.09±0.25 e             | 8.04±0.57 d       |

4. Conclusion and Discussion
Tomatoes grown in Cadmium-contaminated soil have a major safety hazard for human consumption. Therefore, reducing the absorption and accumulation of Cd in tomatoes is important. Studies have found that adding a certain concentration of Se in soil can reduce the accumulation of Cd in tomatoes [8]. In this study, 10 μmol·L⁻¹ concentration of Se was used to irrigate the root system, which can reduce the absorption of Cd, further proving that Se has an antagonistic effect on Cd.

Acknowledgments
This work was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (STEP), Grant No. 2019QZKK0303

References
[1] Shu, S., Tang, Y.Y., Luo, J.Y., et al. (2016) Effects of exogenous 24-supernatant lactones on carbon assimilation and antioxidant metabolism in Tomato leaves under mild low temperature and low light stress. J. J. Plant Physiol., 8: 1295-1304.
[2] Grant, C.A., Buckley, W.T., Bailey, L.D., et al. (1998) Cadmium accumulation in crops. Can J Plant Sci. J. Can. J. Plant Sci., 78(1): 1-17.
[3] Satarug, S., Baker, J.R., Urbenjapol, S., et al. (2003) A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. J. Toxicol. Lett., 137(1-2): 65-83.
[4] Zhang B.C., Wang, L., Fan L.X., et al. (2015) Accumulative properties of lead and cadmium in vegetables and their effects on vegetable growth. J. J. Ecol., 34(10): 2873-2878.
[5] Rotruck, J.T., Pope, A.L., Ganther, H.E., et al. (1973) Selenium: Biochemical Role as a Component of Glutathione Peroxidase. J. Science., 179(4073): 588.
[6] Kumar, M., Bijo, A.J., Baghel, R.S., et al. (2012) Selenium and spermine alleviate cadmium induced toxicity in the red seaweed Gracilaria dura by regulating antioxidants and DNA methylation. J. Plant Physiol. Biochem., 51(2):129.
[7] Ley-Quíñónez, C.P., Zavala-Norzagaray, A.A., Rendón-Maldonado, J.G., et al. (2013) Selected Heavy Metals and Selenium in the Blood of Black Sea Turtle (Chelonia mydas agasiizzi) from Sonora, Mexico. J. Bull. Environ. Contam. Toxicol., 91(6): 645-651.
[8] Alyemeni, M.N., Ahanger, M.A., Wijaya, L., et al. (2018) Correction to: Selenium mitigates cadmium-induced oxidative stress in tomato (Solanum lycopersicum L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. J. Protoplasma., 255(3):985-986.