Bioimaging Application and Growth-Promoting Behavior of Carbon Dots from Pollen on Hydroponically Cultivated Rome Lettuce

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Supporting Information

ABSTRACT: Carbon dots (CDs) obtained from rapeseed pollen with a high production yield, good biocompatibility, good water solubility, low cost, and simple synthesis are systematically characterized. They can be directly added to Hoagland nutrient solution for planting hydroponically cultivated *Lactuca sativa* L. to explore their influence on the plants at different concentrations. By measuring lettuce indices of growth, morphology, nutrition quality, gas exchange, and content of photosynthetic pigment, amazing growth-promotion effects of CDs were discovered, and the mechanism was analyzed. Moreover, the in vivo transport route of CDs in lettuce was evaluated by macroscopic and microscopic observations under UV light excitation. The results demonstrate that pollen-derived CDs can be potentially used as a miraculous fertilizer for agricultural applications and as a great in vivo plant bioimaging probe.

INTRODUCTION

Carbon nanomaterials (CNMs) have found uses in many fields, such as optical devices, superconductor materials, molecular switches (memory switch), quantum computers, and biomedical applications, because of their exceptional mechanical, thermal, optical, and electrical properties. Among these, studies of the fantastic effects of carbon nanotubes (CNTs), carbon nano-onions (CNOs), fullerenes, fullerols, and carbon nanohorns on plant growth have attracted increasing attention in recent years. Taking CNTs as an example, previous research found that tomato plants grown in soil supplemented with 50 μg/L multiwalled CNTs could produce 2 times more fruit than the control group. However, CNTs are not good candidates for this use because of the following shortcomings: high equipment cost coupled with a low yield, cumbersome synthesis steps such as high-temperature reaction, complicated purification with unavoidable heavy metal residues, insolubility in water before further modification, and so forth. These problems push the cost and practicability of using CNTs in agriculture to an unacceptable level. Moreover, increasing the soil carbon pool can enhance both crop productivity and yield stability, which is attributed to the significance of carbon for crop growth. Therefore, finding another CNM with great fertilizer efficiency is essential.

Carbon dots ( CDs) are one of the most promising CNMs, possessing superb properties including low toxicity, good biocompatibility, chemical inertness, good water solubility, low cost, widely available precursors, and eco-friendly preparation, and have inspired extensive research in a wide variety of fields including bioimaging, biosensing, sensors, security, photocatalysis, and optoelectronic devices; however, they have not been used in agriculture, yet they have tremendous potential advantages. First, their low toxicity, good biocompatibility, and eco-friendly preparation ensure safety for crops, the human body, and the environment. Second, the large-scale synthesis and application of CDs in plant cultivation are made possible because of their low cost and widely available precursors. Third, their stability and storability after being added into nutrient solution are guaranteed by their chemical inertness. As a zero-dimensional nanomaterial (particle size ≤ 100 nm) with good solubility in water, they could be absorbed easily by most crops since one-dimensional CNTs can be transported into the cell nucleus of *Arabidopsis*. Additionally, the fluorescence properties of CDs

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provide a tool to track their route after being absorbed by plants and to evaluate their mechanism of action.

Although Tripathi and Sarkar claimed positive effects of CDs on roots of wheat, the maximal particle size of the material they used was near 100 nm, almost beyond the recognized scope of CDs. Also, no emission spectrum was presented in their paper, and the 10 day cultivation period they used made their results extremely controversial. Thus, currently, no one has made acceptable use of CDs in agricultural studies.

Herein, as shown in Figure 1, CDs synthesized on a massive scale from rapeseed pollen by a hydrothermal method were directly added to Hoagland nutrient solution after being characterized. Afterward, Rome lettuce was cultivated hydroponically as a model to explore the influence of CDs on plants at different concentrations. Lettuce indices of growth, morphology, quality, gas exchange, and content of photosynthetic pigment were measured after the plants were harvested to explore the influence of the CDs. Moreover, the in vivo transport routes of the CDs in lettuce were also observed through macroscopic and microscopic observations under UV light excitation, and mechanisms are discussed based on the obtained data.

**RESULTS AND DISCUSSION**

The volume of the reaction system was increased from 400 to 800 mL. The particle size distribution of the CDs is shown in the transmission electron microscopy (TEM) image (Figure 2a,c). The mean diameter \(D_m\) was 5.2 nm (Figure 2c), which is larger than the reported result (1.9 nm). In addition, 52% of the CDs were between 3.1 and 5.1 nm. Clear lattice fringes with interplanar spacings of 0.20 nm were observed in the high-resolution TEM (HRTEM) image (Figure 2b), which demonstrate that these CDs possess a crystalline graphitized core. The individual X-ray photoelectron spectroscopy (XPS) spectrum for C 1s, N 1s, and O 1s binding energies (Figures 2d and S1a–c) revealed that three types of carbon (C−C, C−O/
C=N, and C=O), two types of nitrogen (C−N−C and N−H), and two types of oxygen (C=O and C−O) are present in the CDs. Fourier transform infrared (FTIR) spectroscopy demonstrated the presence of carbonyl, hydroxyl, and amino groups embedded in the CDs (Figure 2e). Specifically, characteristic peaks at 1662, 2976, 3405, and 3405 cm⁻¹ correspond to the stretching vibrations of C=O, C−H, O−H, and N−H, respectively, whereas peaks at 1052 and 1400 cm⁻¹ are attributed to the bending vibrations of C−N−C and N−H,¹²,¹³ which is consistent with the XPS result (Figure S1a-c).¹⁴–¹⁶

In addition, the transmittance of C=O was higher than that of N−H (Figure 2e) and the ratio of C=O was lower than that of C−O (Figure S1a), which conflicts with the original study, verifying the reduction of the carboxyl group. For this reason, the conjugative effect of C=O with aromatic carbon on the surface of the CDs was weakened, resulting in a lower intensity and blue shift of the absorption peak from 280 nm to approximately 270 nm (Figure S1e).¹⁷ Furthermore, the absorption peak is consistent with the typical absorption of an aromatic π system, similar to that of polycyclic aromatic hydrocarbons.¹⁸

The wavelength-dependent fluorescence emission spectra of the CDs are shown in Figure S1d; they exhibited tunable emission from 424 to 503 nm upon excitation from 340 to 440 nm. The optimal excitation wavelength was 360 nm, with the emission peaking at 432 nm. Notably, the intensity of the broad peak under 380 nm was close to the one under 360 nm, which gave proof of the uneven and larger particle size shown in the TEM image (Figure 2a). A larger size always accompanies a reduction in quantum confinement, leading to red shifts in the emission spectra.¹⁸ Consistent with the fluorescent spectrum, the dilute solution of CDs possessed a tawny color under sunlight and showed blue fluorescence under excitation at 365 nm (Figure S1e).

The quantum yield (QY) of the CDs upon excitation at 360 nm was calculated to be 7.7%, using quinine sulfate as the reference, which was a bit lower than the reported result (10.2% at 80 mL).¹³ Interestingly, by analyzing the XPS survey spectra (Figure S1), the C, N, and O contents of the CDs were determined to be 67.3, 7.6, and 25.1%, respectively, whereas they were previously found to be 66.3, 8.2, and 25.5%, respectively. It is clear that this reaction condition decreased the N content but did nothing to the C and O contents. Researchers have found that a larger nitrogen content in CDs leads to a more intense emission,¹⁸ which is the reason for the lower QY in this study.

After suction filtration using a mixed cellulose ester membrane (0.22 μm), the mean volume of the CD solution was 720 mL, with a mean concentration of 8.0 mg/mL. The production yield was 28.8%, close to the original result (30% at 40 mL), indicating that a consistent high yield was obtained after increasing the size of the reaction system. Moreover, the price of rapeseed pollen is only 0.01 dollars/g, which means that the price of the CDs is 0.03 dollars/g, solving the problem of the high cost of large-scale synthesis.

Overall, after enlarging the volume of the reaction system from 400 to 800 mL, the CDs used in this research still possessed the advantages of high production yield, low cost, and a simple synthesis process and they had a larger size and modest fluorescence properties, which means that they can meet the dose requirement of macroscale hydroponic culture. Additionally, synthesis from biomass without any heavy metal content endows these CDs with possible use for vegetable cultivation. More importantly, their excellent biocompatibility and water solubility with great stability have been demonstrated in previous studies.¹⁶,¹³ Thus, using these CDs for the hydroponic cultivation of vegetables is reasonable and feasible.

The growth behavior of lettuce cultivated with different concentrations of CDs was evaluated, and the results indicated that the biomass and leaf area of the lettuce obviously increased as the CD concentration increased (Figures 3 and S2). The best result was obtained for the 30 mg/L treated sample: the biomass of lettuce increased by 48.09% over that of untreated lettuce (0 mg/L), close to the growth rate of shoot fresh weight (FW) and dry weight (DW), whereas it was 25.19% at 10 mg/L (Figure S2l). There was no significant difference between 20 and 30 mg/L; the growth rate for the former was 41.98%. In summary, the increase of the leaf area and leaf number contributed to the promotion of shoot FW and DW together.

Most nutrient qualities were unaffected by the CDs; for example, there was no significant difference among the contents of ascorbic acid (AA) (Figure S3a), soluble sugar (SS) (Figure S3b), and soluble protein (SP) (Figure S3c). However, different CD concentrations remarkably affected the nitrate content (Figure 4a) of lettuce compared with that of the control group; for example, 20 mg/L CD-treated lettuce had the lowest nitrate content, which decreased by 33% . The transpiration rate (Figure 4b) and stomatal conductance (Figure 4c) had the same changing tendency; the trends of other photosynthesis indices (chlorophyll content, net photosynthetic rate, and intercellular CO₂ concentration) are presented in Figures S3 and S4. The pH value of the nutrient solution was controlled at 6.0−7.0; from Figure 4d,e, it can be seen that the CD concentration had no significant effect on the pH and electrical conductivity (EC) of the nutrient solution.

As mentioned above, the obtained CDs can efficiently emit blue light under excitation, which provides the possibility of utilizing the change in the CD emission intensity to monitor the transport systems in lettuce. To test this, 0 and 1 mg/mL CD-treated lettuce samples were measured (Figure S5). Compared with the control group where no fluorescence was exhibited, blue fluorescence was exhibited in the main leaf veins, stems, and especially roots of 1−1 (Figure S5a−c), the obvious fluorescence on the surface of the cells was arresting and looked like a heart (Figure 5d,e), which proved that the CDs were on the cell walls in the experimental group (Figure S5a−c).
5f,g; rip-cut sample). Moreover, breathtaking photos containing root cells with bright blue fluorescence at every pore were taken successfully. The lack of a vascular bundle around the root cells provided persuasive evidence for the transport and uptake of CDs within cells in the treated group compared with the control group. Similar observations were also found in the stems and main leaf veins (Figures S7 and S8). The inner slight fluorescence in cells indicated that the vascular bundle is the channel by which the CDs are transported.

The water in plants is transported through apoplast or cellular pathways, and the absorption kinetics is driven by hydrostatic pressure gradients. More than that, the uptake of anthropogenic organic chemicals, fullerols, fullerenes (C70), and CNOs by plant roots has been proven to be a passive process through the transpiration stream; thus, it is not surprising that greater transpiration leads to increased accumulation of them. Some specific indices varied widely in the Rome lettuce. In summary, shoot FW and DW, leaf width, leaf area, and leaf number showed a nearly linear positive correlation as the concentration of CDs increased from 10 to 30 mg/L (Figure S2), resulting in the best production yield at 30 mg/L. From this data, it is suggested that the addition of CDs could enlarge the leaf area by extending the leaf width and increase the leaf number significantly, which jointly contributed to the enlargement of shoot FW and DW. In addition, the variation trends of other indices did not fit the above data (Figures 4 and S3); that is, the reduction rate of the nitrate content and the growth rates of root DW, stomatal conductance (Gs), and transpiration rate (Tr) reached their maximum values at 20 mg/L. This similar variation trend of Tr and Gs was identical to that of previous studies; i.e., greater Gs generates faster Tr. As well, water bundles, which are responsible for the transportation of water and minerals from the bottom of the plant upward. Hence, most CDs gathered in vessels within main leaf veins, which could be verified because they exhibited the strongest blue light (Figures 5e,g, S7, and S8). Also, the existence of green light in this part, which was mostly caused by the excitation of intermediates from transformation or conjugation reactions with larger size and weaker quantum confinement CDs, strongly demonstrated the metabolism of CDs by lettuce. Some specific indices varied widely in the Rome lettuce. In summary, shoot FW and DW, leaf width, leaf area, and leaf number showed a nearly linear positive correlation as the concentration of CDs increased from 10 to 30 mg/L (Figure S2), resulting in the best production yield at 30 mg/L. From this data, it is suggested that the addition of CDs could enlarge the leaf area by extending the leaf width and increase the leaf number significantly, which jointly contributed to the enlargement of shoot FW and DW. In addition, the variation trends of other indices did not fit the above data (Figures 4 and S3); that is, the reduction rate of the nitrate content and the growth rates of root DW, stomatal conductance (Gs), and transpiration rate (Tr) reached their maximum values at 20 mg/L. This similar variation trend of Tr and Gs was identical to that of previous studies; i.e., greater Gs generates faster Tr. As well, water

Figure 4. Effects of different CD concentrations on the (a) nitrate content, (b) transpiration rate, (c) stomatal conductance, (d) pH, and (e) EC.

Figure 5. Macroscopic observations of shoots (a), roots (b), and plants (c) under UV light excitation. Microscopic observations of roots of control (d, e) and leaves (f, g) under UV−vis light excitation.
absorption would become more efficient through better growth of roots, accommodating the urgent requirement of high-strength Tr. At this point, exploring the reason that the CDs induce the growth of leaf width, leaf number, and Gs is critical. 

Nitrate content was used to being exploring the reason that CDs effect lettuce growth. It is known that nitrate is an important material for osmoregulation of lettuce and the nitrogen source both for Chl and protein of plants. The lack of an increase in the SS and Chl contents indicated that this was not a main contributor. The increase in the growth and morphological indices at 20 mg/L was not the most remarkable one; the greatest result was obtained at 30 mg/L with a higher nitrate content, affirming that it did not play a main role in these indices too. Moreover, the shoot water content was nearly the same, indicating that such a decrease could not be produced by the attenuation of water from greater transpiration. Now that the above possibilities regarding assimilation and dilution have been determined not to be involved, the possibility that osmotic pressure was increased by the addition of CDs was taken into consideration.

In similar research conducted using CNOs as the additive, the sustained and slow release of nutrient ions within the xylem was caused by interactions of surface groups, and these ions play a key role in the observed effects. Because CDs and CNOs both are zero-dimensional CNMs with spherical profiles and ample groups on their surfaces, the possibility of an analogous impact in the xylem by CDs deserves consideration. In this study, CDs possessing vast carboxyl, hydroxyl, and amino groups (Figure 2e) are able to trap nutrient ions by hydrogen bonding and electrostatic interactions for controlled release. When they are accumulated in the vessels of leaf main veins, CDs can release these ions persistently, resulting in increases in leaf width and leaf number with higher ion concentration in tissues. Furthermore, the aforementioned higher ion concentration may be the reason for the increased Gs because it is well-known that the concentration of ions, including K⁺ and Ca²⁺ which are abundant in Hoagland nutrient solution, has an immense impact on its state. For example, a moderate concentration of K⁺ in stomata guard cells would increase Gs, whereas an excess would decrease it. In other words, the proper dose of nutrient ions released from CDs promoted the Gs of lettuce, hence accelerating the transpiration stream containing nutrient ions, which facilitated lettuce growth. In addition, plants adjust their nitrate concentration to maintain their osmotic pressure, which might be destroyed by a high concentration of nutrient ions, presenting as a reduction of nitrate content. However, such ions were excessive for stomata guard cells at 30 mg/L, which led to a slight decrease of Tr by 11.8% (compared with that at 20 mg/L). The relatively faster growing rate (50%) in the relatively faster growing rate (50%) at 30 mg/L was not the most remarkable one; the greatest result was obtained at 30 mg/L with a higher nitrate content, affirming that it did not play a main role in these indices too. Moreover, the shoot water content was nearly the same, indicating that such a decrease could not be produced by the attenuation of water from greater transpiration. Now that the above possibilities regarding assimilation and dilution have been determined not to be involved, the possibility that osmotic pressure was increased by the addition of CDs was taken into consideration.

From Figure S6, CDs could be blamed for inhibition effects possibly related to soil salinity when they are used at high concentration. At a high concentration of 1 mg/mL, which is over 33 times that at 30 mg/L, lettuce could grow for 37 days at least, providing a superb demonstration of the excellent biocompatibility and water solubility of the CDs used in this work. Hence, making use of CDs in cultivation is worth approving.

From another perspective, it is well-known that AA is an important antioxidant component both for human health and vegetable growth. Nitrate can be restored into nitrite, which damages human health, whereas vegetables constitute its major dietary source. SS is not only important for the quality of lettuce but also for its taste. SP comprises functional proteins mostly in the form of enzymes, which also constitute one of the important indices of senescence in vegetables. CDs can reduce the nitrate content without affecting the other three indices; thus, its capacity to improve these quality indices should be recognized as well.

## CONCLUSIONS

In the present work, CDs were hydrothermally synthesized in a simple manner from rapeseed pollen with a high production yield and low cost (0.03 dollars/g). The obtained CDs showed great biocompatibility, good water solubility, larger size, and modest fluorescence properties. Rome lettuce was hydroponically cultivated with different concentrations of the obtained CDs, and the results showed that 20–30 mg/L CDs can increase the production yield strikingly and decrease the nitrate content. It was confirmed that the CDs are transported from nutrient solutions to vessels within the xylem of vascular bundles both by apoplast and cellular pathways along with the transpiration stream. This study indicates that CDs synthesized from rapeseed pollen can act as a perfect material for labeling cells in vivo, affecting plant physiology processes, and increasing plant yields.

## EXPERIMENTAL SECTION

### Hydrothermal Synthesis of CDs

With reference to the original method, 20 g of rapeseed pollen purchased from Taobao was dispersed in 800 mL of deionized water. After being sonicated for 5 min, it was transferred into a 1 L stainless steel autoclave (Xintai GSHA-1) and maintained at 200 °C for 24 h with a stirring rate of 100 rpm. Then, the CD solution was obtained after separating the impurities by vacuum filtration using a mixed cellulose ester membrane (0.22 μm pore size). Afterward, the solution was stored at 2 °C for further characterization and application.

### Hydroponic Cultivation of Rome Lettuce

Lettuce cultivation was carried out in 24 growing channels placed on 4 shelves of a stainless steel rack indoors (Figure S9), which means that there were 6 channels for each concentration of CDs. In fact, each light-emitting diode tube (Jinlei JL-T8PLA001) consisted of 192 lamp beads, with a 6:1 red light to blue light ratio; the peak positions of the red and blue light were 451 and 643 nm, respectively. The photoperiod was 14 h, and the total light intensity per channel was 150 μmol m⁻² s⁻¹. Every concentration contained three repeats with 36 lettuces in each group (Figure S9b,c). Half-strength Hoagland nutrient solution (50 L) with 0, 10, 20, or 30 mg/L CDs was transported to the lettuce for 30 min every 90 min by water pumps. During the first half of the hydroponic cultivation...
period, water was added every 2 days and nutrient solution was replaced every 6 days. Furthermore, the frequency was changed to every day and every 4 days in the second half of the cultivation period. 1 M HNO$_3$ solution and 1 M KOH solution were used to ensure that the pH of all nutrient solutions was between 6.0 and 7.0 for the regular growth of lettuce. The entire hydroponic cultivation time was 25 days, and the temperature was between 18.4 and 21.6 °C with the humidity from 54.0 to 68.6% indoors. All Rome lettuces possessed 5 leaves containing 1 heart leaf before cultivation.

Hydroponic cultivation at high concentration of CDs was carried out for the purpose of finding the fluorescence of CDs in lettuce easily. Hence, 4 lettuces were cultivated for 37 days in this test. After being properly cut out, 4 purified water bottles (555 mL) were used as growth devices. All bottles contained 500 mL of full-strength Hoagland nutrient solution, and two of them contained 1 mg/mL CDs. The control lettuces were named 0 and 1−1 and the experimental ones were named 1−1 and 1−2 (Figure S10). The nutrient solutions were replaced on the 17th day. The frequency of adding water was every 2 days in the first half of the hydroponic cultivation period, and this was changed to every day in the second half. The total light intensity was 95 mol m$^{-2}$ s$^{-1}$. The other conditions were the same as those in the above-described cultivation with a low concentration of CDs.

**Characterization of CDs.** HRTEM and TEM images of CDs were obtained with a JEOLO-2100F microscope operated at 200 kV. FTIR spectrum was measured by a Nicolet 6700 infrared detector (Thermo Fisher Scientific). The fluorescence spectrum was recorded on a HITACHIF F-7000 fluorescence spectrophotometer. UV−vis optical absorption spectrum was taken with a Shimadzu UV-2550 UV−vis spectrophotometer. The QY of the CDs was measured by a fluoroscope fluorescence spectrophotometer and UV−vis spectrophotometer. Photographs were taken with a mobile phone. XPS study was performed on a Kratos Amicus spectrometer equipped with conical anode Mg Kα radiation. Peak positions were internally referenced to the C 1s peak at 284.6 eV. The production yield of the CDs was measured with the help of weighting bottles (25 mm × 25 mm) and a Biocool FD-1A-50 freeze dryer.

**Characterization of Rome Lettuce.** Four lettuces in each repeat group were harvested randomly and wiped with tissues. The shoot FW, shoot and root DW, shoot water content, and specific leaf weight were measured. Shoot DW and root DW were obtained by desiccation in an oven at 75 °C for 3 days. By punching the third leaf (counting from the bottom up without 2 cotyledons) 5 times in each lettuce using a puncher with a radius of 3 mm, 20 wafers were obtained in each repeat group and dried in an oven at 75 °C for 2 days. After calculating the ratio of DW and area, the specific leaf weight was obtained.

For the morphology indices of lettuces of the above biomass indices, the number of leaves longer than 5 cm was counted (without 2 cotyledons). In addition, the leaf length, leaf width, and leaf area of the third leaves were measured by CAD 2016 after taking photos horizontally in the same place with the background of coordinate papers. The leaf length, leaf width, and leaf area of the 11th leaves (counting with the naturally deciduous leaves, without 2 cotyledons) of 4 lettuces with a high concentration of CDs were measured as replacements for the naturally deciduous fifth one.

Quality indices were determined using the third leaf; liquid nitrogen was used after washing, and the material was stored at −16 °C. All tests were measured by UV−vis spectrophotometer and repeated three times. In addition, specific steps are presented in the Supporting Information.

Molybdenum blue colorimetry was used to measure the content of AA with a slight modification.$^{39}$ SS content was obtained by modified anthrone colorimetry.$^{36}$ SP content was measured by Coomassie brilliant blue G-250 staining with a slight modification.$^{40}$ Nitrate content was evaluated by the modified method of Cataldo with a slight modification.$^{41}$

The photosynthetic pigment contents of the right middle parts of the fresh third leaf in each lettuce, containing Chl a, Chl b, TChl, and carotene, were extracted with 25 mL of 80% (v/v) acetone and measured by the method of Lichtenthaler.$^{42}$ The absorbance of the solutions was read at 470, 646, and 663 nm against the solvent blank (80% acetone) by using the UV−vis spectrophotometer. Contents of each pigment were calculated by equations listed in the Supporting Information.

All photosynthesis indices, Pn, Tr, Gs, and Ci, were measured by a PP Systems TDS-2 portable photosynthesis system on the last day of cultivation indoors. The tested parts were the right middle sections of the fifth leaf of 3 lettuces in every repeat group.

The pH and EC of the nutrient solution were tested by a HANNA Instruments HI98129 portable analyzer every 2 days when the pumps had worked for half an hour after adding water or replacing nutrient solution.

For fluorescence microscopy images, after harvest, the lettuces cultivated with a high concentration of CDs were washed with deionized water 5 times. Then, the roots, stems, and leaf veins of the 11th leaf were cut into slices and observed on a Leica DMS000 B fluorescence microscope both in bright field and UV (360 nm). All images were taken at 10 magnification with 6.15 and 64.0 ms of exposure time for bright field and UV, respectively. The exposure time was 3.89 ms for the stems in bright field.

**Data Statistics.** A Nano Measurer 1.2.5 was used to measure the particle size distribution and interplanar spacing of CDs. All of the data were subjected to one-way analysis of ANOVA and Duncan test at the a < 0.05 level by SPSS17.0 to calculate the standard deviation and significant difference, although the latter one could not be implemented at high concentration if there were fewer than 3 study groups. Bars labeled with the different letters are significantly different (a < 0.05) according to the Duncan test.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00657.

Methods for measuring ascorbic acid, soluble sugar, soluble protein, and nitrate content; equations for calculating photosynthetic pigment content; and analytical data (PDF)

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**Notes**

The authors declare no competing financial interest.
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