Plant Physiology and Biochemistry 142 (2019) 137–142

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Compound repair effect of carbon dots and Fe^{2+} on iron deficiency in *Cucumis melon L.*

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ARTICLE INFO

Keywords:
Carbon dots
Fluorescence imaging
Plant iron deficiency
Iron supplement

ABSTRACT

Iron-deficiency is one of the most widespread micronutrient deficiency faced by plants, and proper iron supplementation is essential for the growth of crops and for people to obtain iron from food. In order to explore new methods of iron supplementation, we studied the repair effect of CDs on iron-deficient (*Cucumis melo L.*) muskmelon. Iron-deficient muskmelons were treated with different concentrations of Fe^{2+}, CDs and their complexes. The results showed that CDs significantly increased the iron transport rate and it is noteworthy that 75 mg/L CDs increased the iron transport rate of 0.7 mg/L Fe^{2+} by 134%. The compound treatment reduced the oxidative stress caused by iron deficiency, such as the CAT activity in the leaves of the compound treatment group was 10%–50% lower than that of the iron supplementation alone. Fluorescent imaging results of melon proved that CDs entered into the muskmelon seedlings. In combination with the above results and the adsorption of CDs, we speculated that the way CDs promoted iron absorption and transport was most likely to combine with Fe^{2+} and co-transport in melon, which changed the content of reactive oxygen species and other free radicals, thus causing changes of physiological state of melon. This study confirmed that CDs had a positive effect on the iron deficiency of muskmelon, and improved the growth of muskmelon under the condition of iron deficiency, which has a certain reference value for further optimization of iron supplementation solution.

1. Introduction

Iron, as a vital nutrient element for plant growth, has been widely studied in recent years (Q. Li et al., 2016; Masuda et al., 2017; Nozoye et al., 2017). As a serious agricultural problem, iron deficiency is common in crops, especially for plants in calcareous soil (Jalali et al., 2017; Ramzani et al., 2016). Iron deficiency will affect the photosynthesis of crops (Weng and Guerinot, 2013), which will lead to serious foliar chlorosis and increase the oxidative stress response in plants (Selby-Pham et al., 2017), affect the growth of crops, reduce the yield of crops and the iron content of edible crops, thus reducing the amount of iron that can be obtained by the human body. New solutions to iron supplements for crops have to be explored. Rajaie et al. (Rajaie and Tavakoly, 2017) found that the leaf spray (5 g/L) acidulated Fe^{2+} (EDTA-Fe) could effectively reduce the yellowing of Valencia Orange in calcareous soil. The growth of Oriental plane tree in calcareous soil can be improved by applying ferric sulphate-organic acid on the leaf surface than for soil treatment (Salahi et al., 2017). The application of exogenous NO in different critical growth periods can also alleviate iron deficiency and chlorosis of peanut in calcareous soil (Dong et al., 2018). However, the application of iron fertilizer and gas fertilizer on the leaf surface may cause harm to the environment and human body (NO is a kind of toxic gas).

The emergence of nanomaterial has given researchers new inspirations, such as the application of nanomaterial in biosensors (Sarihi et al., 2019) and as pesticides and fertilizer transporters in agriculture (Rastogi et al., 2019). The small size effect (Roduner, 2006), surface effect (Mesaric et al., 2013), adsorption (Tina et al., 2015) and other properties of nanomaterial provide us with more possibilities to develop it. Among them, Ghafari H et al. (Ghafari and Razmjoo, 2018) treated hard grain wheat by three forms of iron oxide, namely Nano-iron oxide, iron chelate (EDTA) and iron sulphate. The results showed that 2 g/L Nano-iron oxide significantly improved the content of chlorophyll and protein, and had the best effect on the growth of wheat. However, iron oxide nanoparticles will also cause damage to plants and affect their growth (Gui et al., 2015). New ways of iron supplementation still need

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https://doi.org/10.1016/j.plaphy.2019.06.035
Received 28 March 2019; Received in revised form 27 June 2019; Accepted 27 June 2019
Available online 28 June 2019
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to be found. Therefore, in order to explore more effective solutions, we found CDs.

CDs is a kind of biocompatible material (Smart et al., 2006), which have been widely studied in medicine. CDs showed good photoluminescence characteristics in fluorescence imaging and they were used in biological imaging (Edison et al., 2016; Sachdev et al., 2016) and detection (Han et al., 2016). For plants, CDs are showing a positive-impact, too. For example, they can reduce the activity of antioxidant enzymes in *Arabidopsis thaliana*, reduce the toxicity of 1, 3-dinitrobenzene to *Arabidopsis thaliana*, promote plant growth and water absorption in saline environment (Martínez-Ballesta et al., 2016), and the use of CDs as a light conversion material can improve plant photosynthesis (Sai et al., 2018). However, there are not many studies on the metal nutrition of CDs. We hypothesized that the CDs could be used as the carrier of iron supplementation, so we conducted an experimental investigation. Based on the characteristics and advantages of CDs, we applied different iron supplementation treatments to iron deficiency melon. And the effects of different iron supplementation treatments on melon with iron deficiency were discussed.

2. Materials and method

2.1. CDs and Fe^{2+}

The CDs used in the experiment were obtained from Optical Fiber Experimental Center in Wuhan University of Technology, and their physical and chemical characteristics (Lin et al., 2018) were shown in Table S1. Since this experiment was carried out in hydroponic environment, we tested their hydration particle size, as shown in Fig. S1. Fe^{2+} (from $FeSO_4{\bullet}7H_2O$) is chelated with EDTA.

2.2. Treatment method

CDs of three concentrations (50 mg/L, 75 mg/L and 150 mg/L) were used in the preliminary experiment for 3 days culture with 1/4 Hoagland nutrient solution, the plants in the 150 mg/L treatment group had obvious damage, while the other two groups grew well (Fig. S2). Therefore, CDs of 50 mg/L and 75 mg/L were used in formal experiment. In the formal experiment, the concentration of Fe^{2+} was based on the concentration of Fe^{2+} in the Hoagland nutrient solution. The concentration of Fe^{2+} in the full concentration of Hoagland nutrient solution (2.8 mg/L) and the concentration of Fe^{2+} in 1/4 of the Hoagland nutrient solution (0.7 mg/L) were selected. After 2 weeks of iron deficiency culture (hydroponication was used to ensure the relativity of treatment conditions), the muskmelon was treated with iron supplementation. The treatment groups were shown in Table 1 and physiological indicators were detected after 5 days. The group with iron deficiency was the control check (CK).

2.3. Detection method

The water content of the treated melon was detected, the antioxidant enzyme activities of catalase (CAT, refers to Gallego S M et al. (Gallego et al., 1996)), peroxidase (POD, refers to Jingxian Z et al. (Zhang et al., 1995)), superoxide dismutase (SOD, refers to Wang Y et al. (Wang et al., 2004)) and chlorophyll content in root and leaf were detected. Iron content in muskmelon root and leaf was determined by Atomic Absorption Spectrometry (AAS). Fluorescence inversion

Table 1
Iron and CDs concentration in each treatment group.

	0	50 mg/L CDs	75 mg/L CDs
0.7 mg/L Fe^{2+}	Fe1	Fe1C1	Fe1C2
2.8 mg/L Fe^{2+}	Fe2	Fe2C1	Fe2C2

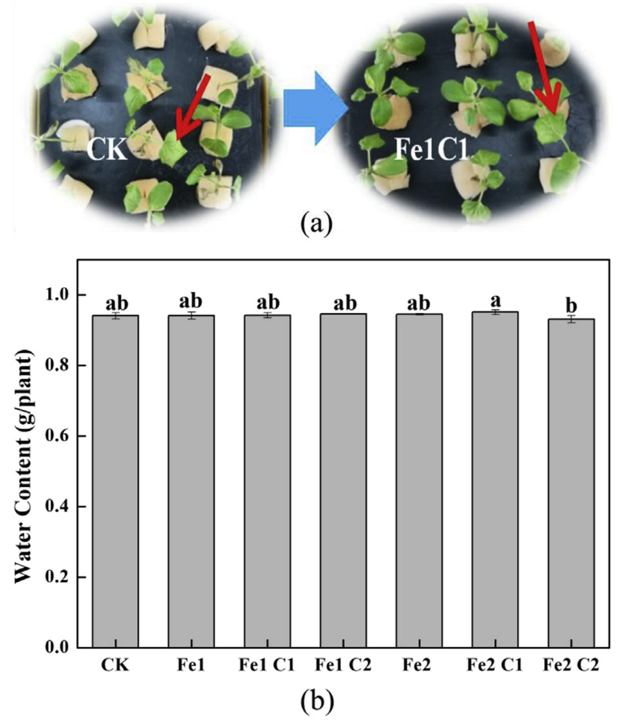

Fig. 1. (a) Growth of muskmelon plants (control and 0.7 mg/L Fe combined with 50 mg/L carbon dots); The leaf indicated by the red arrow appears yellow (b) The water content of muskmelon plant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

microscope was used to image the distribution of CDs in melon (including tissues and cells). Fluorescent images of muskmelon plants were taken in the UV dark box.

2.4. Data analysis

IBM SPSS Version 21 was used for data analysis, and each treatment was repeated three times. One-way ANOVA was used, and Duncan multiple comparisons were made ($p < 0.05$). In origin 8.0, the result was the mean standard deviation (SD).

3. Results

3.1. Growth of muskmelon

According to the growth of melon after treatment, the chlorisis caused by iron deficiency is relatively serious; some leaves of CK have withered (Fig. S4, CK). In the group treated with 0.7 mg/L Fe combined with 50 mg/L CDs (Fe1C1) had the best apparent effect, and the leaves were greener than those in other groups. However, some leaves still showed slight yellowing (Fig. 1(a)). There was no significant difference in water content between different treatment groups (Fig. 1(b)).

3.2. Chlorophyll content and iron transport rate

In order to explore the effect of iron supplementation further, chlorophyll content in the leaves and the iron content in the roots and leaves was detected at the same time. From the perspective of chlorophyll content (Fig. 2(a)), compared with CK, the chlorophyll content increased by 21% only in the case of low concentration CDs participation (Fe1C1), while the difference between other groups and the control group was not significant, which was corresponding to the iron content in the leaves. In terms of iron content in the root (Fig. S3), the

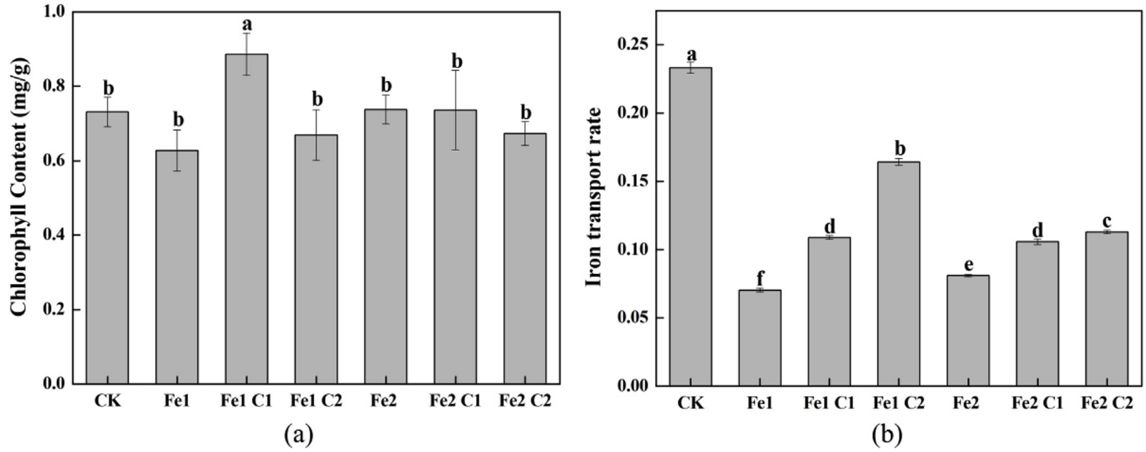

Fig. 2. (a) Chlorophyll content (mg/g) in fresh leaves of muskmelon plants. (b) Iron transport rates from root to leaf.

intervention of CDs affected the absorption of iron by muskmelon, and the iron concentration of Fe1 was 204% higher than that of Fe1C2, and the difference was extremely significant. As for leaves, the iron content in treatment groups was increased compared with CK. At the same time, we figured out the transport rate of iron from root to leaf. The iron transport rates of the groups with added CDs were significantly higher than that of the groups without CDs and increased with the increase of CDs concentration (Fig. 2(b)). The difference between Fe1C2 and Fe1 was the most significant, and the iron transport rate of Fe1C2 was 134% higher than that of Fe1. Because the content of iron in the root and leaf of melon is very low under the condition of iron deficiency, the iron transport rate in the control group (CK) is very high.

3.3. Oxidative stress

In order to evaluate the effect of iron supplementation on muskmelon physiology, the activities of enzyme related to oxidative stress (CAT, POD and SOD activity in fresh tissue) were detected (Fig. 3).

Compared with the control group, CAT activity in roots of each treatment group increased after iron supplementation. The activity of CAT in root was higher in the separate iron treatment groups (Fe1 and Fe2) than in the compound treatment groups (10%–50%), and the effect of low concentration CDs on oxidative stress was positive. CAT activity in the leaves was lower in the treatment group than in the iron-deficient control group (18%–63%), and CDs had no significant effect on CAT activity.

For POD activity, compared with the control group, both compound treatment and individual treatment resulted in the high activity of peroxidase in roots, and the effect of compound treatment was more negative. However, for leaves, the process of iron supplementation still has a certain inhibitory effect on the generation of peroxides in crop leaves, and low-concentration iron treatment is better than high-concentration iron treatment. Compared with direct iron supplementation (Fig. 3, Fe2), the effect of the participation of high concentration CDs was more positive, and POD activity of Fe2C1 and Fe2C2 was significantly lower than that of Fe2.

In both root and leaf, the activity of SOD in iron supplementation groups were significantly lower than that in control group (41%–49%), but the activity of SOD in roots was significantly higher than that in leaves (38%–398%). To some extent, the difference of oxidative stress caused by iron deficiency in different parts of plants was attributed. CDs had no significant effect on SOD activity in leaves.

3.4. Carbon dots tracer – fluorescence imaging

The results of iron transport rate and oxidative stress suggest that the existence of CDs may play a role in the repair of iron deficiency in muskmelon. In order to further prove the mechanism of CDs action, we studied the operation of CDs in melon. CDs can emit blue visible light under the excitation of ultraviolet light (W. Li et al., 2016). According to this property of CDs, photos of the whole muskmelon plant under ultraviolet light are obtained. It can be seen that the fluorescence intensity increased successively according to the different concentrations. In the treatment group with slightly higher concentration (75 mg/L CDs), there were blue highlights in the leaves (Fig. S5). In addition, slices of the root, stem and leaf of muskmelon were observed under fluorescence inverted microscope after treatment for one day (Fig. 4 and Fig. S6), but no fluorescence intensity gradient was found as shown in Fig. S5. In addition, the untreated control group also emitted blue light from ultraviolet light, but with a weak intensity.

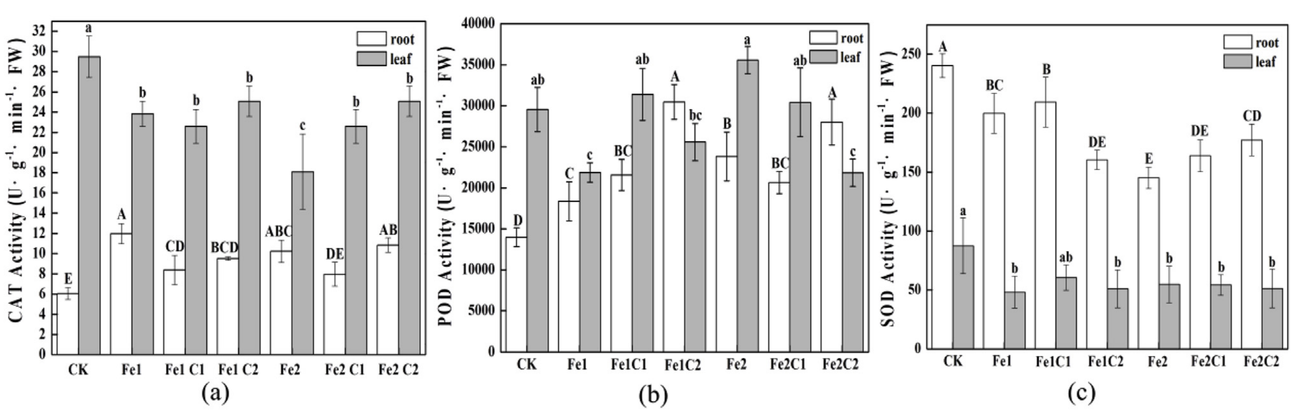

Fig. 3. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity (U/(g·min)) in fresh tissue of melon.

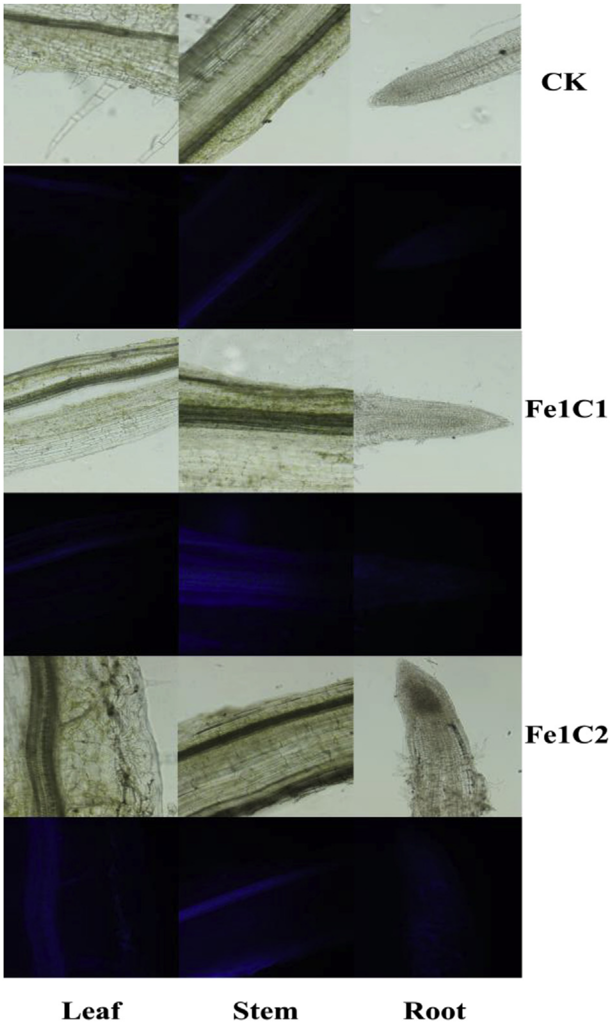

Fig. 4. Fluorescent sections of root, stem and leaf tissues of melon were treated with Fe1C1 (0.7 mg/L Fe^{2+} compound 50 mg/L CDs) and Fe1C2 (2.8 mg/L Fe^{2+} compound 75 mg/L CDs). CK was the control group, and section preparation was conducted one day after treatment.

4. Discussions

Leaf yellowing caused by iron deficiency is a common plant disease (Prasad and Djanaguiraman, 2017). In this experiment, muskmelon still showed the phenomenon of partial leaf yellowing caused by iron deficiency after different combinations of iron and CDs were applied, but there has been a great improvement in the compound treatment group of Fe1C1 compared with the control group. The repair effect of low concentration CDs on iron-deficient muskmelon was better than that of high concentration CDs (Fig. S4). Slight yellowing may be related to a short treatment time. Water content can explain the effect of iron supplementation on the growth of muskmelon. Regarding the water content, the participation of CDs can promote the increase of water content of rice seeds in germination (9.06%–12.76% higher than that of control) and improve the growth and metabolism of rice (Nair et al., 2012). In this experiment, there was no significant difference in water content between different treatment groups (Fig. 1(b)), which may be related to the plant species and stage of growth.

Iron is involved in the formation of non-heme ferritin, which is necessary for plant photosynthesis and respiration, and also affects chlorophyll content (Krohling et al., 2016). In order to quantify the differences in the effects of iron supplementation in each group, the iron transport rate and chlorophyll content of the treated muskmelon were analyzed (Fig. 2). Different from the control group, the iron content in roots of the treatment group was much higher than that in leaves, indicating that iron supplementation was sufficient (Fig. S3). At the same time, the difference in iron content between roots and leaves under the same Fe^{2+} concentration confirmed that the participation of CDs in the condition of adequate iron supplementation inhibited the absorption of iron by muskmelon, but promoted its transport of iron (Fig. 2(b)). This phenomenon may be related to the regulation mechanism of maintaining iron homeostasis in plants (Connorton et al., 2017). The increase of utilization of iron in melon is mainly through reduction strategy (Nikolic and Pavlovic, 2018). In order to deal with the stress caused by Fenton reaction (Fe overdose) (Schützendübel and Polle, 2002), iron absorption and distribution are strictly regulated at the transcriptional level (Connorton et al., 2017). The chlorophyll content corresponding to the iron content was unique in the Fe1C1 group, indicating that the combination of CDs and iron could promote the chloroplast content of muskmelon, especially the combination of low concentration. This is consistent with the promotion effect of CDs on the chlorophyll content of bean sprouts (Wang et al., 2018).

When plants are stimulated by the outside world (salt stress, drought stress, etc.), a series of stress reactions will be produced and produced a large amount of active oxygen free radicals. These free radicals can be cleared by a series of reactions involving antioxidant enzymes such as CAT, POD and SOD (Chaves et al., 2009; Foyer and Noctort, 2010), in which CAT and POD can decompose H_2O_2 generated by stress reaction into O_2 and H_2O (Willekens et al., 1995; Németh et al., 2009). That SOD can convert superoxide anion radical into H_2O_2 (Alscher et al., 2002) might explain the effect of treatment on muskmelon to some extent. In this study, oxidative stress may come from two aspects: one is caused by iron deficiency, and the other one is caused by iron supplementation. For iron deficiency, the difference of SOD activity is obvious. In both roots and leaves, SOD activity of Fe1 was lower than CK (Fig. 3(c)), which based on the experimental concentration of Fe1 was similar to 1/4 Hoagland nutrient solution. In addition, the activities of CAT and POD also have common differences in leaves. In this aspect, iron supplementation has repair effect on oxidative stress caused by iron deficiency, and the application of carbon dots has certain inhibitory effect on the production of peroxides, such as the decrease of SOD activity in Fe1C2. However, there was no significant difference of SOD activity in other treatment, which may be related to the cycle and retention of iron in melon (M Dudley et al., 2012). Another part is iron supplementation (mainly in root), such as the difference in the activity of CAT and POD. The CAT activity of Fe2 is lower than that of Fe1, and the POD activity of Fe2 is higher than that of Fe1 (CAT and POD activity in Fig. 3). The participation of low-concentration CDs has a positive effect on the oxidative stress that iron supplementation may cause. These results indicated that, under the stress of iron deficiency, CAT and POD were more activated, which may be related to the processes of iron in the formation of chlorophyll (Miller et al., 1984) and a series of other physiological process in leaves. Under the iron supplementation treatment, there was also a certain stress reaction, mainly the activation of SOD activity, and the intervention of CDs alleviated the stress reaction caused by iron supplementation to some extent. Damage is still present in some treatment groups, which remain to be further studied and is expected to be reduced by artificial design of CDs (Muthukumar et al., 2014; Wan et al., 2016).

According to the difference of fluorescence intensity, the distribution of CDs in plants can be known. The difference of fluorescence intensity in Fig. S5 is not completely consistent with that observed by fluorescence inverted microscope (Fig. 4), which may relate to the operation of sections, and it is difficult to obtain identical sections. However, being compared with the control group, both intact plants and sections showed significantly stronger fluorescence. One thing that caught our attention was that the large size of CDs in solution in Fig. S1 did not affect the absorption of CDs by melon (the bright blue fluorescence in Fig. S5). In order to further observe the distribution of CDs in

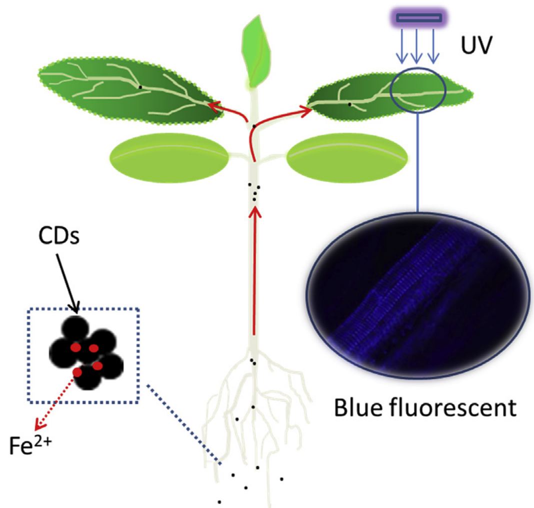

Fig 5. The transport route of CDs in muskmelon and the possible transport mode of iron; Under UV, the leaves of melon were blue fluorescent.

plant cells, we made cell sections, and observed that some CDs did enter into the cells from Fig. S6. And the transfer from root to leaf occurred in melon. This transfer process may be completed by the muskmelon in the process of water absorption through the root system (W. Li et al., 2016).

CDs has the ability to adsorb phenol and aniline (Bo and Baoshan, 2010; Kun and Baoshan, 2010). In the results of this experiment (Fig. 4), CDs can be efficiently transferred from the test solution into melon. Therefore, we speculate that CDs for the promotion of iron transport effect could be due to a co-translocation mechanism (Fig. 5): CDs were efficiently translocated into the melon after combining with Fe^{2+}. This binding force may come from the adsorption of Fe^{2+} by CDs. The transport efficiency of iron also increases with the increase of CDs concentration (Fig. 2(b)). At the same time, due to the change of iron concentration in muskmelon and the entry of CDs, some physiological conditions of muskmelon also changed (Fig. 3).

5. Conclusions

To sum up, we studied the effect with CDs combined Fe^{2+} treatment on iron supplementation of iron-deficient muskmelon and compared compound treatment with that of the individual group. In the results, the promoting effect of CDs on iron transport was very obvious. From the effect of compound treatment, the compound treatment with low concentration (50 mg/L CDs combined with 0.7 mg/L Fe^{2+}) played an obvious positive role in iron supplementation of plants, increasing the chlorophyll content and iron transport rate while reducing the oxidative stress. Fluorescence imaging also showed that CDs work by entering plants. To some extent, this result indicates that the effect of proper compound treatment is better than that of the single treatment, which has important reference value for further optimizing crop iron fertilizer and improving crop fertilizer efficiency.

Conflicts of interest

There are no conflicts of interest to declare.

Contribution

Junli Li defined the research topic and framework of study and provided writing assistance. Daoyong Yang finished the writing work and provided language help. Yuxuan Cheng, Fengting Wan and Ruiliang Jia detected the physiological parameters. Yunqiang Wang provided the experimental site and daily management of plants.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (Grant No. 2018YFD0201300); China Agricultural Research System (Grant No. CARS-25); the Fundamental Research Funds for the Central Universities (Grant No.2017IB006; 2018HSB103; 2019IB005); the Major Technology Innovation of Hubei Province (Grant No.2016ABA095).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.06.035.

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