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1. Materials and Reagents

The tobacco wastes treated by air drying are provided by Zunyi Company of Guizhou Tobacco Company. Tobacco wastes only need to undergo simple grinding operations before use, and do not distinguish between tobacco leaves and stems (the components contained in stems are basically the same as those in tobacco leaves[1]). Lettuce (Lactuca sativa, Chunxiang) seeds were purchased from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. Absolute ethanol (EtOH), acetone, hydrogen peroxide (H_2O_2), hydrogen nitrate (HNO_3), hydrochloric acid (HCl), sodium hydroxide (NaOH), sucrose, potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were all purchased from the Shanghai Macklin Biochemical Co., Ltd. All reagents were used as received without further purification unless otherwise mentioned, and ultrapure water was used throughout our experiments.

2. Preparation of CDs

B-CDs were prepared using a simple one-pot hydrothermal method with tobacco waste debris as precursors. Specifically, tobacco waste debris (0.5 g), H_2O_2 (0.2 g) and water (10 ml) were placed in a polytetrafluoroethylene (PTFE) liner for ultrasonic treatment (300 W; 5 minutes). Then, the PTFE liner was placed into a stainless-steel autoclave and heated at 180 ℃ in an oven for 6 h. After cooling to room temperature, the obtained brown solution was filtered, and the residue was washed with water until the dripping filtrate was clear and transparent. The filtrate was filtered through a 0.22 μm filter membrane, dialyzed (MW: 100-500) in ultrapure water for 12 hours, and the filtrate in the dialysis bag was evaporated on a rotary evaporator (50 ℃) until a dried, solid, brown-black B-CDs were obtained. An additional vacuum drying in the oven or freeze-drying can also be applied. The solvent obtained from the rotary evaporation can be collected and reused. The preparation of G-CDs (10 ml of EtOH) and R-CDs (10 ml of acetone) is similar to that of B-CDs by replacing 10 ml of water with the indicated solvent and by using the same solvent to wash the residue.

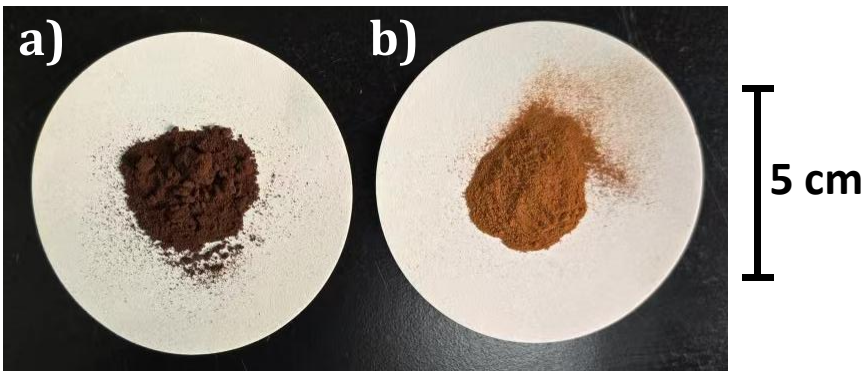

Fig. S1. Digital photographs of the synthesized (a) B-CDs, and (b) R-CDs.

3. Characterization of CDs

The UV-Vis absorption experiments were conducted on the UV-2550 UV-Vis spectrometer (Shimadzu, Kyoto, Japan), with a range of 200-800 nm and a resolution of 1 nm. The fluorescence measurements were carried out by using a RF-5301 spectrophotometer (Shimadzu, Kyoto, Japan), and the slit width of the excitation and emission were fixed at 5 nm (range: 220-800 nm, resolution: 1 nm). The UV-Vis absorption and fluorescence spectra were also measured using the same instrument for subsequent CDs@Chl. All spectra were collected at 25 ℃, using the 10 mm fluorescence cuvette. The transmission electron microscopy (TEM) was performed on FEI Talos F200X (Thermo Scientific, Massachusetts, US) under 100 or 200 kV accelerating voltage. The scanning electron microscope (SEM) was conducted on SU8020 (Hitachi, Tokyo, Japan) with an operating voltage of 3 or 5 kV. The morphology was also observed under the same instruments for subsequent CDs@Chl. The X-ray diffraction (XRD) pattern was collected using a D8 ADVANCE instrument (Bruker, Saarbrücken, Germany), which has a Cu-Kα source monochromator for single crystal analysis at 40 kV and 40 mA. Fourier transform infrared spectroscopy (FTIR) was performed on a Nicolet IS10 spectrometer (Thermo Scientific, Massachusetts, US) using the KBr tablet pressing method in the range of 400-4000 cm^{-1}. The Raman spectra were taken on a InVia Reflex spectrograph (Renishaw, London, UK). The thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) analysis were determined by a Netzsch sta449f3 thermogravimetric analyzer (Netzsch, Bavaria, Germany) in the 25 to 800 ℃ range with a heating rate of 10 ℃/min under nitrogen atmosphere. The X-ray photoelectron spectroscopy (XPS) experiments were performed using a Escalab 250XI X-ray photoelectron spectrometer (Thermo Scientific, Massachusetts, US), and a monochromatic Al-Kα X-ray source was excited by 1486.6 eV. The calibration of binding energy was based on C_{1s} at 284.8eV. The fluorescence images of CDs in lettuce tissues and CDs@Chl were obtained using Zeiss LSM 800 (Zeiss, Oberkochen, Germany) confocal laser scanning microscope (CLSM). The measurement of fluorescence lifetime was carried out using the standard time-correlated single-photon counting method on a Quantaurus-Tau C16361-2 fluorescence lifetime tester (Hamamatsu Photonics, Hamamatsu City, Japan). The exciting light was a portable diode laser. Laser beam was guided into the samples, and fluorescence was detected at the emission maximum of the corresponding sample. The bandwidth for the excitation as well as for the emission was < 2 nm. The Zetasizer Nano ZS90 (Malvern Instruments, Malvern City, UK) was used to measure the zeta potential (ζ) of each sample at a 90° angle. The absorbance test of the sample was conducted on a D-8PC UV-Vis spectrophotometer (Pkiles, Nanjing, China). The chlorophyll fluorescence parameters (Table S2) were determined on the IMAG MAX/L chlorophyll fluorescence imaging system (Walz, Affectrich, Germany). The net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (Ts) and intercellular CO_2 concentration (Ci) of lettuce leaves (Table S3) were measured using a Li-6400 portable photosynthesis system (Li Cor Biosciences, NE, US).

4. Optical Properties and Quantum Yield (QY) Measurements

The QY measurement of B-CDs was performed using quinine sulfate (0.5 M H_2SO_4 as solvent; QY = 54.6%) as standard. The QY of R-CDs was obtained by using rhodamine 6G (EtOH as

solvent, QY = 95.0%) as standard. The QYs of our samples were determined by the reference point method[2].

$$\varphi_x = \varphi_{st} (I_x / I_{st}) (\eta_x^2 / \eta_{st}^2)(A_{st} / A_x) \quad (1)$$

Where φ is the QY, I is the measured integrated emission intensity, η is the refractive index of the solvent, and A is the optical density. The subscript "st" refers to standard with known QY and "x" for the sample. In order to minimize re-absorption effects, absorption in the 10 mm fluorescence cuvette was kept below 0.10 at the excitation wavelength.

Table S1. The absorbance, PL integrated area and QY of characteristic emissions from B-CDs (in water) and R-CDs (in ethanol) as compared to quinine sulfate and rhodamine 6G reference dyes.

	Quinine sulfate	B-CDs (λ_{ex}=375)	Rhodamine 6G	R-CDs (λ_{ex}=551)	Rhodamine 6G	R-CDs (λ_{ex}=585)	Rhodamine 6G	R-CDs (λ_{ex}=617)
Abs.	0.0508	0.0684	0.9083	0.9892	1.9304	1.8523	1.2479	1.1621
Integ. PL	8946.2180	2270.5845	35915.1305	1990.3680	29926.3855	1347.2510	29140.7805	1099.2250
QY (%)	54.6	10.2	95.0	4.8	95.0	4.4	95.0	3.8

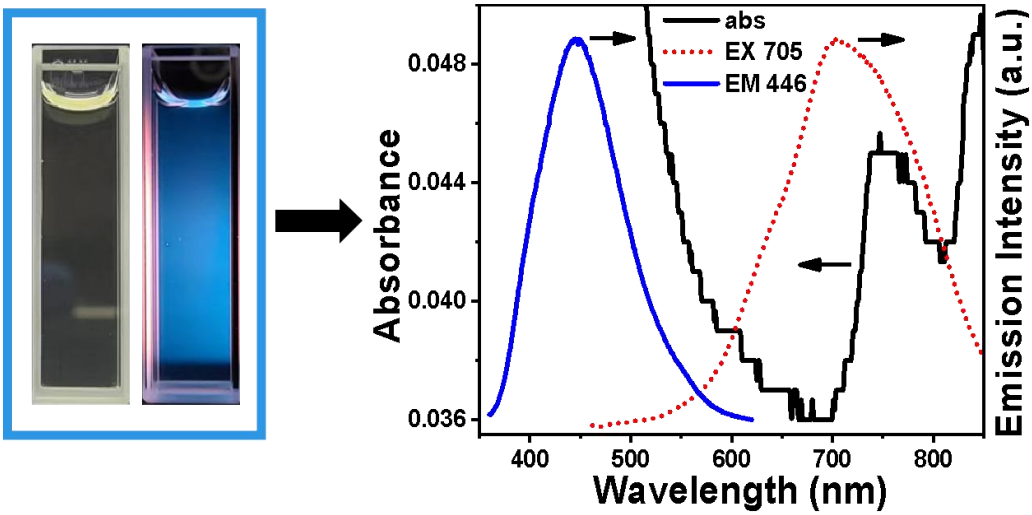

Fig. S2. Up-conversion properties of the aqueous solution of B-CDs (λ_{ex} = 705 nm, λ_{em} = 446 nm) at the concentration 0.24 mg/mL.

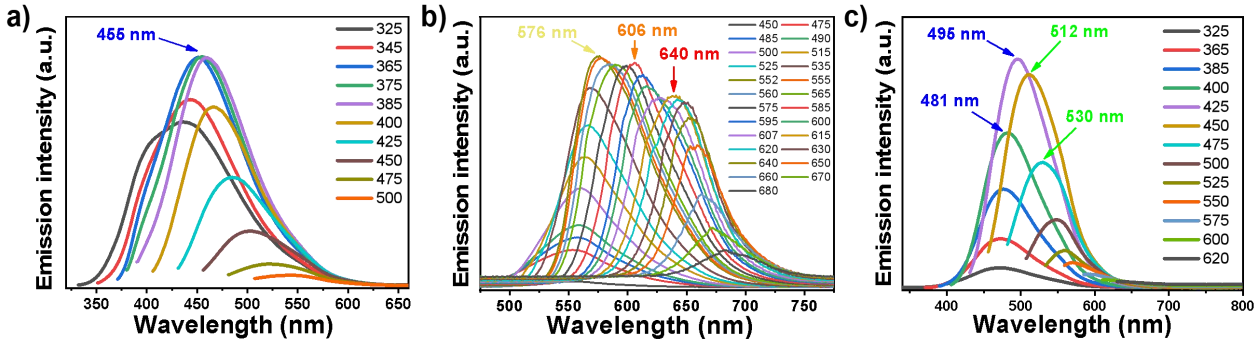

Fig. S3. The emission spectra of (a) aqueous solution of B-CDs (0.24 mg/mL), (b) acetone solution of R-CDs (3.50 mg/mL) and (c) aqueous solution of B-CDs (3.50 mg/mL) recorded at the gradually increasing excitation wavelengths.

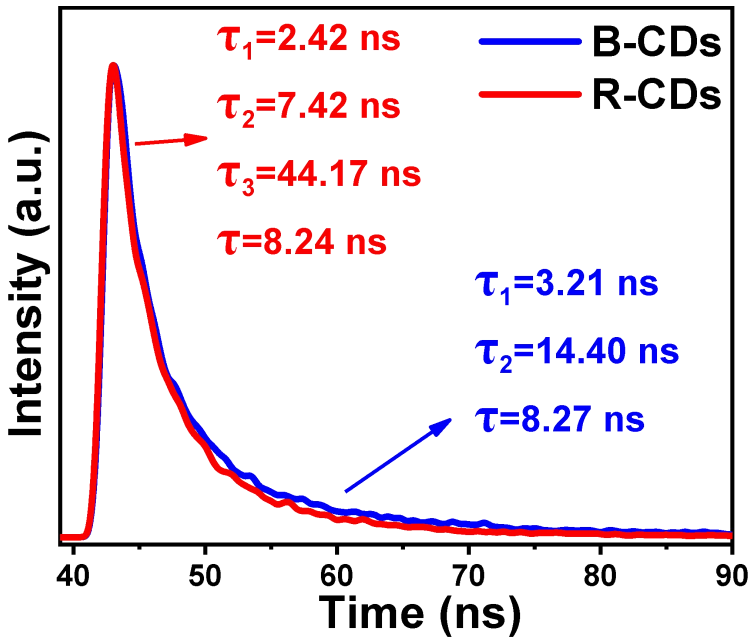

Fig. S4. Fluorescence lifetime measurements of aqueous solution of B-CDs (0.24 mg/mL, blue) and acetone solution of R-CDs (3.50 mg/mL, red).

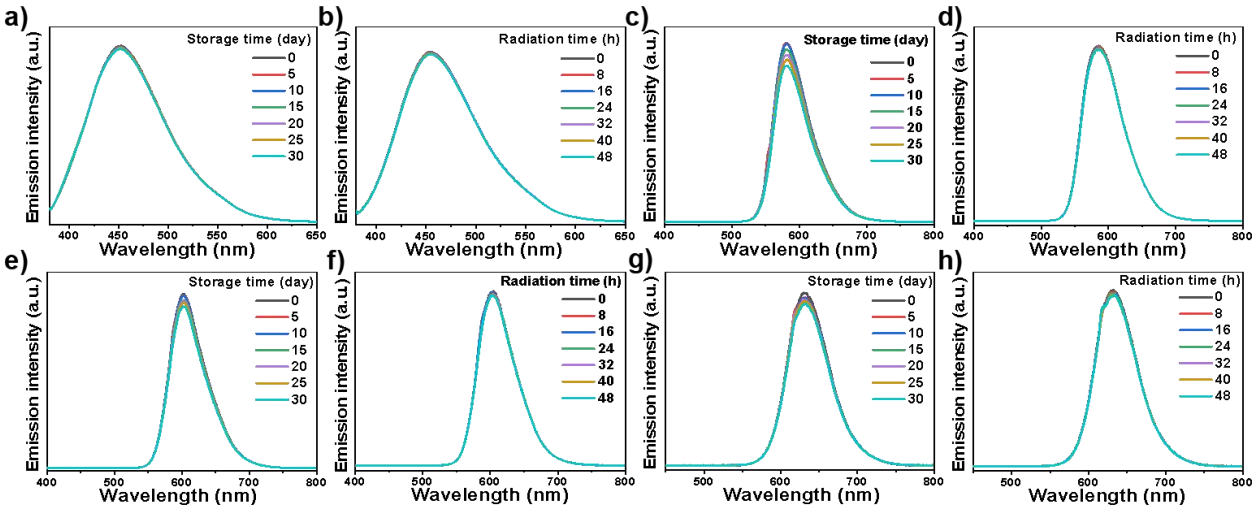

Fig. S5. Fluorescence intensity of B-CDs (a, b) during storage at ambient conditions and the UV irradiation, respectively (λ_{ex} = 375 nm). The fluorescence intensity of R-CDs (c, d, e, f, g, h) during storage at ambient conditions and the UV irradiation, respectively (c/d: λ_{ex} = 551 nm, e/f: λ_{ex} = 585 nm, g/h: λ_{ex} = 617 nm). The samples were stored at ambient conditions for 30 days and their emissions spectra were determined every 5 days. The samples for the UV irradiation (365 nm, 16 W) were stored for 48 hours and their emissions spectra were measured every 8 hours.

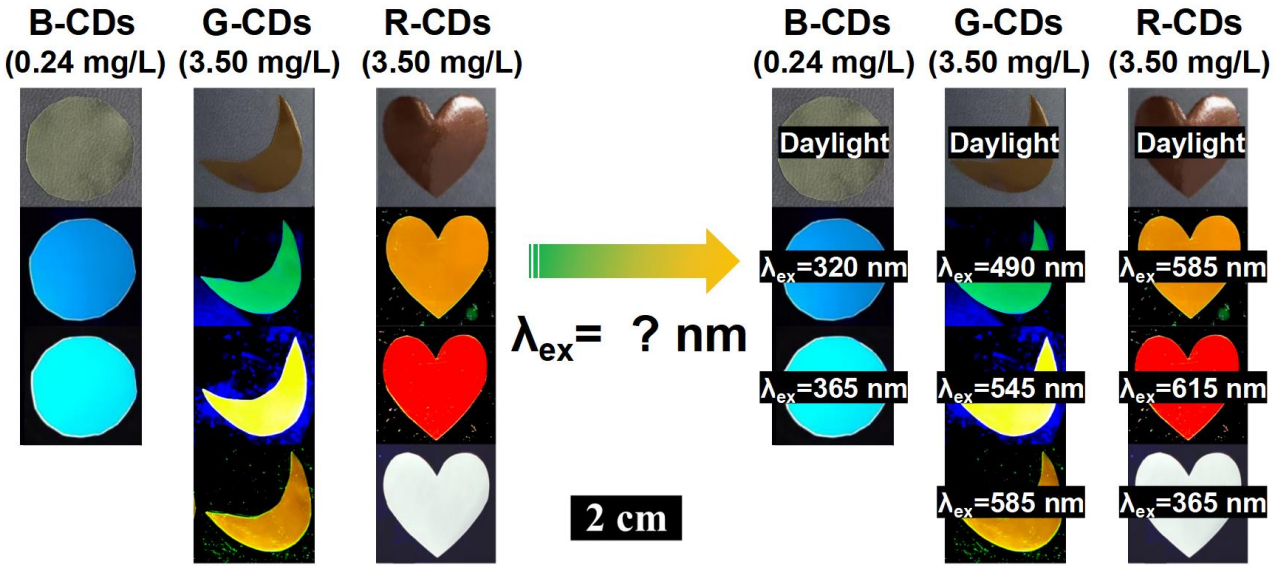

Fig. S6. Digital photographs of polyvinyl alcohol (PVA) films containing CDs under daylight and different excitation wavelengths.

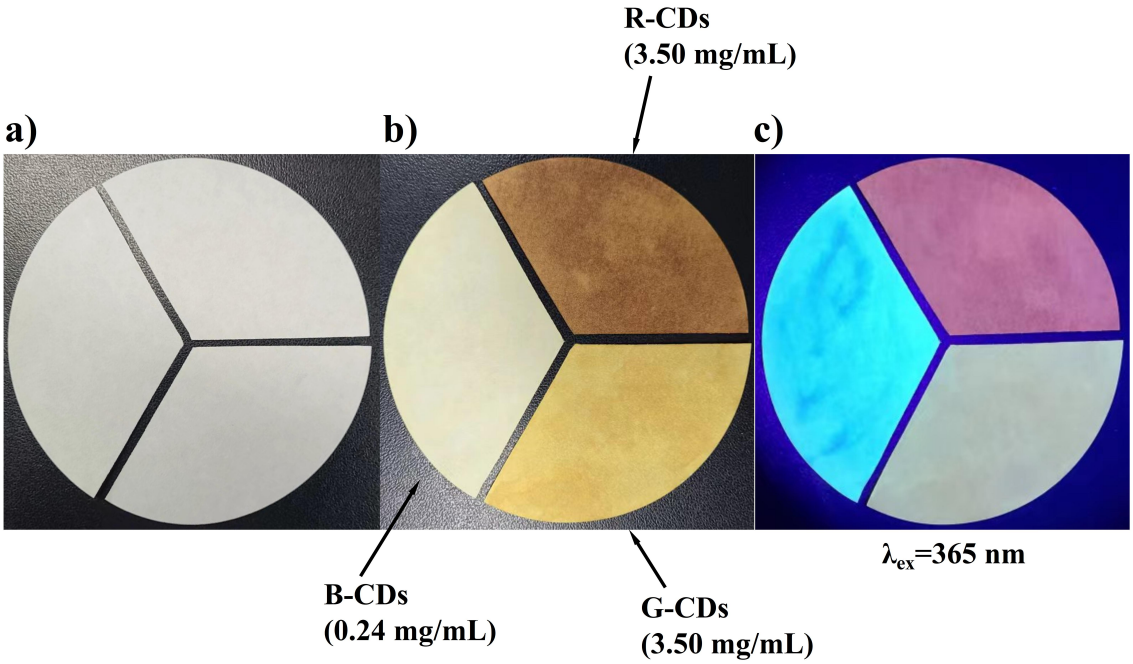

Fig. S7. Digital photographs of blank filter paper (a) and filter paper coated with CDs solution under (b) daylight and (c) λ_{ex} = 365 nm.

5. Thermal Properties

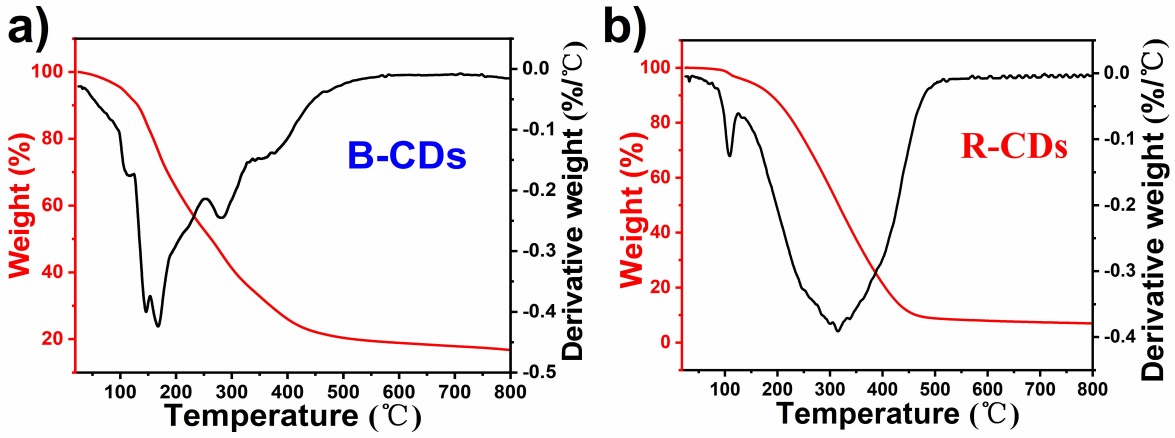

Fig. S8. TG (red) and DTG (black) analysis of (a) solid B-CDs and (b) R-CDs.

6. Photosynthetic Activity Measurements

6.1 Isolation of Chloroplasts

Due to the similarity in structure and function of chloroplasts among different types of plants[3], chloroplasts were isolated from a fresh lettuce (purchased from supermarkets) as models to study the light conversion of CDs on photosynthesis and the morphology of CDs@Chl. Isolation of chloroplast from the lettuce was carried out using gradient centrifugation method[4]. Fresh and cleaned lettuce leaves (20 g) and 30 mL of precooled sucrose buffer (0.4 M/L sucrose, 10 mM/L KCl, 30 mM/L $Na_2HPO_4 \cdot 12H_2O$, 20 mM/L KH_2PO_4, pH = 6.63) were added to an electric mixer and ground into a slurry, which was then filtered through four layers of gauze. Subsequently, the filtrate was centrifuged at 1000 rpm for 3 minutes, and the obtained supernatant was further centrifuged at 3000 rpm for 3 minutes. The precipitate was collected and resuspended in the aforementioned sucrose buffer to obtain chloroplast suspension. All the above operations were carried out in the dark at 0-4 ℃, and chloroplast suspension was stored in a refrigerator at 4 ℃ for subsequent use. The concentration of chlorophyll can be determined by recording the absorbance of the suspension at 652 nm in a mixed solution of acetone and water (8:2, v/v).[5] The concentration of chloroplast suspension represents the chlorophyll content. Via the method one can obtain a mixture of fragmented chloroplasts (i.e. thylakoids) and some intact chloroplasts.[3,4,6] The calculation equation for chlorophyll concentration (mg mL^{-1}) is as follows (2):[7]

$$C(\text{mg}\cdot\text{mL}^{-1}) = \frac{OD_{652}\times 50}{34.5} \qquad (2)$$

where C represents the chlorophyll concentration and OD_{652} is the absorbance at 652 nm. The initial concentration of chloroplast suspension extracted is 3.96 mg mL^{-1} in our study.

6.2 Preparation of CDs@Chl

Chloroplast suspension was mixed with CDs (B-CDs: 0.24 mg/mL or R-CDs: 3.50 mg/L, C_{Chl}:C_{CDs}=1:1) in the sucrose buffer solution and incubated continuously at 4 ℃ for 5 hours in the dark. Subsequently, the mixture was centrifuged at 4 ℃ and 3000 rpm for 3 minutes in order to remove uncombined CDs or chloroplasts and to collect the formed complexes (B-CDs@Chl, and R-CDs@Chl). Chloroplast complexes mixed with different proportions of B-CDs and R-CDs were obtained by adjusting the volume ratio of B-CDs@Chl and R-CDs@Chl. For example, BR-CDs(1:1)@Chl is $V_{B\text{-}CDs@Chl}$:$V_{R\text{-}CDs@Chl}$ = 1:1. The complexes were resuspended in sucrose buffer and stored in a refrigerator at 4 ℃. According to the reported methods,[8,9] SEM, TEM, and CLSM were used to observe the distribution of CDs in chloroplasts. The UV-Vis absorption spectra, the fluorescence emission spectra, and the fluorescence lifetime were also measured.

6.3 Hill Reaction

The Hill reaction was performed to investigate the effect of CDs on chloroplast photosynthetic activity using the reduction rates of DCPIP and potassium ferrocyanide. The Hill reaction was carried out according the reference.[3,6] The optimal concentration ratio in CDs@Chl was determined through preliminary experiments (B-CDs:Chl = 2:1, and R-CDs:Chl = 1:1). Firstly,

2,6-dichlorophenolindophenol (DCPIP, 60 μM) was added to both CDs and in the dark. The same operations were performed for CDs and individual chloroplasts. The obtained mixture was immediately irradiated under a xenon lamp (600 lux) at room temperature, and the absorbance at 600 nm was recorded at 1-minute intervals. The reduction experiment of potassium ferrocyanide is similar to DCPIP. The samples were mixed with potassium ferrocyanide (final concentration of 250 μM) in the dark at room temperature. After irradiation under a xenon lamp (3000 lux) for 2 minutes or a UV analyzer (365nm, 16W) for 5 minutes, the mixture was added with trichloroacetic acid (final concentration of 2%) to stop the reaction. Subsequently, after centrifugation at 3000 rpm for 3 minutes at 4 ℃, the supernatant was collected to measure the absorbance at 420 nm. Each of the above treatments is repeated three times to ensure the accuracy of the results. The decrease in DCPIP or potassium ferrocyanide reflects the Hill activity of chloroplasts. The absorbance variations of DCPIP and potassium ferrocyanide are calculated using the following formula (3):

$$\text{The absorbance variations of DCPIP or potassium ferrocyanide} = A_t - A_0 \quad (3)$$

where A_0 and A_t represent the absorbance of the mixture at 0- and t-minute, respectively. The standard curve (Fig. S26) is used to match the concentration of reduced DCPIP or potassium ferrocyanide.

6.4 Cultivation of Lettuce and Foliar Spraying of CDs

Lettuce seeds are disinfected for 20 minutes by using a 2 wt% sodium hypochlorite solution and rinsing with ultrapure water. Then, the seeds were placed on a germination tray and kept at a constant temperature of 30 ℃ in the darkness. After germination, the seedlings were transplanted into a 4 L hydroponic tank and transferred to a greenhouse (25 ℃, relative humidity of 70%, irradiation intensity of 205.875 μmol m^{-2} s^{-1}, 12 hours of illumination/12 hours of darkness, the spectrum of the light source is shown in Fig. S27), and the initial dosage of nutrient solution was commercial hongland nutrient solution with 30% concentration (Shanghai yuanye Bio-Technology Co., Ltd, Shanghai, China). During the cultivation period, seedlings need to change water every three days. Subsequently, lettuce seedlings of 7 days old with the same growth were selected and transplanted into four hydroponic tanks containing nutrient solution of normal concentration, with a density of six seedlings per pot. The nutrient solution also needs to be replaced every three days. On the second day after transplantation (three leaves and one heart), aqueous solutions (5 mL·pot^{-1}) of B-CDs, R-CDs, and BR-CDs (250 mg/L each for B-CDs and R-CDs) with a concentration of 500 mg/L were sprayed onto the leaves of lettuce seedlings, respectively. The operation of leaf spray needs to be carried out once a day for 15–20 days depending on the growth trend. The concentration of the mixed solution of B-CDs and R-CDs with different concentration ratios (Fig. S20) is also 500 mg/L [BR-CDs(1:5) = B-CDs(83.33 mg/L):R-CDs(416.67 mg/L), BR-CDs(1:2) = B-CDs(166.67 mg/L):R-CDs(333.33 mg/L), BR-CDs(1:1) = B-CDs(250.00 mg/L):R-CDs(250.00 mg/L), BR-CDs(2:1) = B-CDs(333.33 mg/L):R-CDs(166.67 mg/L), BR-CDs(5:1) = B-CDs(416.67 mg/L):R-CDs(83.33 mg/L)]. The plant height data of lettuce was measured after 7 days of application. During the spray, it should be proceeded evenly and slowly to avoid runoff and shake the lettuce leaves. Spraying ultrapure water was simultaneously done for the control group. The above experimental treatment was repeated three times. After the spraying experiment,

the biomass, plant height, root length, number of leaves, and leaf width of six lettuce plants in each treatment group were measured. The top two leaves of six lettuces from each treatment group were collected for measuring chlorophyll content. The leaves from the same position (the third leaf from the periphery to the inside) in each treatment group were selected for measurement of photosynthesis instrument, chlorophyll fluorescence imaging system, electron microscopy, confocal laser scanning microscope (CLSM), genes, soluble sugar content, soluble protein content, malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, Rubisco activity, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) content, etc. The measurements of the above relevant indexes were repeated three times.

6.5 Chlorophyll Fluorescence Measurement

Before testing, lettuces treated with CDs for 20 days were placed in darkness for 12 hours to balance the redox state of Photosystem II (PS II). Then, the leaves of each lettuce were collected for measurement of chlorophyll fluorescence parameters (Table S2) under photosynthetically available radiation (PAR) of 213 μmol m^{-2} s^{-1}. The six lettuces from each treatment group were all measured and 4 points from each leaf were selected to ensure accuracy. Furthermore, the lettuce leaves of the ultrapure water treatment group were subjected to a parallel testing. The measurement of the above parameters was repeated three times.

6.6 Observation of CDs@Chl and CDs in Lettuce Tissues

Before the test, CDs@Chl was stored in a dark environment at 4 ℃. The fluorescence images of the distribution of CDs in the CDs@Chl were observed by CLSM respectively, and the specific excitation wavelengths were detailed in the annotations of Fig. 4g. The entire measurements need to be completed as soon as possible to avoid inactivation of chloroplast. After 15 days of foliar spraying of CDs, the lettuces were harvested, and the root, stem, and leaf tissues of lettuce from each treatment group were selected for frozen sections treatment. Three sections of each tissue were prepared for ensuring the accuracy of the data CDs absorption and translocation across lettuce tissues was monitored. The specific excitation wavelengths are shown in Fig. S16–19.

6.7 Determination of Photosynthetic Pigments

Any book related to plant physiology measurement can be referenced for the measurement of photosynthetic pigment content.[10] The surface of 0.5 g fresh lettuce leaves was wiped clean, then cut into small pieces (removing the midrib), and 3 samples were prepared for each treatment. Fresh samples were placed in agate mortar, then a small amount of quartz sand, calcium carbonate powder, and 3 ml ethanol solution (95%) were added to grind into a homogeneous slurry. Subsequently, 10 ml of ethanol solution (95%) was added to the homogenate, further ground until the tissue turned white, and then let stand for 3–5 minutes. The extracted solution was filtered into a 25 mL brown volumetric flask. For the accuracy of the results, the mortar, grinding rod, filter paper and residue used in the experiment were washed with 95% ethanol solution until there was no green color. Finally, the volumetric flask was filled to 25 mL with 95% ethanol solution, shaken well and poured into a cuvette with a light path of 10 mm. The absorbance of pigments was

measured using a UV-Vis spectrophotometer at 470, 649, and 665 nm, respectively. The formula for calculating pigment content is as follows:

$$\text{Chlorophyll a (mg/L)} = 13.95\ A_{665} - 6.88\ A_{649} \quad (4)$$

$$\text{Chlorophyll b (mg/L)} = 24.96\ A_{649} - 7.32\ A_{665} \quad (5)$$

$$\text{Carotenoid (mg/L)} = (1000\ A_{470} - 2.05\ C_a - 114.8\ C_b)/245 \quad (6)$$

$$\text{Total chlorophyll content (mg/L)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (7)$$

where A_{470}, A_{649} and A_{665} represent the absorbance of the extraction solution at 470, 649 and 665 nm, respectively.

6.8 Plant Physiology Measurement

Rubisco activity was measured using the Rubisco activity detection kit (cat no. UPLC-MS-4263, UPLC-MS, China). The contents of ATP and NADPH were measured using ATP assay kit (cat no. UPLC-MS-4576, UPLC-MS, China) and $NADP^+$/NADPH assay kit (WST-8 method) (cat no. S0179, Beyotime, China), respectively. The soluble sugar and protein content were measured using a plant soluble sugar content detection kit (cat no. BC0030-50T/48S, Solarbio, China) and a plant soluble protein content detection kit (cat no. BC3180-50T/48S, Solarbio, China), respectively. All reagents, samples, and standards were prepared strictly according to the manufacturer's protocol, and the calculation of the results was carried out according the provided reference.

6.9 Quantitative Real-Time (qRT) PCR Analysis

The gene expression level of psbA was determined by qRT-Polymerase Chain Reaction (PCR) analysis using M-Actin as the internal reference. The gene sequences were retrieved from the National Center for Biotechnology Information (NCBI) and primers were designed using Primer Premier software version 5 (Table S4). The six lettuce leaves from each treatment group were divided into three groups, which frozen in liquid nitrogen immediately and stored in a refrigerator of -80 °C. According to the manufacturer's protocol, the RNArep Pure Plant total RNA extraction kit (cat no. TSP411, Tsingke Biology, China) and the reverse transcription kit (cat no. Superscript III, Invitrogen, US) were used to extract total RNA from these lettuce leaves and perform reverse transcription into cDNA, respectively. Then, the qRT-PCR analysis was performed using the standard protocol on a fluorescence quantitative PCR instrument (cat no. StepOne Software, Applied Biosystems, US). Finally, the $2^{-\Delta\Delta C}$T method was applied to calculate the relative gene expression levels.[8] Lettuce leaves sprayed with ultrapure water were used as a control.

7. Phytotoxicity Measurements

The content of MDA was determined using the Gunderson method. The method of reducing nitroblue tetrazolium (NBT) was used to determine SOD activity.[6,11,12] Three repeated measurements were applied to each treatment.

8. Cell Counting Kit-8 (CCK-8) Assay for the Cell Cytotoxicity

A 100 μL suspension of HeLa cells was seeded into a 96-well plate and then incubated in a 5% CO_2 atmosphere (37 °C) for 24 hours. Subsequently, The cells were exposed to the another 100 μL culture medium that contained same concentration CDs (B-CDs=R-CDs=BR-CDs: 100 μg/mL) for 24 h after the removal of original culture medium. Herein, the concentration of BR-CDs used is B-CDs (50 μg/mL) and R-CDs (50 μg/mL). Then, the culture medium was discarded, and the cells were washed three times with Dulbecco's Phosphate-Buffered Saline (DPBS). 100 μL of culture medium containing 10 μL of CCK-8 (the addition of CCK-8 was performed rapidly) was added to each well, followed by incubation for 2 hours. Finally, the absorbance of each well was measured at a wavelength of 450 nm using a microplate reader. Cell viability was measured using a CCK-8 (cat no. PH1759-500T, Phygene, China). A control group was set up without the addition of CDs.[13]

9. Statistical Analysis

The measurement data presented in this article were represented as mean ± standard deviation (SD), and each treatment was repeated three times. One-way analysis of variance (ANOVA) and Duncan multiple comparison test ($p<0.05$) were used to determine statistical significance using IBM SPSS Statistics 20.

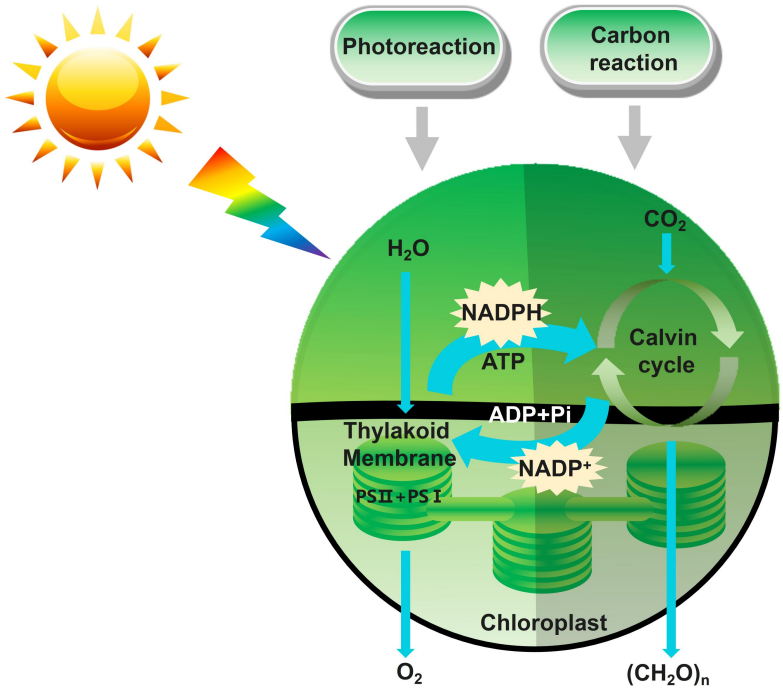

Fig. S9. The photoreaction and carbon reaction of chloroplast photosynthesis. PS I: photosystem I, PS II: photosystem II.

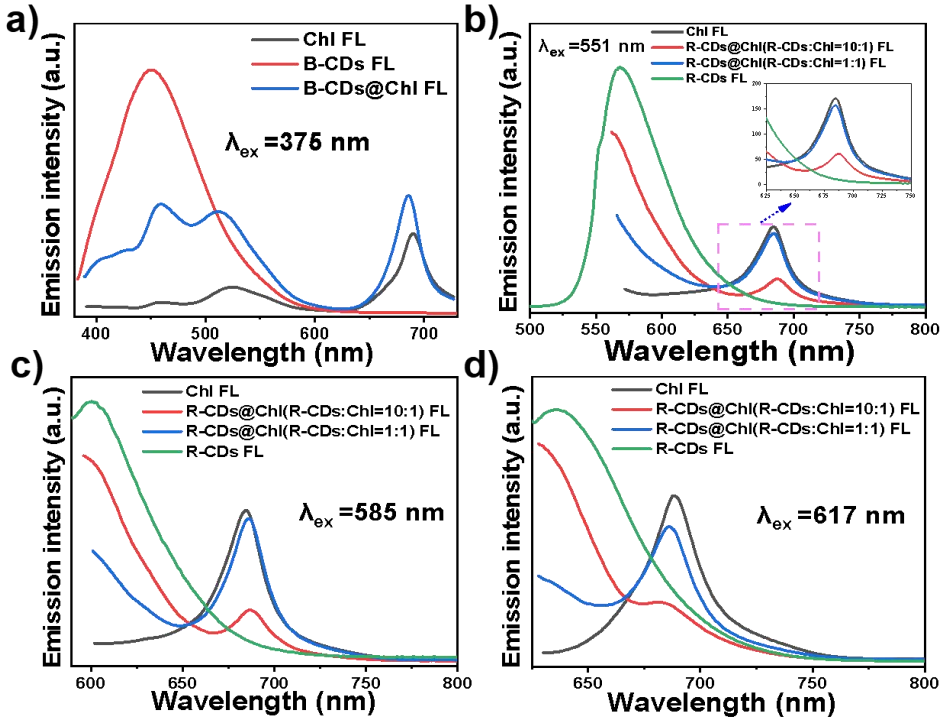

Fig. S10. (a) The emission spectra of individual chloroplast, B-CDs (0.24 mg/mL) in aqueous solution, and B-CDs@Chl (λ_{ex} = 375 nm). (b, c, d) The emission spectra of individual chloroplast, R-CDs (3.50 mg/mL) in acetone solution, and R-CDs@Chl (λ_{ex} = 551/585/617 nm). Chloroplast and CDs@Chl were measured in sucrose buffer solution.

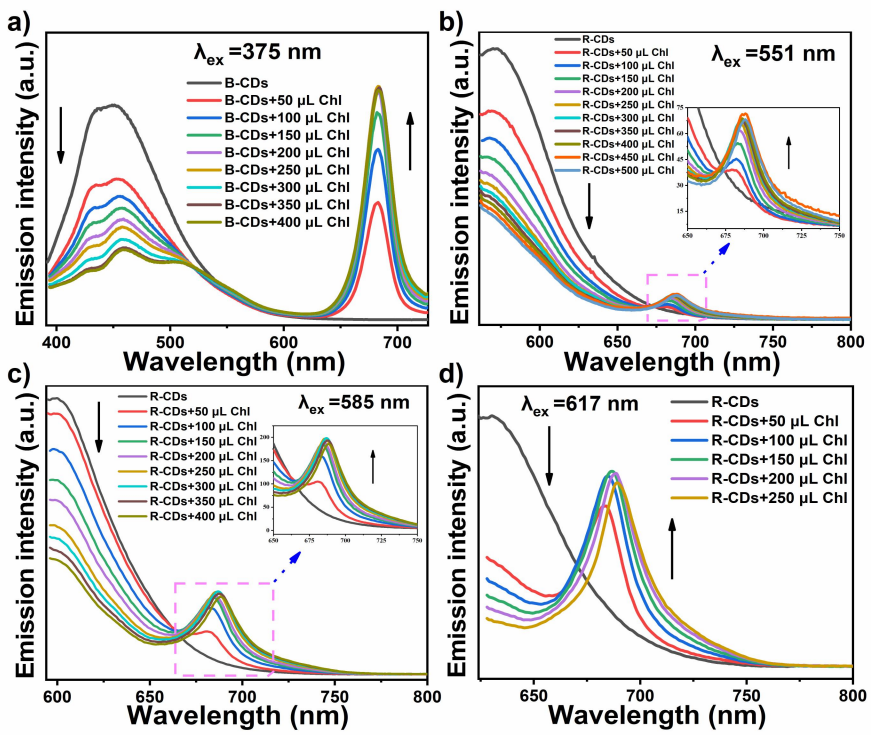

Fig. S11. (a) The fluorescence emission spectra of B-CDs (0.24 mg/mL) in sucrose buffer solution with chloroplast added dropwise at λ_{ex} = 375 nm. The fluorescence emission spectra of R-CDs (3.50 mg/mL) in sucrose buffer solution with chloroplast added dropwise at (b) λ_{ex} = 551 nm, (c) λ_{ex} = 585 nm and (d) λ_{ex} = 617 nm. The sucrose buffer solution of individual CDs was set as the control.

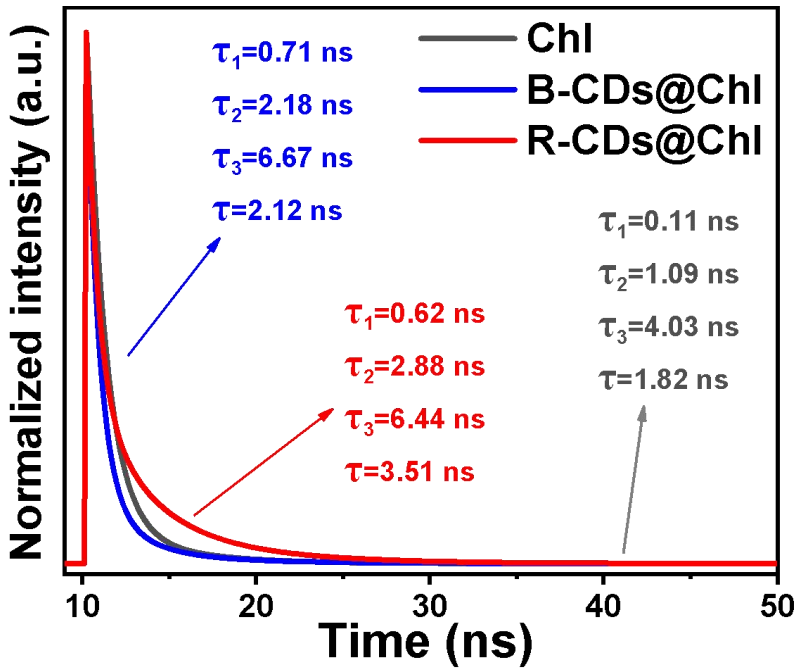

Fig. S12. The fluorescence lifetimes of individual chloroplasts (gray), B-CDs@Chl (0.24 mg/mL, blue), and R-CDs@Chl (3.50 mg/mL, red). The above combinations are all dissolved in sucrose buffer solution.

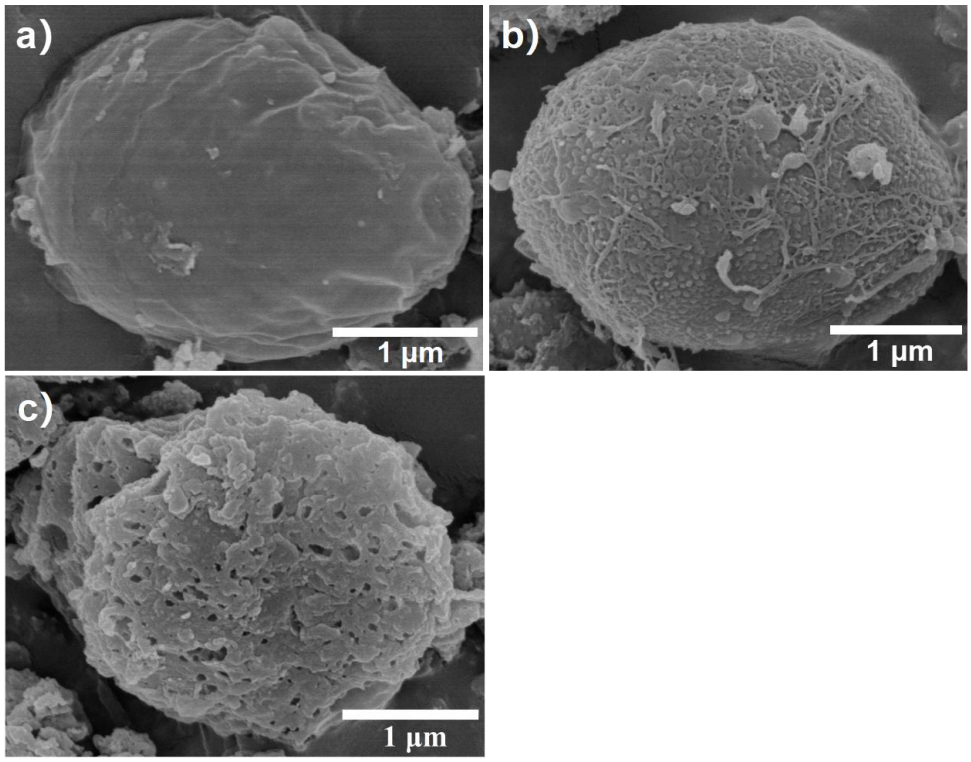

Fig. S13. SEM images of (a) untreated chloroplast, (b) B-CDs@Chl, and (c) R-CDs@Chl.

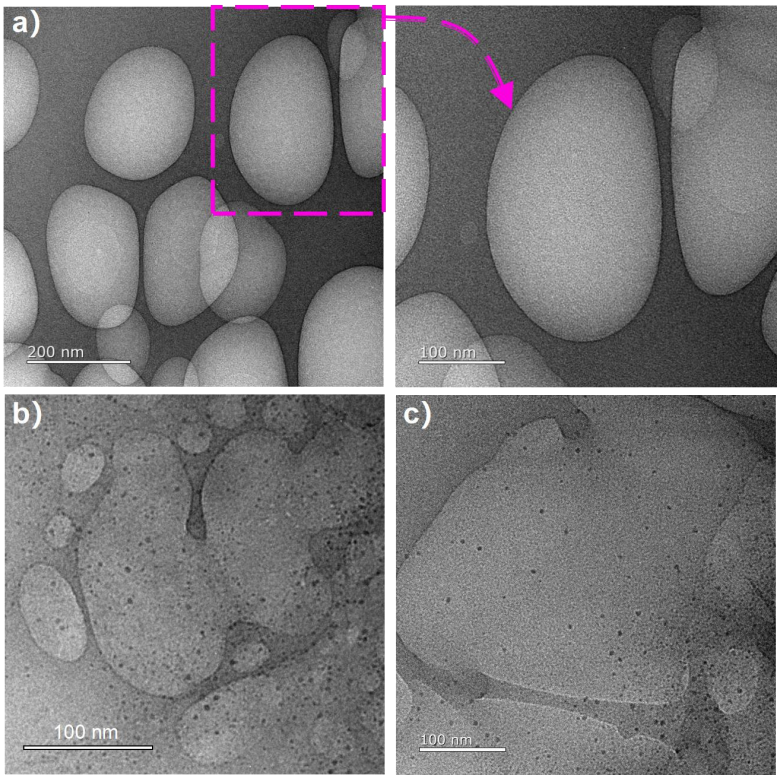

Fig. S14. (a) TEM images of untreated chloroplasts, and high-resolution TEM images of chloroplasts treated with (b) B-CDs, and (c) R-CDs.

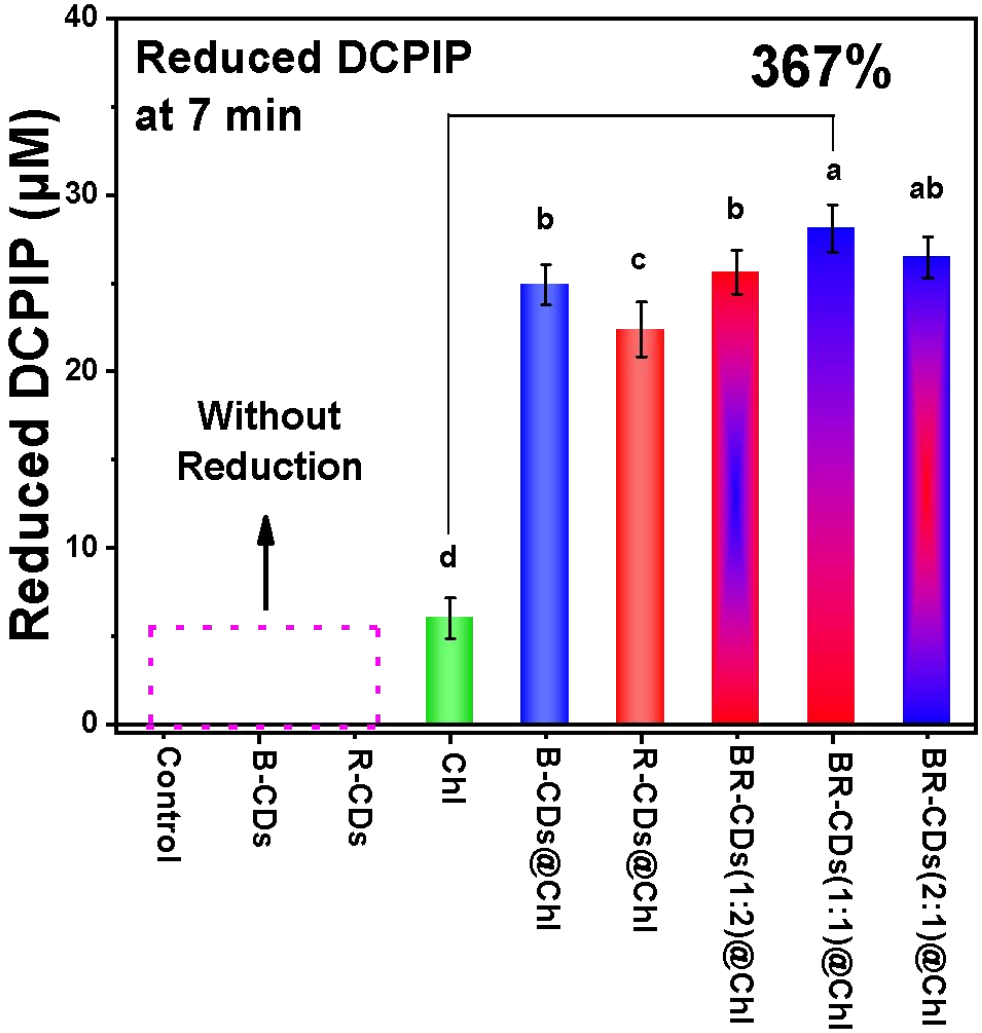

Fig. S15. The effect of bio-optical hybrid photosynthesis systems with different proportions of B-CDs and R-CDs on chloroplast photosynthetic activity. The error bars correspond to the standard deviation (n = 3). Different lowercase letters indicate significant differences among the treatments, based on the Duncan test ($p < 0.05$).

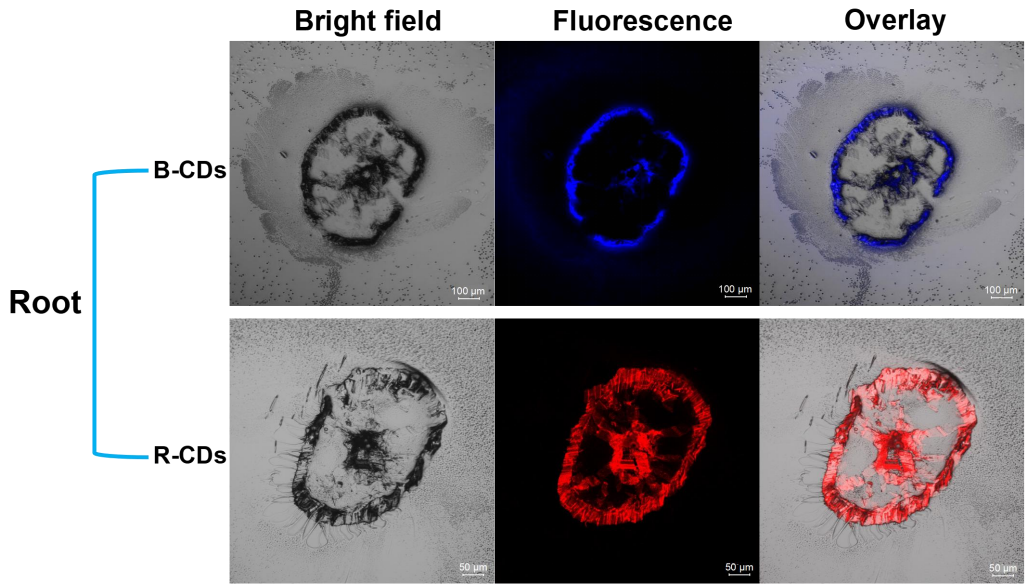

Fig. S16. Confocal images of lettuce roots after 15 days of foliar spraying with CDs. The confocal images of B-CDs were collected at $\lambda_{ex/em}$ = 375/455 nm. The confocal images of R-CDs were collected at $\lambda_{ex/em}$ = 617/640 nm. The roots of lettuce are processed by frozen sections treatment horizontally.

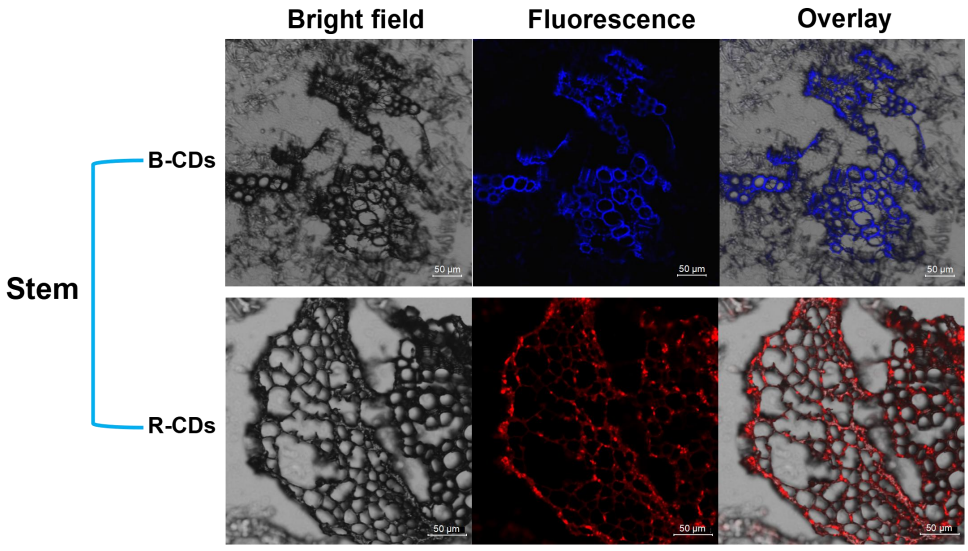

Fig. S17. Confocal images of lettuce stems after 15 days of foliar spraying with CDs. The confocal images of B-CDs were collected at $\lambda_{ex/em}$ = 375/455 nm. The confocal images of R-CDs were collected at $\lambda_{ex/em}$ = 617/640 nm. The stems of lettuce are processed by frozen sections treatment horizontally.

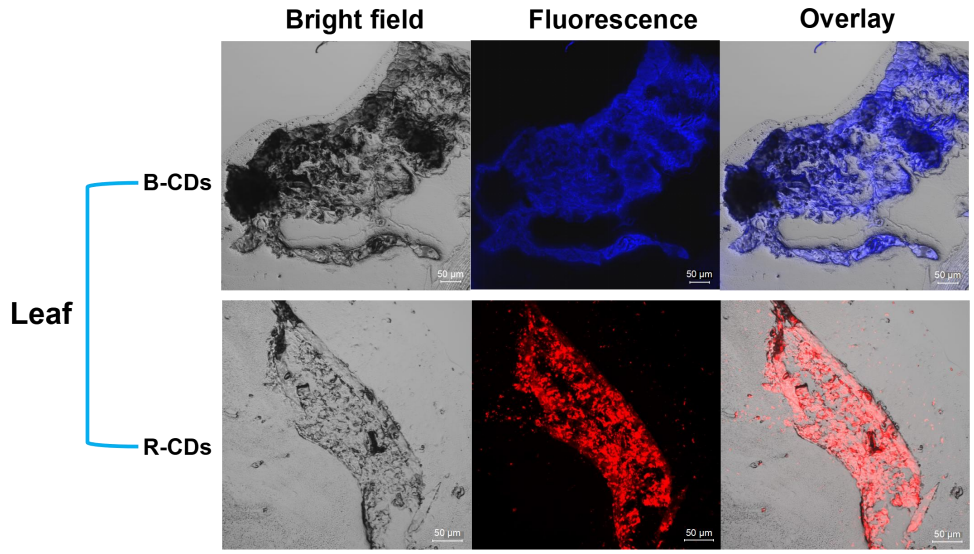

Fig. S18. Confocal images of lettuce leaves after 15 days of foliar spraying with CDs. The confocal images of B-CDs were collected at $\lambda_{ex/em}$ = 375/455 nm. The confocal images of R-CDs were collected at $\lambda_{ex/em}$ = 617/640 nm. The leaves of lettuce are processed by frozen sections treatment horizontally.

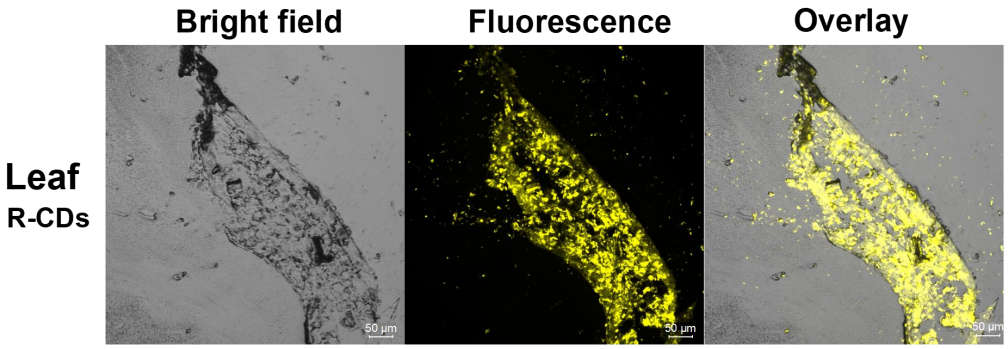

Fig. S19. Confocal images of lettuce leaves after 15 days of foliar spraying with R-CDs. The confocal images of R-CDs were collected at $\lambda_{ex/em}$ = 551/576 nm. The leaves of lettuce are processed by frozen sections treatment horizontally.

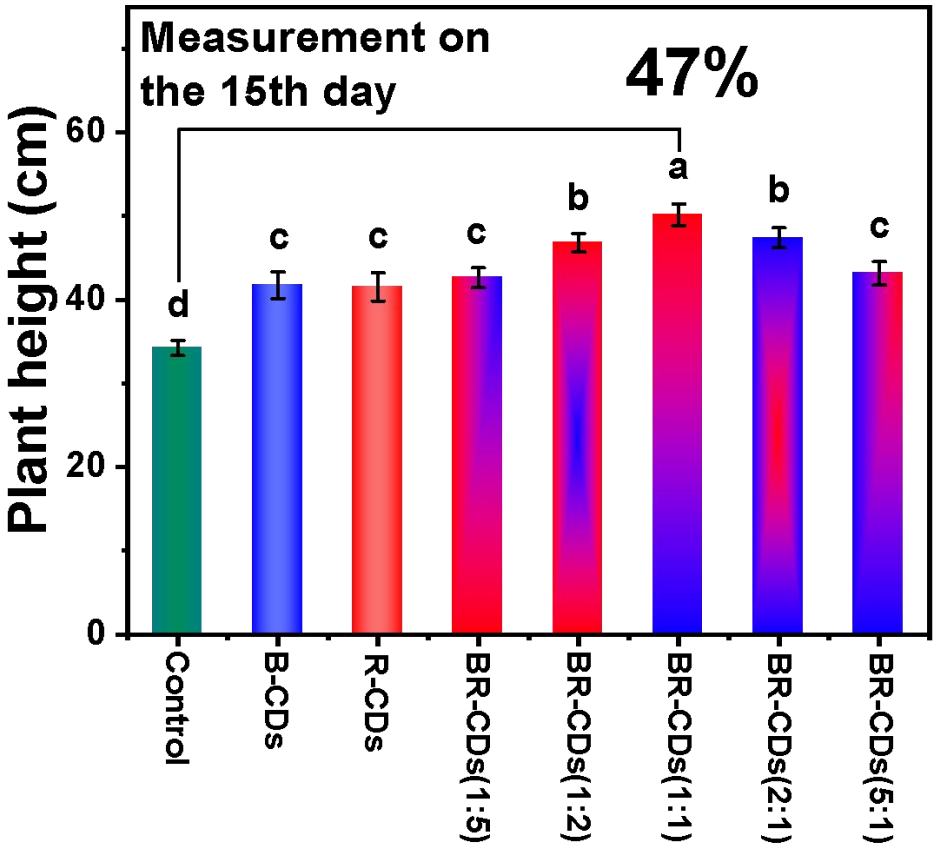

Fig. S20. The effect of spraying a mixed solution of B-CDs and R-CDs with different concentration ratios on lettuce plant height. The error bars correspond to the standard deviation (n = 3). Different lowercase letters indicate significant differences among the treatments, based on the Duncan test ($p < 0.05$).

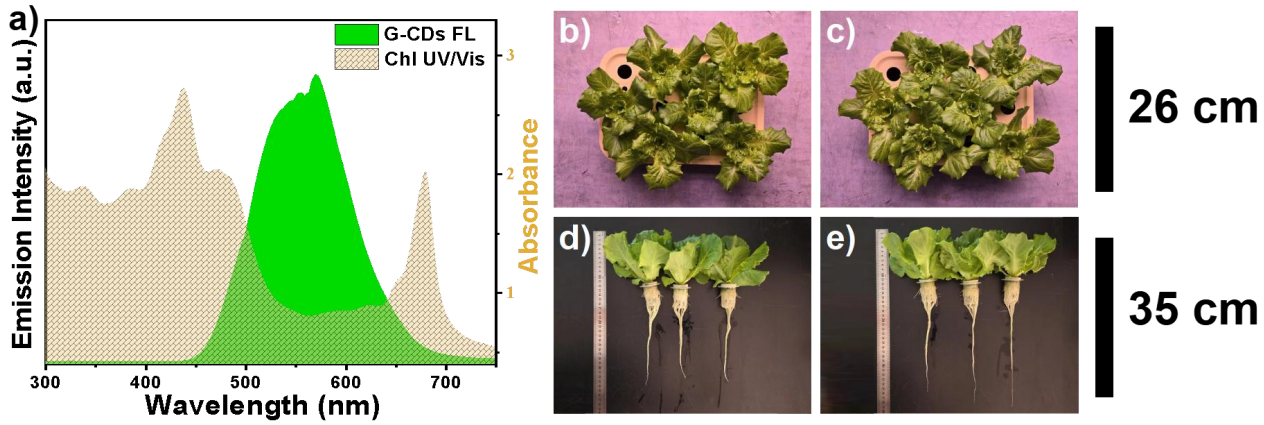

Fig. S21. The absorption spectrum of chloroplast is compared with the emission spectrum of (a) G-CDs (3.50 mg/mL) in ethanol or water. The effects of spraying (b) ultrapure water and (c) 500 mg/L of G-CDs aqueous solutions for 20 days on the growth of lettuce. The three lettuces in d and e were randomly selected.

Table S2. Definition of chlorophyll fluorescence parameters.

Parameter	Definition
Y (II)	The actual quantum yield of PS II
Y (NO)	Quantum yield of non-regulated non-photochemical energy loss in PS II.
Y (NPQ)	Quantum yield of regulated non-photochemical energy loss in PS II.
F_v/F_m	An important physiological parameter to describe the oxidative stress in plants, ranges between 0.8 and 0.84 in normal plant leaves.
NPQ	Non-photochemical quenching: estimates the rate constant for heat loss from PS II.
qP	Photochemical fluorescence quenching parameter estimating the fraction of open PS II centers based on a puddle model, reflecting the photosynthetic activity.
qL	Estimates the fraction of open PS II centers based on a lake model.
ETR	Electron transport rate in PS II.

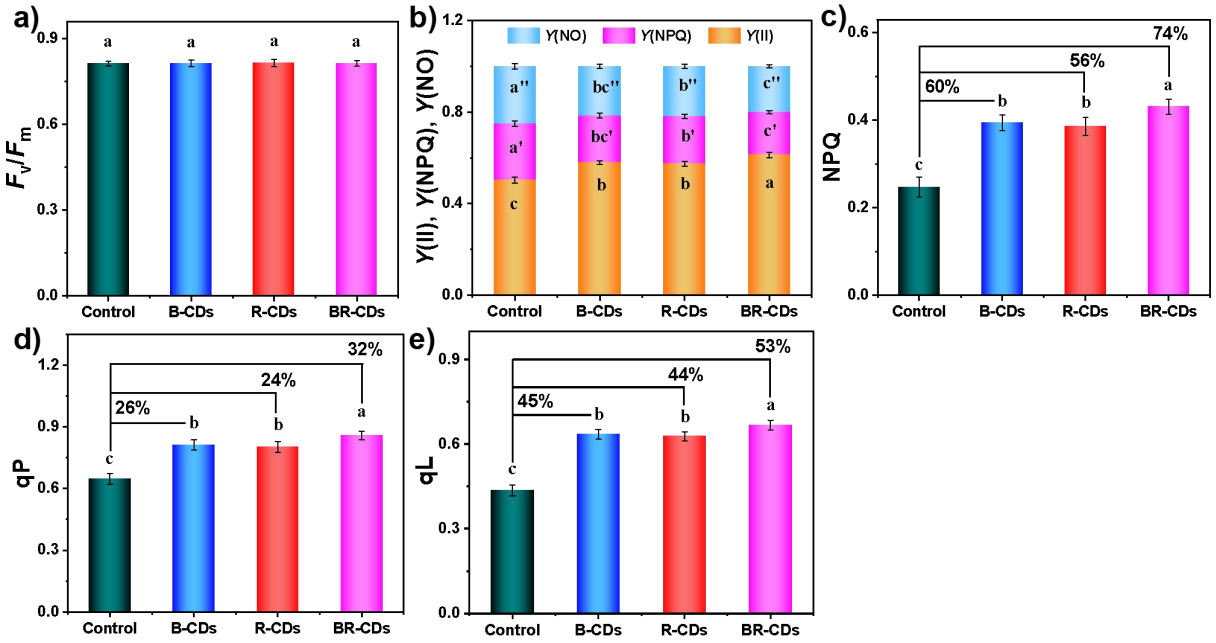

Fig. S22. The effects of ultrapure water and CDs on various chlorophyll fluorescence parameters of lettuce leaves. (a) The maximum photochemical efficiency of PS II (F_v/F_m), (b) the effective quantum yield of PS II [*Y* (II)], the quantum yield of the non-regulated energy dissipation in PS II [*Y* (NO)], and the quantum yield of the regulated energy dissipation in PS II [*Y* (NPQ)], where *Y* (II) + *Y* (NO) + *Y* (NPQ) = 1, (c) the non-photochemical quenching (NPQ), (d) the photochemical quenching based on the puddle model (qP), and (e) the lake model (qL). The error bars correspond to the standard deviation (n = 3). Different lowercase letters indicate significant differences among the treatments, based on the Duncan test ($p < 0.05$).

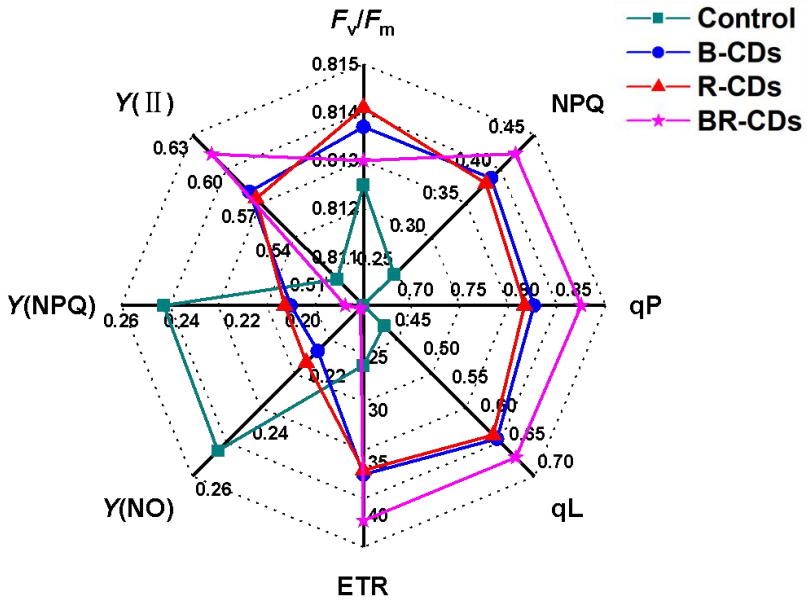

Fig. S23. The F_v/F_m, Y (II), Y (NO), Y (NPQ), NPQ, qP, qL and ETR of lettuce leaves in the control group and CDs treatment group under 213 μmol m^{-2} s^{-1}.

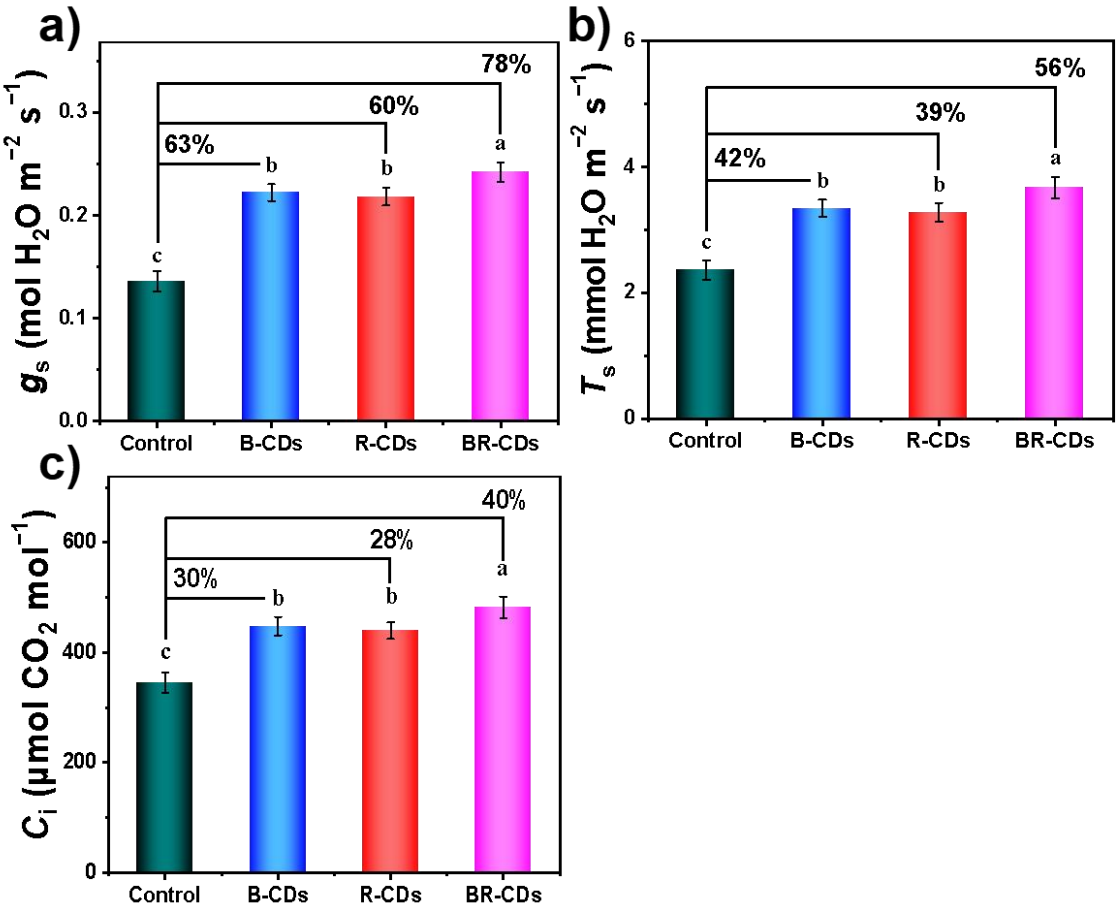

Fig. S24. The effects of spraying ultrapure water and the same concentration (500 mg/L) of B-CDs, R-CDs, and BR-CDs aqueous solutions on the (a) g_s, (b) T_s, and (c) C_i of lettuce. The PAR is 500 μmol m^{-2} s^{-1}. The error bars correspond to the standard deviation (n = 3). Different lowercase letters indicate significant differences among the treatments, based on the Duncan test ($p < 0.05$).

Table S3. Definition of photosynthetic indicators.

Parameter	Definition
P_n	It means the net amount of organic matter synthesized by plants per unit time after deducting the organic matter consumed by plant respiration from the organic matter produced during photosynthesis. It is an important indicator for describing plant growth and the degree of photosynthesis activity.
g_s	It represents the degree of stomatal opening, which affects photosynthesis, respiration, and transpiration of plant.
T_s	It represents the amount of water per unit leaf area that a plant transpires over a certain period of time.The normal process of transpiration is beneficial for plants to absorb and transport water passively, reduce their own temperature, and enhance CO_2 assimilation.
C_i	It is a parameter commonly used in the study of photosynthetic physiology and ecology. Generally, there is a positive correlation between C_i and net photosynthesis, with higher C_i indicating a higher photosynthetic rate.

Table S4. List of primers used for qRT-PCR amplification of selected genes.

Target Genes	Primer Sequence	
M-Actin	Forward	CATGAGGCCACGTACAACTC
	Reverse	TCATGGCAGTTCATGTATTG
psbA	Forward	TCGCTGCTCCTCCAGTAGAT
	Reverse	CGCATACCCAGACGGAAACT

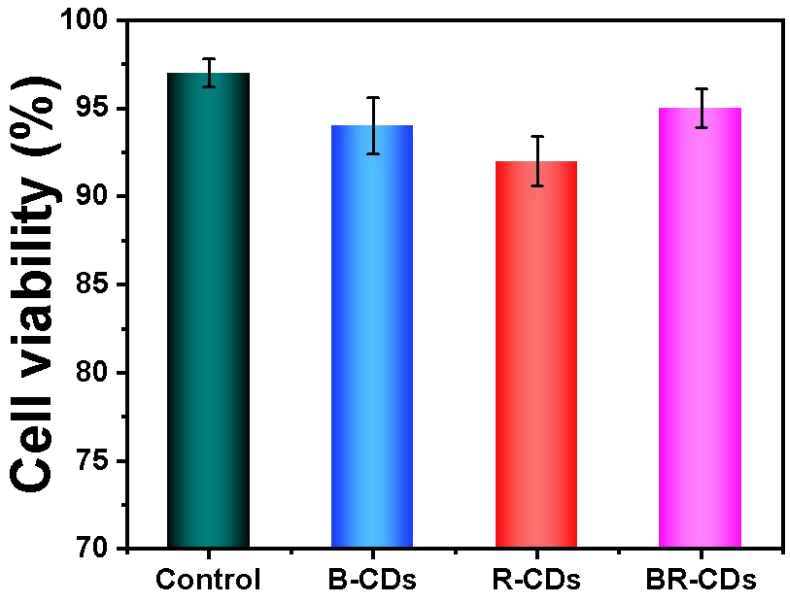

Fig. S25. The viability of HeLa cells after incubating with same concentration CDs (B-CDs=R-CDs=BR-CDs: 100 μg/mL) for 24 h. The error bars correspond to the standard deviation (n = 3).

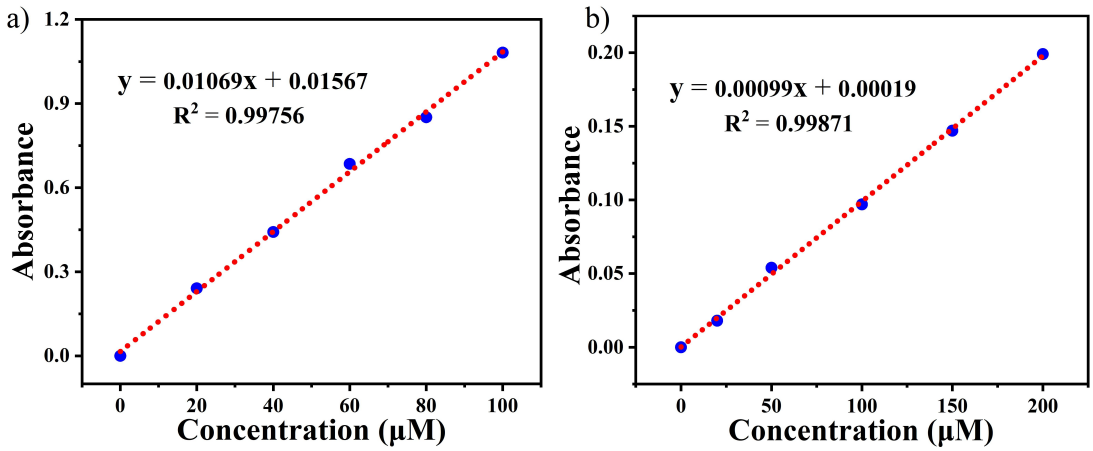

Fig. S26. (a) The relationship between the concentration and absorbance of DCPIP at λ_{ex} = 600 nm. (b) The relationship between the concentration and absorbance of potassium ferrocyanide at λ_{ex} = 420 nm.

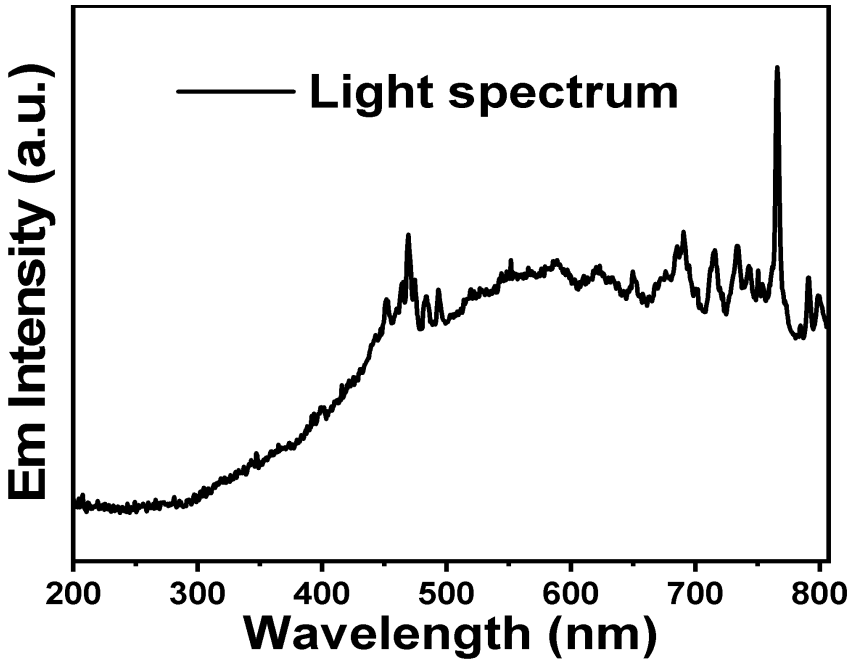

Fig. S27. The spectrum of light sources used in lettuce cultivation.

10. References

1. B. Yan, S. Zhang, W. Chen and Q. Cai, *Journal of analytical and applied pyrolysis*, 2018, **136**, 248-254.
2. S. Zhu, L. Wang, N. Zhou, X. Zhao, Y. Song, S. Maharjan, J. Zhang, L. Lu, H. Wang and B. Yang, *Chemical communications*, 2014, **50**, 13845-13848.
3. Y. Li, X. Pan, X. Xu, Y. Wu, J. Zhuang, X. Zhang, H. Zhang, B. Lei, C. Hu and Y. Liu, *Journal of Hazardous Materials*, 2021, **410**, 124534.
4. Y. Wang, S. Li, L. Liu, F. Lv and S. Wang, *Angewandte Chemie International Edition*, 2017, **56**, 5308-5311.
5. W. Zheng, N. Shan, L. Yu and X. Wang, *Dyes and Pigments*, 2008, **77**, 153-157.
6. W. Li, S. Wu, H. Zhang, X. Zhang, J. Zhuang, C. Hu, Y. Liu, B. Lei, L. Ma and X. Wang, *Advanced Functional Materials*, 2018, **28**, 1804004.
7. H. Yuan, L. Liu, F. Lv and S. Wang, *Chemical Communications*, 2013, **49**, 10685-10687.
8. Y. Li, X. Xu, B. Lei, J. Zhuang, X. Zhang, C. Hu, J. Cui and Y. Liu, *Chemical Engineering Journal*, 2021, **422**, 130114.
9. S. Chandra, S. Pradhan, S. Mitra, P. Patra, A. Bhattacharya, P. Pramanik and A. Goswami, *Nanoscale*, 2014, **6**, 3647-3655.
10. X Wang, Principles and techniques of plant physiology and biochemistry experiments. Higher Education Press, 2018, pp. 131-133.
11. H. K. Lichtenthaler and A. R. Wellburn, *Biochemical Society Transactions*, 1983, **11**, 591.
12. I. Durak, Z. Yurtarslanl, O. Canbolat and Ö. Akyol, *Clinica Chimica Acta*, 1993, **214**, 103-104.
13. S. Zhao, H. Wang, J. He, L. Dong, T. Xie, Y. Luo, J. Li, P. O. Lartey, K. Guo and J. Liu, *Aggregate*, 2024, e625.