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Assessment of the Phytotoxicity of Gold Nanoclusters on Soybean

Jingang Mo[a], Yanjun Guan[b], Yumeng Bai[c], Xuemei Liu[d], Yunbi Zou[e] and Kai Song[*]

School of Life Science, Changchun Normal University, Changchun, P.R.China

*Corresponding author e-mail: 37357077@qq.com, [a]mojingang@163.com, [b]2364865312@qq.com, [c]1761110108@qq.com, [d]xuemei2000@126.com, [e]zouyunbi@qq.com

Abstract. In this work, the effect of gold clusters on seed germination and physiological changes in soybean was assessed. The results show that seed germination was were promoted by gold clusters solution. However, with the increase of gold clusters concentration, the root length and steam length of soybean decreased trough seed germination. Meanwhile, the fresh and try weight of soybean also decreased. The further study found that content of MDA in soybean increased obviously, and the content of soluble sugar in soybean decreased obviously under the stress of high concentration gold cluster solution.

1. Introduction

Nanomaterials have certain toxic effects on microorganisms, aquatic and terrestrial animals and plants. Plant is an important part of ecosystem. On the one hand, nanomaterials may affect the development and growth of plants, on the other hand, the metabolic activities of plants will affect the migration and transformation of nanomaterials in the environment and their transfer in the food web [1-3]. However, there is still a lack of research on the interaction between nanomaterials and plants, most of which are limited to the apparent phenomena caused by the interaction [4, 5].

In this work, we used the small size gold nanoclusters composed of only a few dozen atoms as the experimental materials to study their effects on the growth behavior of soybean. The changes of soybean germination index, antioxidant system enzyme activity and other physiological indexes were investigated, and the effects of nanomaterials on plant growth were discussed. This work can provide a theoretical basis for systematically evaluating the response and feedback of plants to nanomaterials.

2. Experimental section

2.1. Materials

The gold nanocluster material was prepared in our laboratory with a particle size of about 2nm. Soybeans seeds were purchased from Ouya supermarket.

2.2. Method

For germination, 420 beans of uniform size and full color were randomly selected and soaked in 75% ethanol for one minute, then cleaned immediately with sterile water until ethanol was washed. Each 70 soybeans were placed in a germination box, and 10 mL gold clusters solution were added to the box. Finally, the soybeans were cultured in an artificial climate box in dark at 25℃. The gold clusters solution should be replaced every day, and the number of seed germination should be observed to record the daily growth of soybean seeds [6].

After ten days of cultivation, the root length, stem length, fresh weight and dry weight of soybean seeds were measured. Malondialdehyde (MDA) was determined by thiobarbituric acid color reaction. The content of soluble sugar was determined by anthrone method.

3. Results and discussion

The data listed in Table 1. Can be seen that gold nanoclusters do not inhibit the germination of soybean seeds, which may be due to the protective effect of seed coat, so pests and harmful exogenous substances will not destroy the embryos of seeds. In addition, many studies show that some nanomaterials promote plant growth and development [7, 8]. they can promote the germination of peanut, wheat, pea and onion seeds at low concentration, On the contrary, at high concentration, it can significantly inhibited the germination rate of seeds. The results of this experiment confirm the similar mechanism.

Table 1. Effect of gold nanoclusters solution on Germination of soybean seeds.

Concentration of gold nanocluster solution (mg/L)	Germination Rate (%)	Germination potential (%)
0	91.4	51.4
10	94.3	68.6
20	95.7	57.1
50	97.1	67.1
100	94.3	61.4
200	97.1	77.1

As shown in Figure 1, the root length of soybean seedlings decreases with the increase of the concentration of gold nanoclusters. The root length of soybean seedlings is the shortest when the concentration of gold nanoclusters is 200 mg/L, which is 32% lower than that of the control group. In addition, stem length at 50mg/L and 100 mg/L concentrations was higher than that at 10 mg/L and 20 mg/L concentrations because of errors in measurement. By observing that the total stem length was smaller than that of the control group, we concluded that gold nanoclusters also inhibited the stem length of soybean. Further analysis showed that the stem length of soybean seedlings was higher than the root length. The main reason was that the young root of soybean lost its protection after breaking through the seed coat and contacted with the solution of gold nanoclusters directly, which resulted in the root damage was higher than the stem damage[9].

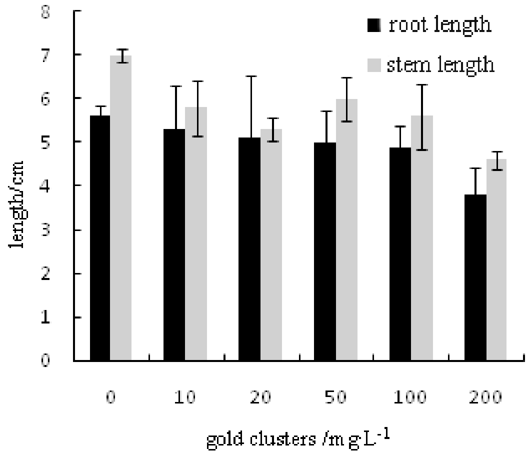

Figure 1. Root and stem lenght of soybean treated with different concentration of gold nanoclusters

Compared with the water control group, the fresh and dry weight of soybean were inhibited by different concentrations of gold nanoclusters. Among them, 20 mg/L and 200 mg/L concentration of gold nanoclusters on soybean fresh weight and dry weight inhibition effect is more obvious, and 200 mg/L concentration of gold nanoclusters solution on soybean fresh weight and dry weight inhibition is the largest (Fig. 2). At 50 mg/L and 100 mg/L, the dry weight and fresh weight of soybean seedlings were higher than that of 10 mg/L and 20 mg/L. This may be due to the fact that when soybean seedlings were exposed to gold nanocluster solution, the nanoparticles themselves blocked part of the absorption structure, which caused a certain degree of stress, resulting in the weakening of water absorption function of soybean. The soybean itself may have adapted to the environment or initiated some mechanism to cope with it, so the weight began to increase.

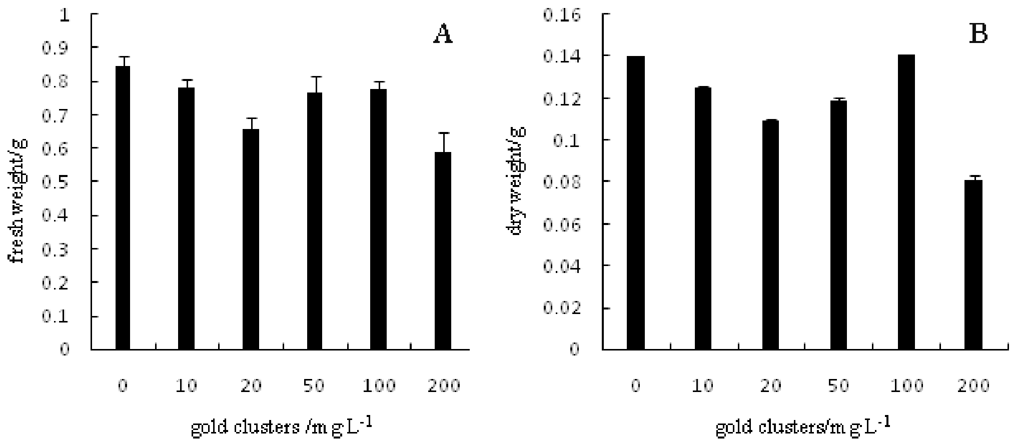

Figure 2. Fresh (A) and dry (B) weight of soybean treated with different concentration of gold nanoclusters

Malondialdehyde (MDA) is the final product of membrane lipid peroxidation, and the content of MDA in plants can reflect the degree of injury under adverse environment. MDA is a cytotoxic substance that can destroy the plasma membrane and cause damage to the cell membrane system. Malondialdehyde content will increase when plants are in an unfavorable environment. As shown in Figure 3, the malondialdehyde content of soybean treated with 10 mg/L gold nanoclusters was not significantly different from that of the control group. With the increase of the concentration of ZnO nanoparticles solution, the content of malondialdehyde also increased. The maximum malondialdehyde content reached at 200 mg/L concentration. The results showed that in the environment of gold nanocluster solution, soybean was subjected to stress. This is consistent with the conclusion of Xu's research about the effects of CuO nanoparticles on the growth and physiological characteristics of Arabidopsis leaves [10]. Similar results were also reported by Guo on the phytotoxicity of ZnO nanoparticles [11].

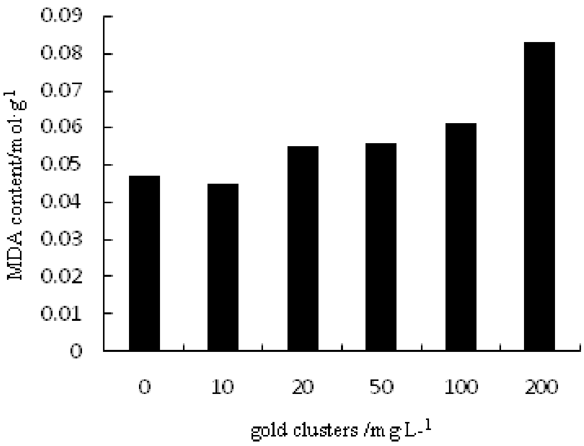

Figure 3. MDA content of soybean treated with different concentration of gold nanoclusters

In plant tissues, soluble sugar plays a role in osmotic regulation. The higher the content of soluble sugar in plant tissues, the higher the osmotic regulation function of plant cells, and the lower the damage of plasma membrane. It can be concluded that the soluble sugar content in plants can reflect the degree of stress. The content of soluble sugar in Soybean Treated with 10 mg/L, 20 mg/L, 50 mg/L, 100 mg/L and 200 mg/L gold nanocluster solution was 0.368%, 0.375%, 0.345%, 0.255% and 0.225% respectively. Compared with the control group, the content of soluble sugar in soybean seedlings at 10 mg/L was lower than 74% of the control group, and was higher at 200 mg/L. The results showed that gold nanoclusters could inhibit the soluble sugar content of soybean seedlings significantly (Fig. 4). This indicates that after the treatment of gold nanoclusters, the plasma membrane damage of soybean increased [12, 13].

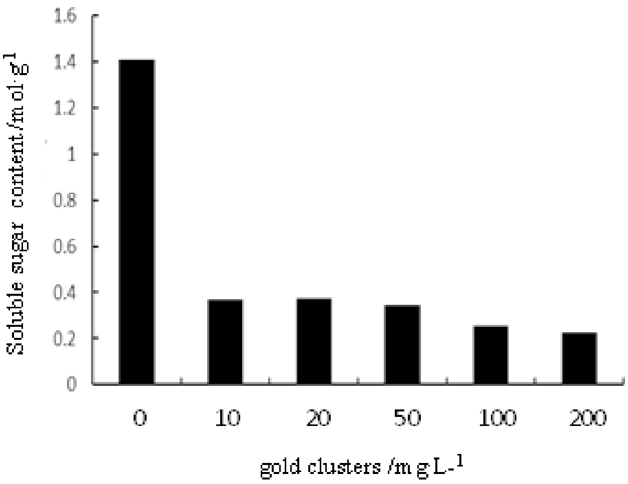

Figure 4. Soluble sugar content of soybean treated with different concentration of gold nanoclusters

4. Conclusion
The gold nanoclusters solution promoted the germination of soybean seeds. With the increase of concentration, the root length and stem length of soybean decreased, while the fresh weight and dry weight decreased. Under the stress of high concentration of gold nanoclusters solution, the content of soluble protein and malondialdehyde in soybean seedlings increased significantly, and the content of soluble sugar in soybean decreased significantly. This indicates that the gold nanoclusters can stimulate the mechanism of stress tolerance in plants and present a dose-dependent manner.

Acknowledgments
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