Effects of exogenous glutathione on antioxidant system of cucumber seedlings under cadmium stress

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Abstract: With the development of social economy, environmental protection has become a hot topic. Excessive emissions of agricultural chemicals increase the accumulation of the heavy metal cadmium (Cd) in soil. When plants are stressed by high concentrations of heavy metals, their antioxidant capacity and detoxification capacity will decrease, which will lead to damage the cell mechanism of plants. Glutathione (GSH), as an important water-soluble antioxidant in the organism, can enhance plant resistance in stress. This study used cucumber seedlings as test material, using hydroponics, to research the effects of exogenous GSH on antioxidant system under Cd stress. The study showed that exogenous GSH could enhance the antioxidant capacity of cucumber leaves under Cd stress and increase the activity of SOD and POD, and reduce the content of MDA. However, the contents of reduced GSH and GSSH were reduced when exogenous glutathione was applied under Cd stress.

1. Introduction

In recent years, the heavy metal pollution caused by waste water, waste gas, irrigation of farmland sewage and other factors have become a serious environmental pollution problem. When the content of Cd reaches a certain concentration, it can damage plant roots, increase membrane permeability, and destroy the photosynthetic system and antioxidant system. Li et al. [1] found that under the Cd stress, the contents of soluble sugar, soluble protein, VC and free amino acid in garlic decreased correspondingly. Zhang et al. [2] pointed out that when Cd reached a certain concentration in melon, the synthesis of plant antioxidant enzymes and its own activity would be inhibited. Song et al. [3] found that when Chinese cabbage was under the Cd stress, chloroplast expanded obviously, matrix lamellar structure was destroyed, direction was disturbed, cell wall was irregularly thickened, and plasmal wall was separated.

Cucumber is an annual vine and climbing herb, which is cultivated in large area and has high yield and wide planting range in vegetable cultivation in China. Although Cd is not an essential element of plants, it can be easily absorbed by roots. And it can be transported by the xylem, so it accumulates in the aboveground part of the plant, which is manifested in stunted plants, susceptible to infection, growth retardation, leaf green loss and other phenomena [4].

Glutathione (GSH) is a kind of sulfhydryl compounds containing cysteine residues, soluble in water [5], has very wide range of effects on the plant cells. It also plays an important role in a variety of biological reactions, such as enzyme activity, metabolism, material transport, plant cell programmed death, etc. GSH is important in detoxified substances of plant cells, and has a strong affinity to metal ion. GSH can invade plant cells to chelate toxic heavy metal ions and compounds, which can reduce or eliminate the toxicity of heavy metals. In the detoxification process, GSH participates in the catalytic
reaction of substrates, clears the content of reactive oxygen species, reduces the toxicity caused by reactive oxygen stress to plant bodies [6].

In this study, it studied the effect of exogenous GSH on antioxidant system of cucumber seedlings under heavy metal stress, which could provide a theoretical reference and basis for the application of exogenous GSH in vegetable production and promote the sustainable development of environmental ecology.

2. Materials and methods

2.1. Plant materials and treatments
The study was carried out in the facility greenhouse of Sichuan Agricultural University and the laboratory of facility department. The study selected full and uniformity of cucumber seeds, with gauze wrap, in 55 °C warm water for 15 min, and turned to soak in the normal temperature water for 6 h. Germination was accelerated for 24 hours in a 28 °C incubator, and the seedlings were raised by hydroponic method after more than 2/3 of the seeds were exposed. The seeds were placed in a sponge tank with 2.5 L 1/4 Hoagland nutrient solution in a hydroponic container and cultured under a plastic greenhouse. The concentration of Cd was set at 100 μmol/L, configured with Cd chloride (CdCl₂ · 2.5 H₂O). GSH concentration was set at 5 mmol/L. When the seedlings grow into one leaf and two leaves, selecting with similar shape, the same growth rate and no bacterial infection to transfer into a plastic bucket containing 10 L 1/2 Hoagland nutrient solution. The pH of the nutrient solution was adjusted to 6.0 with NaOH or HCl, and the nutrient solution was aerated in the gap. Then change the nutrient solution every 2 d. After delayed seedling, different experimental treatments were carried out. And the study was divided into 4 treatments, as follows: 1) CK (blank control); 2) Cd (cadmium stress control); 3) GSH (glutathione treatment); 4) Cd + GSH (cadmium and glutathione treatment). For each 3 pots, 9 plants per pot, a total of 12 pots, repeat 3 times. Methods of GSH treatment: cucumber seedlings were pretreated before stress, and GSH was sprayed at 21:00 PM. The amount of GSH was sprayed until the leaf began to drip. After 2 d, Cd stress was applied, and GSH was sprayed again. After 5 d under Cd stress, GSH was sprayed the last time. The method was the same as above. Under the Cd stress, the second fully expanded leaf at the growth point was taken at the 0 d, 5 d and 10 d, respectively, to determine relevant indexes.

2.2. Determination of enzyme
Content of MDA were determined by thiobarbituric acid method [7]. Vitality of SOD, POD and CAT were determined by reference to Jiao et al. [8].

2.3. Determination of glutathione content
The reduced and oxidized GSH content was determined by reference to Lihua Yin[9]. Weigh 0.2 g samples by frozen liquid nitrogen to place them into a precooled mortar, then add 2 ml of 5% (w/v) sulfosalicylic acid extract and grind until homogenized, volume to 4 mL. Then centrifugation by 12000 g for 10 min at 4 °C to take the supernatant to determine reduced and oxidized GSH content. Use Excel to organize data; SPSS data processing software was used for significance analysis (Duncan's new complex range method).

3. Results

3.1. Effects of exogenous GSH on MDA in cucumber seedlings under Cd stress
Figure 1. Effects of exogenous GSH on MDA in cucumber seedlings under Cd stress.

In figure 1, on the 5th day of Cd stress, MDA content of Cd stress is 1.6 times that of CK. On the 10th day of Cd stress, the MDA content of Cd stress reached the maximum, was 36.611 nmol/g and 1.5 times as much as CK content, which indicated that the cell membrane of the plant was damaged seriously. However, applying exogenous GSH, MDA content were all reduced. Applying exogenous GSH, MDA content without Cd and MDA content with Cd was 22.934 nmol/g and 24.034 nmol/g, respectively, which showed no significant difference compared with CK.

3.2. Effect of exogenous GSH on antioxidant activity of cucumber seedlings under Cd stress

From the figure 2, with the increase of treatment time, SOD content showed a decreasing trend but both the content of POD and CAT showed an increasing trend in Cd stress treatment. Compared with the control, the SOD content was higher with applying exogenous GSH. On the 10th day of Cd stress, POD content of Cd+GSH decreased by 26.2%. On the 5th day of Cd stress, there was no significant difference in CAT content in cucumber leaves. However, on the 10th day of Cd stress, CAT content of Cd+GSH showed the lowest value, 9.507 U·g⁻¹·min⁻¹, which decreased by 34.2%.

3.3. Effects of exogenous GSH on GSH in cucumber seedlings under Cd stress

In figure 3, GSH content and GSSG content increased by 62.5% and 50% in leaves treated with exogenous GSH under no stress; under Cd stress, GSH content and GSSG content decreased by 9.1%
and 8.3% respectively. Compared with Cd stress, exogenous GSH was treated alone that could reduce GSSG level and increase GSH level. Compared with CK, GSH + GSSG content of Cd stress increased about 1.6 times. Under Cd stress, the GSH+GSSG content of exogenous GSH decreased by 8.7%. Compared with CK, GSH/GSSG content of Cd stress decreased by 21.2%. Under no stress, the GSH/GSSG increased by 6.7% when exogenous was applied.

4. Discussion and conclusions
Under normal physiological conditions, plants can maintain low ROS levels in cells to maintain normal cell function and signal transduction. After a long period of evolution, a series of complete ROS scavenging mechanisms have evolved in plants, so that the production and scavenging of ROS are always in a dynamic balance [10]. The results of this study showed that MDA content in cucumber leaves increased gradually. This showed that with the extension of treatment time, Cd could further cause serious damage to plant cell membrane, accelerate the process of membrane peroxidation of cells and trigger the accumulation of toxic substances such as MDA. According to previous studies, GSH can directly react with O²⁻ and OH⁻ to join non-enzymatic reaction to remove active oxygen [11]. And the activity of SOD showed no significant change at the 5th and 10th day, which was higher than SOD of Cd stress, which was consistent with the research results of Gao et al. [12]. These showed that SOD, as an inducible enzyme, played an important role in the process of plant stress resistance [13].

GSH is not only an antioxidant, but also plays an irreplaceable role in signal transduction. GSH can be oxidized to GSSG by H₂O₂ and GSSG can be reduced to GSH, which is in a dynamic equilibrium [14]. The results of this test showed that Cd stress increased the GSH, GSSG, GSH + GSSG content, and decreased the GSH/GSSG ratio. Jiang et al. [15] believed that GSH/GSSG signal plays a key role in regulating photosynthesis and CO₂ fixation cycle. Therefore, the application of exogenous GSH can improve the antioxidant capacity of cucumber seedlings under the stress of Cd and promote the sustainable development of environmental ecology.

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