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Nano-Ferric Oxide Promotes Watermelon Growth

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Abstract

With the rapid growing of nanotechnology, the effects of nanomaterials released into the environment on plants have drawn more and more attention. Iron is an element essential for plant growth and development. Iron is involved in chlorophyll formation; iron deficiency will cause a plant disorder known as chlorosis. However, whether iron in nano-ferric oxide can be absorbed by plants were rarely concerned. Nano-ferric oxide might promote the growth and development of plants in a suitable concentration. An experiment was designed to evaluate whether nano-ferric oxide can be used to treat chlorosis and the physiological changes of plants in nano-ferric oxide environment. Watermelon was chosen as the experimental plant. Seedlings of watermelon plants were grown in full nutrient solution without iron for 2 weeks until the leaves got yellow. Then the seedlings were treated with different concentrations of nano-ferric oxide (0, 20, 50, 100 mg/L) and 50 mmol/L of EDTA-Fe(II) for a month. The control group seedlings were still grown in full nutrient solution without any iron. Indicators such as activity of antioxidase like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and content of malondialdehyde (MDA) and soluble protein were studied to measure the physiological effects nano-ferric oxide might have on watermelon. It was observed that the leaves reverted green. Experimental data showed that watermelon absorbed iron from nano-ferric oxide, and nano-ferric oxide promoted watermelon growth in some ways in a suitable concentration.

Keywords

Nano-Ferric Oxide, Antioxidase, Watermelon, Physiological Effects

1. Introduction

Nanotechnology comprises synthesis of nano-sized (1 - 100 nm) materials and their manipulation to generate

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materials or devices that can be used for various applications [1]-[3]. Nanomaterials have become an important ingredient of analytical methodologies, catalytic processes, DNA labels, biosensors, medicine, food industries, nutraceutics and agriculture [4]-[6]. Metal oxide nanoparticles are receiving increasing attention in a large variety of applications; efforts are underway in many laboratories to understand the potential for ecotoxicity [7]-[9]. nano-ferric oxide is one of the most extensively used metal oxide nanoparticles. It is widely used in magnetic-materials, transparent pigments, catalysts and diagnose reagents among others. With the increasing production and application of nano-ferric oxide, it will inevitably be released into the environment. Iron is one of the essential micronutrients for plants, animals and many other forms of life, and plays a crucial role in a variety of processes such as oxygen transport, energy production, and DNA synthesis [10]. Plants are producers of ecosystems. Iron deficiency of plants will cause chlorosis and have further impact on animals through food chain. Researches of the effects nano-ferric oxide might have on the ecosystem are of great importance.

The impacts of nanomaterials on plants vary greatly not only dependent on the physical and chemical natures of the nanomaterials themselves but also relative to the species studied. Some reports showed that some kinds of nanomaterials could be correlated with major toxicological problems of certain kinds of plants. Lin and Xing reported that ZnO NPs caused phytotoxicity in ryegrass (*Lolium perenne*), radish (*Raphanus sativus*) and rape (*Brassica napus*), displayed as a reduction in root elongation [11]. Zhao *et al*. reported that ZnO NPs produced toxicity in the corn (*Zea mays*) golden variety, by affecting chlorophyll production when the soil was amended with alginate, a natural organic matter. Studies have shown that NPs induce oxidative stress in plants [12]-[14]. Several other workers have mentioned the influence of NPs on rubisco, photosynthetic activity and antioxidant expression profile of plants [15]-[17].

Other reports showed that some kinds of nanomaterials had relatively weak impact or even positive effects on certain kinds of plants. Reports demonstrate that TiO_2 nanoparticles can promote the seed germination of wheat in suitable concerntration and increase shoot and seedling length at 2 and 10 ppm concentration [18]. Studies have shown that ZnO nanoparticles could significantly increase root elongation of green peas, and at all concentrations of ZnO nanoparticles CAT was significantly reduced in leaves, while APOX was reduced in both roots and leaves [19]. Superparamagnetic iron oxide nanoparticles were reported to increase chlorophyll levels, with no trace of toxicity. It was found that physicochemical characteristics of the SPIONs had a crucial role on the enhancement of chlorophyll content in subapical leaves of soybean [20]. Reports by Rosa and López-Moreno showed that germination in cucumber increased by 10% at 1600 mg/L ZnO NPs. Carbon nanotubes, gold nanorods and titanium dioxide nanoparticles can enhance seed germination, root elongation as well as seedling growth as reported. Physical and chemical natures of nanomaterials can also affect its effect towards plants. [21] reported the contribution of the crystalline structure of TiO_2 NPs to their toxicity, two TiO_2 allotropic forms had completely different effects. Rutile and anatase are two TiO_2 allotropic forms and have different surface properties: the first one is lipophilic, while the second one is hydrophilic [22]-[24]. A greater toxic effect was reported for anatase, in comparison with rutile.

In aqueous media nano-ferric oxide can form a suspension with particles that aggregate in clusters of particular sizes. A certain amount of iron also dissolves, and the equilibrium solution depends on the composition of the medium. The toxic effects of metal oxide nanoparticles can be due to the effects of the dissolved ionic metal, or there may be special hazards from nanoparticles themselves due to enhanced bioactivity.

The aim of this research is to find out whether nano-ferric oxide can be absorbed by watermelon and determine the physiological effects of nano-ferric oxide towards watermelon. Watermelon is a garden vegetable consumed worldwide, and its consumption is increasing annually. The watermelon plant has large leaf area, high transpiration rate, and requires more water than grain crops, which could represent higher nanoparticles uptake. Indicators such as activity of antioxidase like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and content of malondialdehyde (MDA) and soluble protein are studied.

2. Materials and Methods

2.1. Materials

$FeCl_3 \cdot 6H_2O$, $Fe_2SO_4 \cdot 7H_2O$, nitroblue tetrazolium, thiobarbituric acid and Coomassive brilliant blue G-250 were bought from Aladdin, Jingchun Biochemical Technology Co., Ltd. Seeds of watermelon (*Citrullus lanatus*) were acquired from Hubei Academy of Agricultural Sciences, China.

2.2. Preparation of Nano-Ferric Oxide

Coprecipitation method was used in the preparation of nano-ferric oxide. Under nitrogen, 2.7 g of $FeCl_3 \cdot 6H_2O$ and 1.4 g of $Fe_2SO_4, \cdot 7H_2O$ were dissolved in 200 ml of deionized water in 500 ml three-necked flask with 60˚C oil bathing and 600 r/min stirring for 5 min to make it fully dissolved. Then 20 ml of 15 ml diluted to 40 ml ammonia were added. 30 minutes later, oil bathing was stopped and leave the reaction system cure at room temperature for 30 min. The mixture was separated through magnetic separation, washing with absolute ethanol for three times, lyophilization, and eventually nano-ferroferric oxide were obtained. 0.3 g of nano-ferroferric oxide were dispersed in 200 ml of deionized water in 500 ml three-necked flask with 90˚C oil bathing and 900 r/min stirring for 30 min, then 10 ml of 3 ml diluted to 40 ml hydrochloric acid were added to the mixture. Air was bubbled continuously for 6 hours. The mixture was then washed with acetone for three times, centrifuged, lyophilized, and eventually about 0.2 g nano-ferric oxide was obtained.

2.3. Treatment of Watermelon Plant

Seeds of watermelon (*Citrullus lanatus*) were acquired from Hubei Academy of Agricultural Sciences, China. The seeds were immersed in distilled water, forcing of germination at 28˚C. Then they were transplanted to spongy with Hoagland solution without any iron for one week. Watermelon seedlings were then divided into five groups. Three groups were treated with 20 mg/L, 50 mg/L, 100 mg/L of nano-ferric oxide, one group with 50 mmol/L EDTA-Fe(II) and one group with Hoagland solution without iron as control. Parameters of cultivate environment: light intensity 2000lx, ratio of light and dark 16 h: 8 h, temperature 28˚C. Indicators of antioxidase and contents of MDA and soluble protein were determined every seven days for three times. A statistical analysis of the results was presented as the mean ± SD (standard deviation).

2.4. Preparation of the Test Solution

For enzyme assays extraction, 0.5 g of fresh leaves were grounded and extracted with 6 ml pH = 7.8 phosphate buffer and 0.01 g PVP in prechilled pestle and mortar. The mixture was centrifuged at 12000 g, 4˚C for 10 min, and the supernatant was diluted to 10 ml.

2.4.1. Superoxide Dismutase (SOD) Activity Assay

Superoxide dismutase (SOD) activity was measured by nitroblue tetrazolium (NBT) method [25]. SOD in supernatant can inhibit the photochemical reduction of nitroblue tetrazolium, thus absorbance can be measured with a spectrophotometer. 3.5 ml of PBS (0.05 mol/L, pH = 7.8), 0.5 ml of Methionine solution (130 mmol/L), 0.5 ml of NBT (750 μmol/L), 0.5 ml of EDTA-Na (100 μmol/L), 0.5 ml of lactochrome (20 μmol/L), 0.2 ml of deionized water, 0.3 ml of supernatant were added into 10 ml transparent buret. The control was prepared using PBS instead of supernatant. The experimental group was illuminated in 4000 lx and the control was placed in the dark for 15 min after uniform mixing. Absorbance was measured at 560 nm.

2.4.2. Peroxidase (POD) Activity

Peroxidase (POD) Activity was estimated by guaiacol colorimetric method (Y. Wang and Z. Yang). 28 μl of guaiacol was added to 50 ml of PBS (0.1 mol/L, pH = 7.0) and dissolved by heating. 19 μl of hydrogen peroxide was added after cooling to prepare the reaction mixture. 1.0 ml of supernatant and 3.0 ml of reaction mixture were added into 5 ml tube as assay mixture, the control used 1.0 ml of PBS (0.05 mol/L, pH = 7.8) instead of supernatant. Start the timer as the hydrogen peroxide was added. Absorbance was measured at 470 nm erery 30 s for 270 s. The POD activity was expressed as the change in absorbance per minute (ΔA470/min/g*FW).

2.4.3. Catalase (CAT) Activity

Catalase (CAT) Activity was measured by the capacity of CAT to decompose hydrogen peroxide [26]. 3.0 ml of PBS (0.05 mol/L, pH = 7.8), 2.0 ml of deionized water and 0.4 ml of supernatant were added into 10 ml tube. 0.6 ml of hydrogen peroxide (0.1 mol/L) was then added and uniformly mixed. Start the timer as the hydrogen peroxide was added. Absorbance was measured at 240 nm erery 30 s for 270 s. The POD activity was expressed as the change in absorbance per minute (ΔA240/min/gFW).

2.4.4. Malondialdehyde (MDA) Content

Malondialdehyde content was determined by thiobarbituric acid method [27]. 0.5 g of watermelon leaves were homogenized in 6.0 ml of PBS (0.05 mol/L, pH = 7.8). The homogenates were centrifuged at 12000 g for 10 min, and the supernatants were diluted to 10 ml. 1 ml of the supernatant was added to 5 ml of 0.5 mol/L thiobarbituric acid (TBA) in 20% TCA in 10 ml tube. The mixture was placed in boiled water bath for 30 min and cooled down quickly with ice bath, and then centrifuged at 12000 g for 15 min. Absorbance of the supernatant was measured at 600 nm, 532 nm, 400 nm. MDA content was expressed in μM/g of fresh weight (FW).

2.4.5. Chlorophyll Content

Extraction of Chlorophyll: 0.5 g of fresh leaves were grounded and extracted with 6 ml 80% acetone solution in prechilled pestle and mortar. The mixture was centrifuged at 12000 g, 4˚C for 10 min, and the supernatant was diluted to 10 ml. Absorbance of the supernatant was measured at 665 nm, 652 nm, 646 nm, 470 nm.

2.4.6. Soluble Protein Content Assay

The protein content was measured by the dying method with Coomassive brilliant blue G-250. 10 mg of bovine serum albumin (BSA) was added into 5 mL of 95% ethanol and 12 mL of 85% phosphoric acid and diluted to 100 ml to make standard solution. The standard curve and equation was obtained using standard solution. 0.1 ml of the supernatant and 0.9 ml of deionized water were added to 5 ml of 0.1% Coomassive brilliant blue G-250 and uniformly mixed. Absorbance of the mixture was measured at 595 nm.

3. Results and Discussion

3.1. Activity of Antioxidase

Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are very important antioxidases that exist widely in nature plants, the activity of which will change when adapting to the environment. Superoxide dismutase scavenges superoxide ion free radicals produced during the course of organism supersession by catalyzing them to disproportionate. Peroxide and catalase catalyze decomposition of hydrogen peroxide to oxygen and water. A of **Figure 1** shows catalase activity of watermelon plant tissues, B for superoxide activity and C for peroxide activity. As seen, antioxidase activity of the control group is less than that of any other group, group of 20 mg/l gets a better result than groups of 50 mg/L and 100 mg/L, group of EDTA-Fe(II) is the highest of all groups. The control group absorbed no iron from the nutrient solution which affected its normal metabolism, resulting in low antioxidase activity. 20 mg/L of nano-ferric oxide is the appropriate dosage for watermelon to absorb and use iron from it, while higher concentrations of nano-ferric oxide of 50 mg/L and 100 mg/L can stimulate watermelon

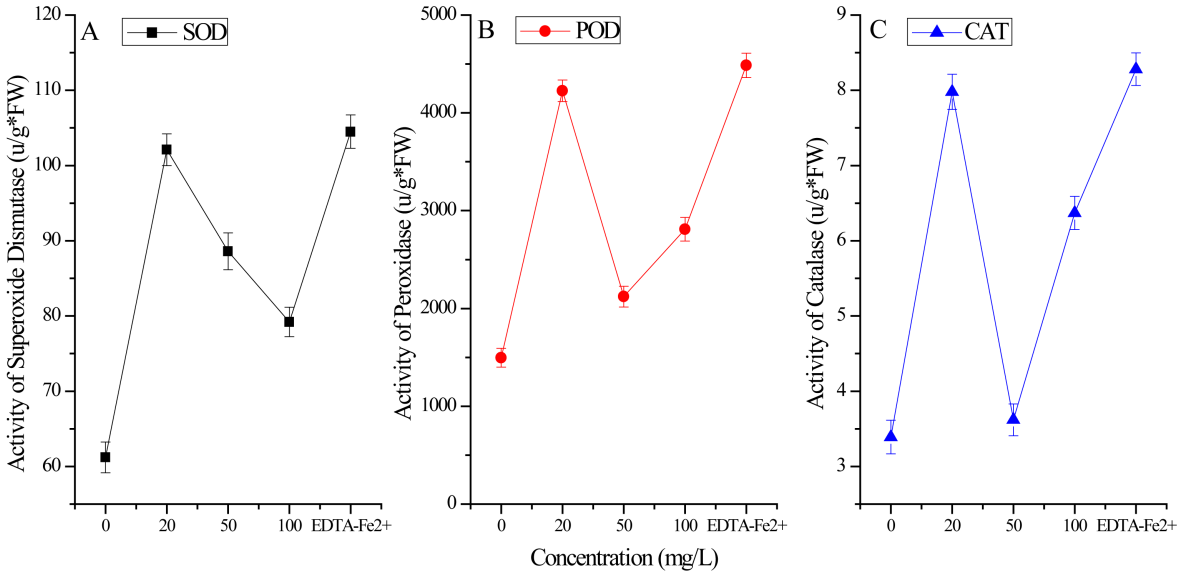

Figure 1. Activity of enzymes after treatment.

tissues to produce more free radicals than the plant can bear and accordingly causes sod activity decrease.

3.2. Content of MDA (Malondialdehyde)

Environmental stress will induce excessive production of free radicals which attack lipid membranes system causing lipid membrane peroxidation and leakage of intracellular electrolyte. Peroxidation of unsaturated fatty acids in the cell plasma membrane produces malondialdehyde (MDA). Thus malondialdehyde can be used as a indicator of membrane peroxidation. As seen in **Figure 2**, group of EDTA-Fe(II) has the lowest level of MDA content, and the control group highest. MDA content of group of 20 mg/L of nano-ferric oxide is the lowest comparing to groups of 50 mg/L and 100 mg/L of nano-ferric oxide. That clearly indicates that iron deficiency causes peroxidation. Watermelon plants treated with EDTA-Fe(II) and 20 mg/L of nano-ferric oxide got a better supply of iron, thus MDA contents are among the lowest. Higher dosages of nano-ferric oxide (50 mg/L, 100 mg/L) promote peroxidation comparing to 20 mg/L of nano-ferric oxide.

3.3. Content of Soluble Protein

Protein is the basis of life. Protein will lose its physiological activity when denatured and cellular aging. The content of soluble protein was utilized as an indicator of protein denaturation. As is shown in the **Figure 3**, watermelon plants grown in 100 mg/L of nano-ferric oxide have the highest level of soluble protein content indicating more serious protein denaturation in plant tissues. A large dose of nano-ferric oxide can cause protein denaturation and accelerate cellular aging significantly. Soluble protein content of EDTA-Fe(II) group is the lowest showing the minimum level of protein denaturation. Group of 20 mg/L has a lower result of soluble protein content comparing to groups of 50 mg/L, 100 mg/L and the control.

3.4. Content of Chlorophyll

Chlorophyll plays indispensable roles in light harvesting and energy transfer during photosynthesis. Iron is an essential element for the synthesis of chlorophyll. Iron deficiency of plants will cause chlorophyll decrease resulting in reducing of photosynthesis rate. Lack of iron leads to chlorosis, even death. As shown in **Figure 4**, the control group has the lowest level of chlorophyll. Phenomenon of watermelon plant chlorosis was observed in the assay as well. Plants grown in EDTA-Fe(II) have the highest level of chlorophyll. Nano-ferric oxide can promote chlorophyll content to some extent at a suitable dose. Plants do absorb iron from nano-ferric oxide, but large doses can reduce the effect. That may be due to the experimental environment was aquatic, while natural growth environment was edaphic. The advantage of nano-ferric oxide to ferrous iron is that it is more stable in soil.

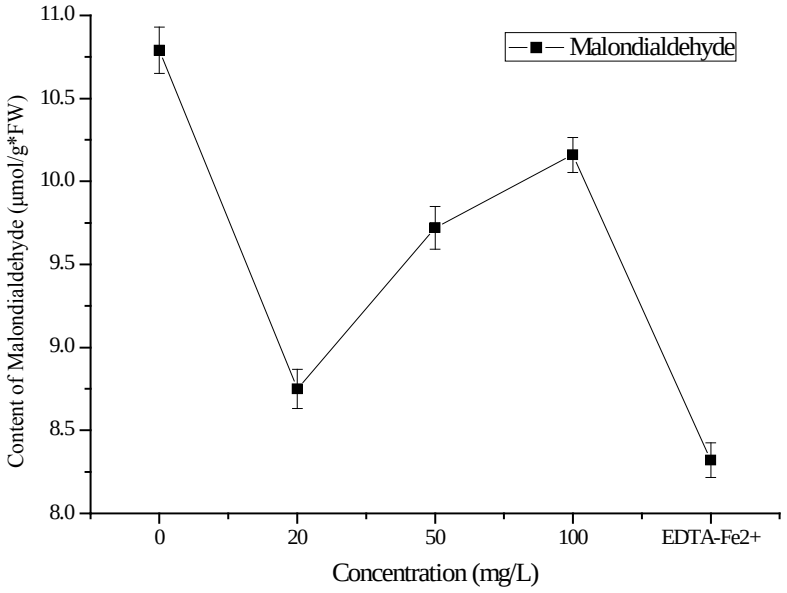

Figure 2. Content of MDA after treatment.

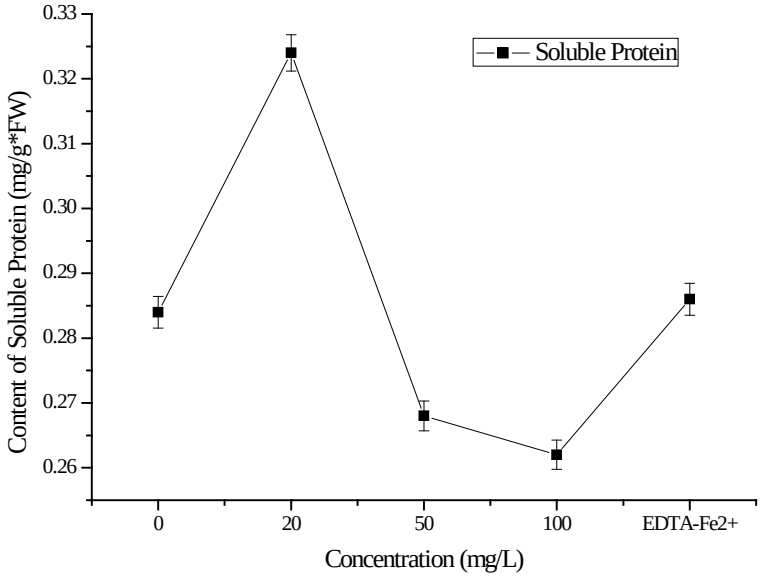

Figure 3. Content of Soluble Protein after treatment.

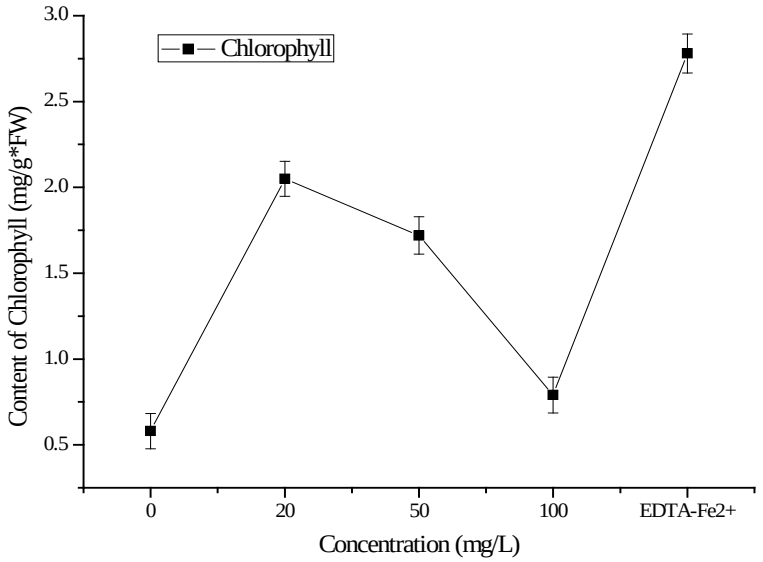

Figure 4. Content of Chlorophyll after treatment.

4. Conclusion

In summary, nano-ferric oxide can be absorbed by watermelon plants and can promote growth of the plants to some extent. We designed this experiment to study the Activity of antioxidases, ferric reductase and content of malondialdehyde, soluble protein, and chlorophyll of watermelon plants treated with different concentration of nano-ferric oxide. Results of antioxidase activity assay shows watermelon plants with 20 mg/L of nano-ferric oxide treatment have a good capacity scavenging oxygen radicials and surviving from environmental stress. And watermelon plants treated with 20 mg/L of nano-ferric oxide have relatively lower MDA content and soluble protein content and relatively higher content of chlorophyll which indicates a better cell health condition. But we also find that large dose of nano-ferric oxide can be harmful to watermelon plants.

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