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Carbon dots improve the nutritional quality of coriander (*Coriandrum sativum* L.) by promoting photosynthesis and nutrient uptake

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Supporting information

Supporting information (15 pages) includes: materials and methods, 7 figures and 2 tables.

Materials and methods

Rubisco activity

Under the action of the catalysis of rubisco, one ribulose-1,5-diphosphate (RuBP) molecule binds to one CO_2 molecule to produce two 3-phosphoglycerate (PGA) molecules, which can be removed by addition of 3-Glycerate kinase and glyceraldehyde-3-phosphate dehydrogenase to produce glyceraldehyde-3-phosphate and oxidize the reduced coenzyme I (NADH). The oxidation rate of NADH is used to evaluate the rubisco activity by measuring the absorbance at 340 nm.[1]

The rubisco activity test kit includes one solution (solution one) and four reagents, including reagent 1 (liquid), reagent 2 (powder), reagent 3 (powder) and reagent 4 (powder). Reagent 1 was used for preparation of other three reagents prior to use. The reagents 2 & 3 were prepared by adding 12.5 mL reagent 1 separately. Reagent 4 was prepared by adding 1.25 mL reagent 1. The working solution was a mixture of reagents 2 & 3 with a volume ratio of 1:1. First, 0.1 g leaves were homogenized in an ice bath with 1 mL solution 1. The mixture was then homogenized by a probe ultrasonicator (S-250D, Branson, USA) for 1 min in an ice bath at 200 W with a working/pausing duration cycle of 3 s/7 s. The mixture was then centrifuged for 10 min at 10956 g and 4 °C to separate the insoluble residue. The supernatant (50 μL) was mixed with 50 μL reagent 4 and 900 μL working solution. Absorbance of the mixture was measured at 340 nm at 20 s and 2 min 20 s, respectively.

Chloroplast isolation

Chloroplasts were isolated from coriander leaves using differential centrifugation, following the instruction of a chloroplast isolation kit (Beijing Leagene Biotechnology Co., Ltd. China). The coriander

leaves were homogenized in a pre-chilled mortar and pestle in ice-cold isolation buffer. The slurry was filtered through 3-layer cheese cloth, followed by centrifugation for 2 min at 300 g and 4 ^{o}C. The supernatant was centrifuged again for another 2 min at 1,000 g and 4 ^{o}C. The supernatant was carefully discarded and the pellet was suspended again in an extraction buffer and used for spectroscopic analysis. Chlorophyll was extracted in chilled 80% acetone and estimated spectrophotometrically at 652 nm, to correct for the suspension concentration difference of chloroplasts used for spectrometric analysis.[2] For further use, chloroplast suspension was equivalent to 10 μg chlorophyll/mL.

Measurement of chlorophyll content

Leaf tissues (0.1 g) were homogenized in 95% ethanol, and all samples were stored in the dark for 14 h to extract chlorophyll from chloroplasts.[3] After standing for 30 min, absorbance of supernatant at 649 and 665 nm was measured. The total chlorophyll content was calculated using equations as follows:

C_a (Chlorophyll a) = $13.95A_{665} - 6.8A_{649}$

C_b (Chlorophyll b) = $24.96A_{649} - 7.32A_{665}$

C_t (total chlorophyll) = $C_a + C_b = 18.16A_{649} + 6.63A_{665}$

Measurement of organic nutrients

Soluble sugar content. Soluble sugar content in plant leaves was measured using sulfuric acid anthrone[4] with a plant soluble sugar content test kit (Nanjing Jiancheng Technology Co., Ltd. China) with glucose as standard. Briefly, 0.3 g leaves were cut and ground with 10 mL phosphate buffer (PBS, pH 7.8). The mixture was centrifuged for 10 min at 1878 g, and the supernatant in each treatment was stored at 4 °C until further use. Then 0.2 mL supernatant, 0.1 mL substrate solution and 1 mL

concentrated sulfuric acid were mixed and heated in a boiling water bath for 10 min. After cooling down by flowing water, the absorbance at 620 nm was measured by a Thermo Multiskan GO microplate reader (USA).

Soluble protein content. Soluble protein content was measured by a staining method with Coomassie brilliant blue[5] using a total protein quantitative assay kit (Nanjing Jiancheng Technology Co., Ltd. China). Briefly, 0.05 mL supernatant was mixed with 3 mL Coomassie Brilliant Blue solution and allowed to stand for 5 min, and then absorbance at 595 nm was measured.

Vitamin C content. Briefly, 0.3 g leaves were cut and ground with 10 mL 2.0% (w/v) metaphosphoric acid.[6] The mixture was centrifuged for 20 min at 1057 g, and absorbance of the supernatant at 243 nm was measured.

Sorption experiment

To test sorption of nutrient elements to CDs and the associated influence on plant uptake; 0, 10, 20, 30, and 40 mg/L CDs were dispersed in 50% Hoagland's nutrient solution (with a volume of 50 mL). These tubes were shaken on a rotary shaker for one week. The mixture was then filtered with a 3 kDa Amicon Ultra-4 Centrifugal Filter Unit. We separately confirmed that the nutrients were not sorbed to the filter. Concentrations of the nutrients including K, Ca, Mg, P, Mn and Fe in the filtrate were measured using ICP-OES as described in the main text.

Analysis of antioxidant response

Extraction of crude enzyme. Briefly, 0.3 g root or shoot tissues were cut and ground with 10 mL phosphate buffer (PBS, pH 7.8). The mixture was centrifuged for 10 min at 5752 g, and the supernatant in each treatment was stored at 4 °C until use.

SOD activity. SOD activity was measured by its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT).[7] The reaction system contained 0.5 mL of the above supernatant, 0.5 mL of 130 mM methionine, 0.5 mL of 750 mM NBT, 0.5 mL of 100 mM EDTA-Na_2, 0.5 mL of 20 mM lactochrome, and 3.5 mL of 0.05 M phosphate buffer (pH 7.8). The well-mixed samples were illuminated for 20 min, but the untreated control prepared in Milli-Q water was placed in the dark. The absorbance at 560 nm was measured.

CAT activity. CAT activity was measured by adding 0.5 mL supernatant to 3 mL of 100 mM phosphate buffer (pH 7.0) that contained 0.01% H_2O_2. The absorbance at 240 nm was measured every 1 min for 4 min.[8]

POD activity. POD activity was estimated using the guaiacol colorimetric method.[9] Briefly, 28 μL of 0.05 M guaiacol was added to 50 mL of 100 mM phosphate buffer (pH 7.0). After completely dissolving and cooling to ambient temperature, 19 μL of 30% H_2O_2 was added and the POD reaction mixture was obtained. POD activity was determined by adding 0.1 mL of the above supernatant to 3 mL of POD reaction mixture and 0.9 mL of 100 mM phosphate buffer (pH 7.0). The absorbance at 470 nm was measured every 1 min for 4 min.

APX activity. APX activity was measured by adding 0.1 mL of the above supernatant to 1.7 mL of 0.1 mM EDTA-Na_2, 0.1 mL 5 mM AsA, and 0.1 mL of 20 mM H_2O_2. The absorbance at 290 nm was recorded every 1 min for 4 min.[10]

MDA content. Briefly, 0.3 g of root or shoot tissues were ground in 2.0 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged for 10 min at 1878 g. Then 1 mL supernatant was added to 2.0 mL of 0.6% thiobarbituric acid (TBA). After boiling in a water bath for 15 min, the mixture was cooled

immediately in an ice bath. After centrifugation for 10 min at 1878 g, absorbance of the supernatant at 450, 532 and 600 nm was measured.[11]

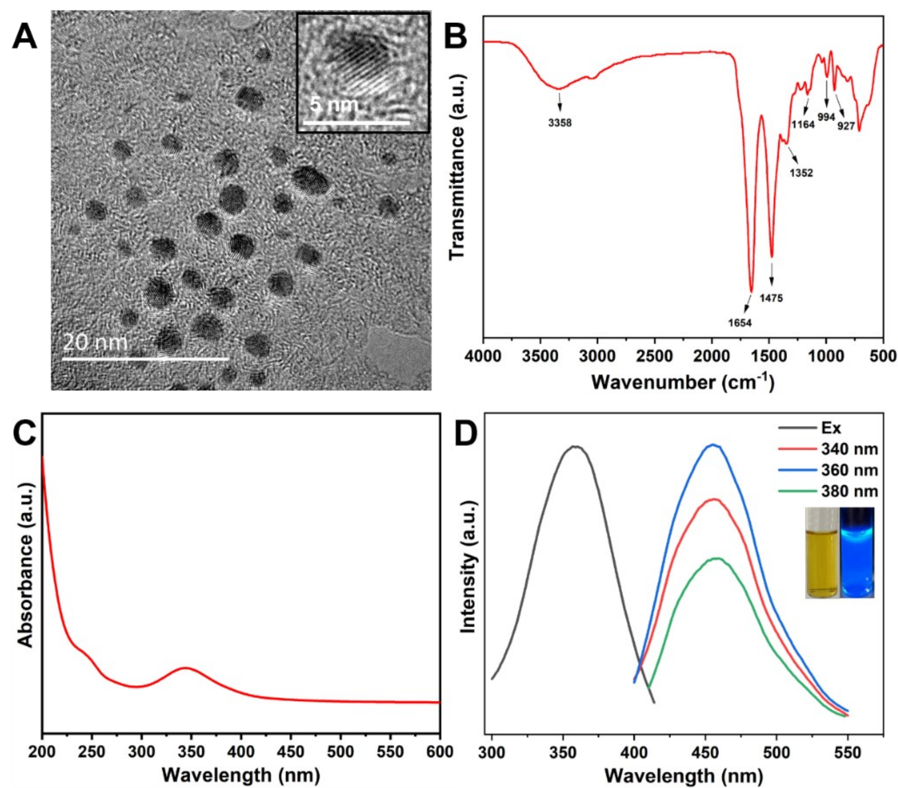

Fig. S1 Characterization of CDs. (A) TEM image (inset: a high-resolution TEM image of individual CDs particles). (B) FTIR spectrum of CDs. (C) UV-Vis spectrum of CDs. (D) Excitation spectrum of CDs monitored at 460 nm and emission spectra of CDs excited at 340, 360 and 380 nm, respectively (inset: the photograph of CDs under visible light and UV light at 370 nm, respectively).

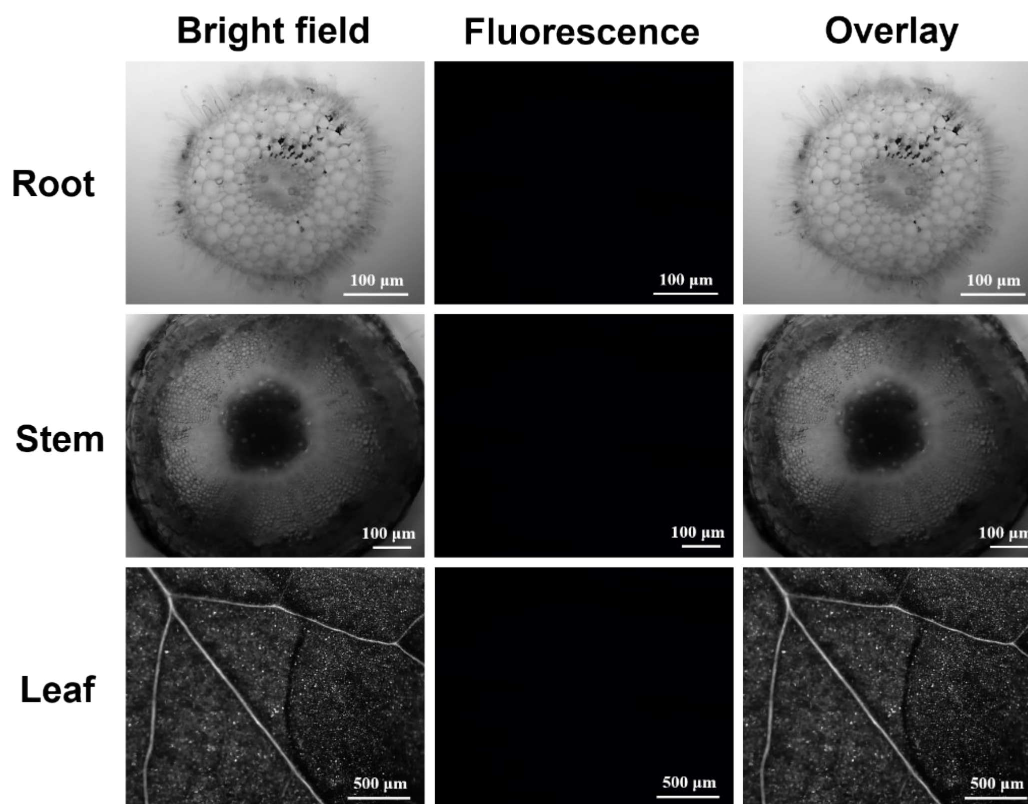

Fig. S2 Fluorescence microscopy images of cross sections from coriander root and stem, as well as leaf squashing from the unamended controls.

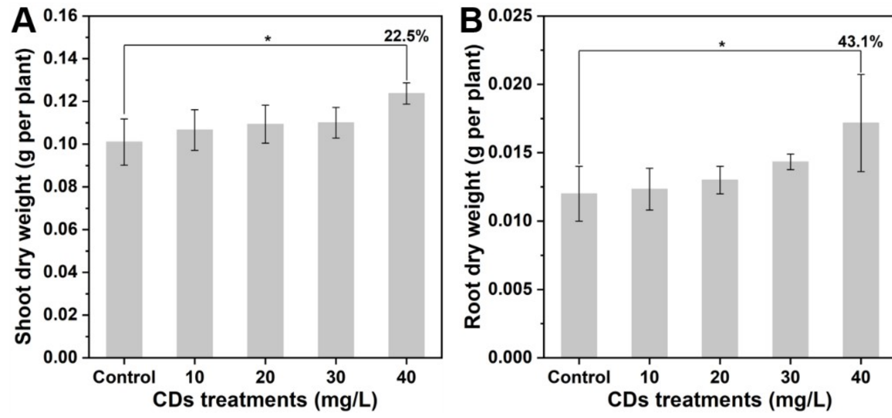

Fig. S3 Effect of CDs at different concentrations on the dry weight of coriander shoots (A) and roots (B). Data are means of three replicates ± SD. Values marked with * are significantly different at $p \leq 0.05$.

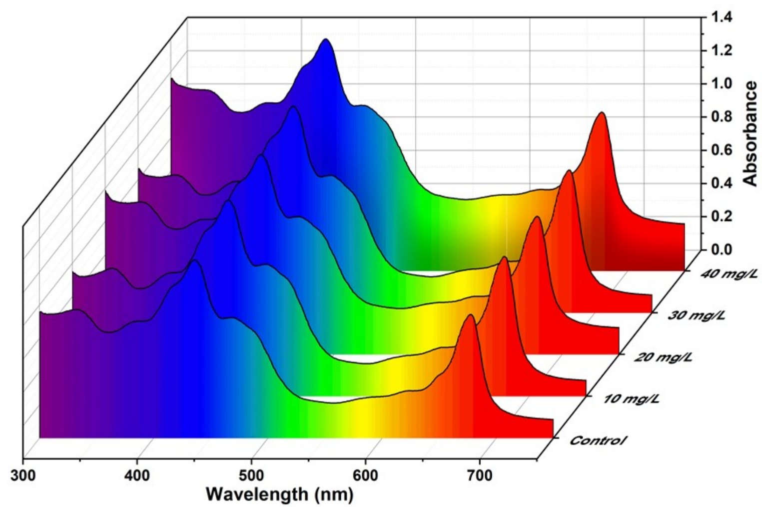

Fig. S4 Effects of CDs at varying concentrations on UV-Vis spectra of chloroplasts isolated from coriander leaves.

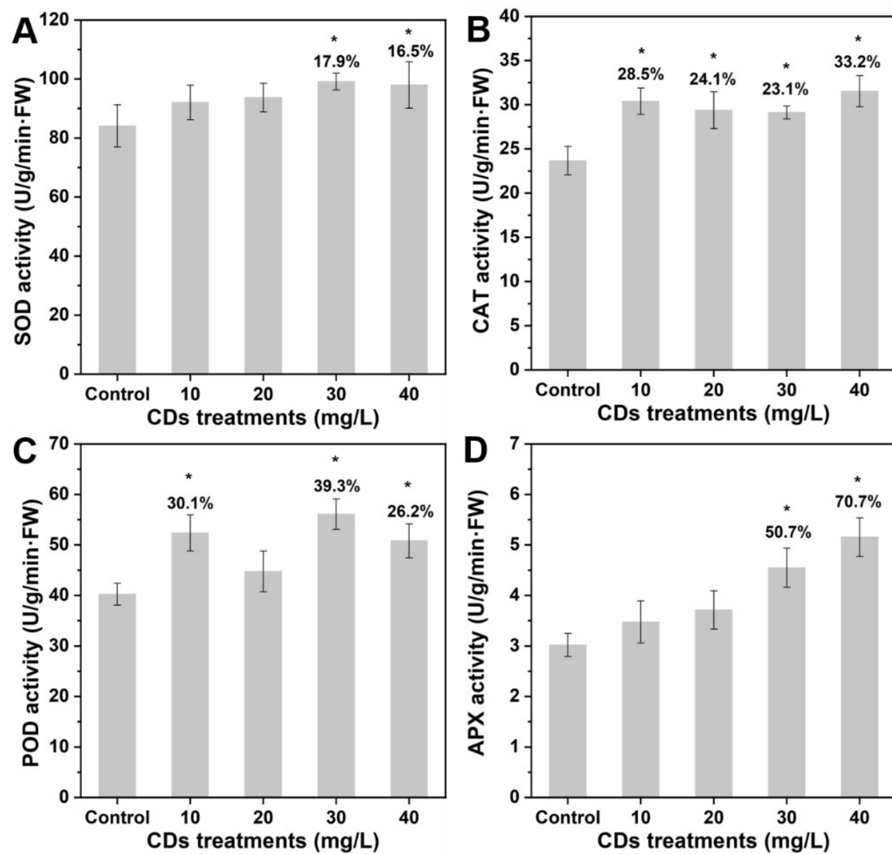

Fig. S5 Effects of CDs on the activity of SOD (A), CAT (B), POD (C) and APX (D) in coriander shoots.

Data are means of three replicates ± SD. Values marked with * are significantly different at $p \leq 0.05$.

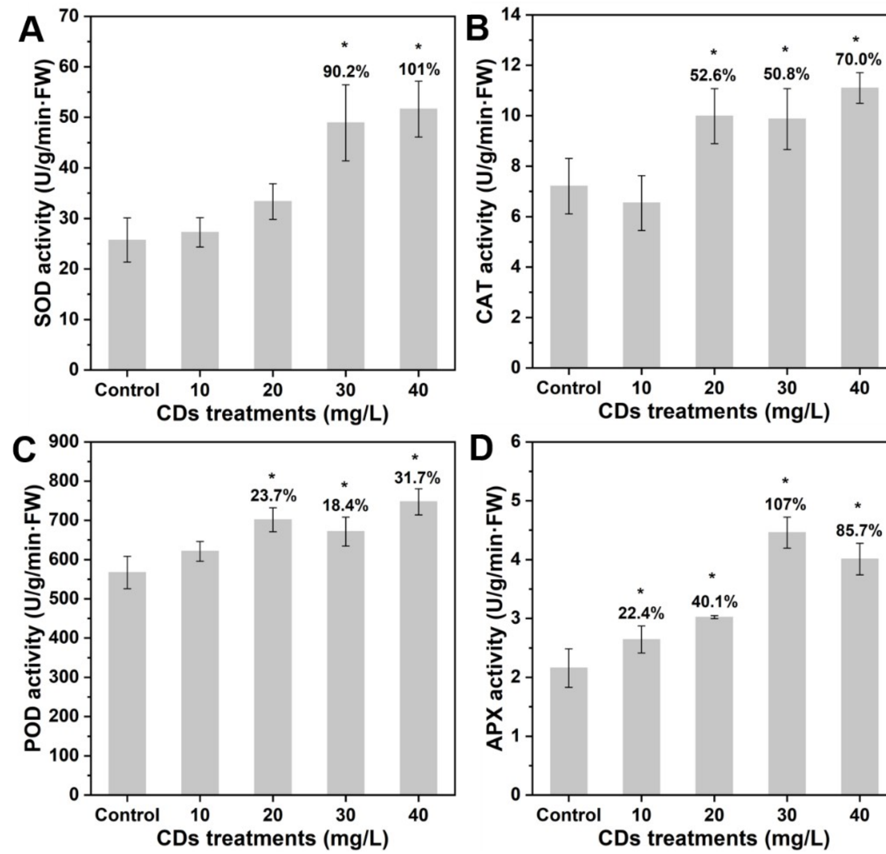

Fig. S6 Effects of CDs on the activity of SOD (A), CAT (B), POD (C) and APX (D) in coriander roots.

Data are means of three replicates ± SD. Values marked with * are significantly different at $p \leq 0.05$.

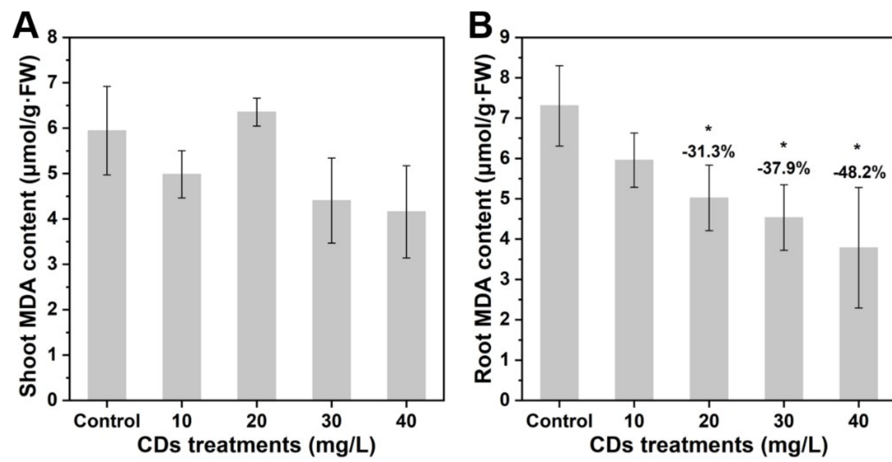

Fig. S7 Effects of CDs on the MDA content in shoots (A) and roots (B) of coriander plants. Data are means of three replicates ± SD. Values marked with * are significantly different at $p \leq 0.05$.

Table S1. Detection limit of nutrients by ICP-OES

Line	Mean	Dev.	DL	Unit
K 766.491	0.0556	0.0005	0.0014	µg/mL
K 769.897	0.0768	0.0015	0.0045	µg/mL
Ca 317.933	< 0.0000	0.0007	0.0021	µg/mL
Ca 396.847	< 0.0000	0.0008	0.0024	µg/mL
Ca 422.673	< 0.0000	0.0008	0.0025	µg/mL
Ca 315.887	< 0.0000	0.0017	0.005	µg/mL
Mg 279.553	< 0.0000	0.0004	0.0012	µg/mL
Mg 285.213	< 0.0000	0.0004	0.0013	µg/mL
Mg 279.078	< 0.0000	0.0041	0.0123	µg/mL
P 213.618	0.1858	0.015	0.045	µg/mL
P 253.561	0.1597	0.0405	0.1215	µg/mL
P 214.914	0.1996	0.036	0.1079	µg/mL
Mn 257.610	< 0.0000	0.0002	0.0005	µg/mL
Mn 259.372	< 0.0000	0.0002	0.0005	µg/mL
Fe 259.940	< 0.0000	0.0006	0.0018	µg/mL
Fe 239.563	< 0.0000	0.0009	0.0028	µg/mL

Table S2. K, Ca, Mg, P, Mn and Fe concentration (mg/L) in 50% Hoagland's nutrient solution amended with 0, 10, 20, 30 and 40 mg/L CDs for one week[a]

Treatment	K	Ca	Mg	P	Mn	Fe
Control	123	60.2	21.4	15.5	3.37	0.78
10 mg/L	122	60.0	21.5	15.5	3.39	0.79
20 mg/L	119	58.7	20.9	15.0	3.28	0.67*
30 mg/L	118	58.6	21.0	15.0	3.26	0.60*
40 mg/L	118	58.4	20.9	14.9	3.23	0.50*

[a] Data are means of three replicates ± SD. Values marked with * are significantly different at $p \leq 0.05$.

References

1. H. Li, J. Huang, Y. Liu, F. Lu, J. Zhong, Y. Wang, S. Li, Y. Lifshitz, S. T. Lee and Z. Kang, Enhanced RuBisCO activity and promoted dicotyledons growth with degradable carbon dots, Nano Res., 2019, 12, 1585-1593.
2. H. Yuan, L. Liu, F. Lv and S. Wang, Bioluminescence as a light source for photosynthesis, Chem. Commun., 2013, 49, 10685-10687.
3. H. K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods Enzymol., 1987, 148, 350-382.
4. J. Chen, Y. Tian, X. Zhang, C. Zheng, Z. Song, A. Deng and W. Zhang, Nighttime warming will increase winter wheat yield through improving plant development and grain growth in north China, J. Plant Growth Regul., 2014, 33, 397-407.
5. H. X. Ren, L. Liu, C. Liu, S. Y. He, J. Huang, J. L. Li, Y. Zhang, X. Huang and N. Gu, Physiological investigation of magnetic iron oxide nanoparticles towards Chinese mung bean, J. Biomed. Nanotechnol., 2011, 7, 677-684.
6. J. P. Zheng, Determination of the Vitamin C in Fruits and Vegetables—Discussion on UV Spectrophotometry, Chinese J. Spectroscopy Lab., 2006, 23, 731-735.
7. Y. Wang, X. Wang, Y. Ying and J. Chen, Transgenic arabidopsis overexpressing Mn-SOD enhanced salt-tolerance, Plant Sci., 2004, 167, 671-677.
8. S. M. Gallego, M. P. Benavídes and M. L. Tomaro, Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress, Plant Sci., 1996, 121, 151-159.
9. J. Zhang, S. Cui, J. Li and M. B. Kirkham, Protoplasmic factors, antioxidant responses, and chilling resistance in maize, Plant Physiol. Biochem., 1995, 33, 567-575.
10. V. Dixit, V. Pandey and R. Shyam, Differential antioxidative responses to cadmium in roots and leaves of pea *(Pisum sativum* L. cv. Azad), J. Exp. Bot., 2001, 52, 1101-1109.
11. R. L. Heath and L. Packer, Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation, Arch. Biochem. Biophys., 1968, 125, 189-198.