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Photosynthesis Enhancement in Maize via Nontoxic Orange Carbon Dots

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ABSTRACT: The sustained increase in leaf photosynthesis may increase crop yield. Due to many limitations, plants use much less photosynthetic capacity than is theoretically possible. Plant nanobionics investigates nanoparticle application in living plants, which improves certain plant functions. We synthesized and tested nontoxic orange carbon dots (o-CDs) for the photosynthetic efficiency increase in maize (*Zea mays* L.). We applied o-CDs foliarly or by adding to the growth solution. The photosynthetic parameters and content of photosynthetic pigments were recorded. The total phenolic content (TPC) and total antioxidant activity (TAA) were measured to monitor the plant antioxidant response to o-CDs. The photosynthetic parameters' values were higher for foliar than for solution application. The 1 mg/L o-CDs applied foliarly and 5 mg/L in solution increased photosynthetic parameters in leaves. The o-CDs raised photosynthetic pigments. The TAA and TPC results indicate reduced antioxidant activity in the plant organs more exposed to o-CDs, depending on the way of application.

KEYWORDS: *antioxidative activity, carbon dots, crop yield, maize, phenolic content, photosynthesis*

1. INTRODUCTION

Carbon dots (CDs) are a group of spherical carbon-based nanoparticles (NPs) with a diameter less than 10 nm.[1] Compared to other carbon-based nanomaterials, the discovery of CDs is relatively late; the concept "carbon dots" was first mentioned by Sun et al. (2006).[2] Due to many excellent physicochemical properties including the core−shell structure, ease of preparation, high photoluminescence (PL), water dispersity, biocompatibility, abundant and tunable surface functionalities, and nontoxicity,[3] CDs have been widely used in various applications where other NPs can or cannot be applied. For instance, considering the difference in the structural composition, CDs are a green alternative of the traditional metal-based quantum dots (QDs), and the lack of heavy metals endows CDs with nontoxicity, reliability, and a huge potential to serve multiple biomedical purposes such as bioimaging, sensing, and drug delivery.[4−6] Besides, CDs can be also applied as a promising polluted water remediation agents.[7] However, CDs also have some limitations such as a short PL emission wavelength, which triggers the rapid development of long-emissive CDs.[8,9] The long-emissive CDs can bring many benefits especially to bioimaging inside the biological matrix to avoid the autofluorescence generated from the biological structures.[10] Furthermore, the optical properties of CDs revealed by the ultraviolet−visible (UV/vis) absorption and fluorescence emission spectra suggest that CDs have high light-harvesting capability, which could be used to improve the photosynthesis efficiency of plants.

Due to many limitations, plants generally own a much lower photosynthetic capacity than they should theoretically, which is an evolutional property.[11] In actual sunlight, only 45% of the light is within the photosynthetically active wavelength range; thus, the theoretical maximum efficiency of solar energy conversion is approximately 11%. Besides, plants do not absorb all incoming sunlight and do not convert all harvested energy into biomass due to reflection, photorespiration, the need for optimal solar radiation levels, absorption of light by non-photosynthetic pigments, and photochemical inefficiency, which results in a maximum overall photosynthetic efficiency of 3−6%.[12] If photosynthesis is inefficient, excess light energy must be dissipated to avoid damages to the photosynthetic apparatus, in the form of heat (non-photochemical quenching) or chlorophyll fluorescence. On the contrary, an increase in the photosynthetic efficiency can lead to an increase in crop production, which may be a promising strategy for sustainable crop production that meets global food demands in the future without the need to increase the cultivatable land and cause damages to unique habitats and a decrease in biodiversity.[13]

It has been recently demonstrated that the use of NPs in plants is a promising methodology to transfer energy to photosynthetic reaction centers,[14] which will increase light absorption.[15] This has been confirmed by applying metal-based NPs,[16,17] which, however, poses a risk of metal toxicity. Meanwhile, organic NPs were explored to increase the photosynthetic capacity, either by applying NPs in *in vitro* systems on the isolated chloroplasts[18,19] or *in planta* (single-walled carbon nanotubes, SWNT),[16] but none of them could

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be visualized inside the plants due to the overlap of their PL emissions with the autofluorescence of plants. Herein, in this study, we synthesized a unique type of CDs, orange CDs (o-CDs) with citric acid and *o*-phenylenediamine (OPD) as precursors by an uncommon ultrasonication approach. Various physicochemical characterizations have been performed on the o-CDs. In our previous studies, they have exhibited an excitation-independent PL, yellow PL emission, moderate fluorescence quantum yield, amphiphilicity, small particle size (~2 nm), abundant surface fucntional groups such as −COOH and $-NH_2$, tunable surface functionality, and nontoxicity.[20] The emission range of o-CDs is partly overlapped with the absorption peaks of chlorophyll. Thus, in the interactions with chloroplasts, through emission, the o-CDs may pass the absorbed photons to the photosynthetic system and enhance the photosynthetic efficiency. Furthermore, the high water dispersity, small size, and amphiphilicity of the o-CDs facilitate their penetration into the cell wall pores and prevent their aggregation on the cell wall or their binding to the cell wall-building polymers. Having all these properties in mind, in this work, our aim was to study if the use of o-CDs may enhance the photosynthetic efficiency of maize, which served as a model plant and agricultural species. We applied o-CDs in two ways—by spraying them on the plant leaves (foliar treatment) or adding them to the hydroponic growth medium (solution treatment). To see if the o-CDs are translocated from the roots to the leaves in the hydroponic treatment, we observed their presence in leaves using fluorescence microscopy. We measured photosynthetic parameters [photosynthetic rate (PR), transpiration rate (TR), and water use efficiency (WUE)] and content of photosynthetic pigments, chlorophyll and carotenoids, in the leaves of treated plants. We analyzed the morphological and biochemical parameters (total antioxidant activity—TAA and total phenolic content—TPC) in the plants after the treatment, to investigate whether the o-CDs showed toxicity to plants. Also, most importantly, finding that these CDs have a photosynthesis-augmenting potential without harming plants opens new possibilities in their uses in agricultural applications, such as increasing the plant productivity.

2. MATERIALS AND METHODS

2.1. Materials. Citric acid (99.5−100%) was purchased from VWR (West Chester, PA, USA). The deionized (DI) water applied was purified using a Modulab 2020 water purification system bought from Continental Water System Corporation (San Antonio, TX, USA). It has a pH of 6.62 ± 0.30 at 25.0 ± 0.5 °C with a resistivity and surface tension of 18.2 MΩ·cm and 72.1 mN/m, respectively. The GE Healthcare Sephacryl S-300 (Uppsala, Sweden) was utilized in size exclusion chromatography (SEC) as the stationary matrix. OPD (99.5%), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), H_2O_2, horseradish peroxidase type II (HRP)−150−250 units per mg of solid, KH_2PO_4, Na_2CO_3, gallic acid, ascorbic acid, 2 N Folin−Ciocalteu reagent, and CH_3OH were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). In the end, all the chemicals were used without any further treatment.

2.2. Preparation of o-CDs. o-CDs were synthesized with 0.02 g of citric acid and 0.28 g of OPD as a carbon source and a N dopant, respectively with a molar ratio of 1:25. Initially, citric acid and OPD were completely dissolved in 10 mL of DI water mediated by 5 min of ultrasonication at a frequency of 42 kHz. The ultrasonication was further applied to sonicate the mixture for another 1 h at a frequency of 42 kHz under the protection of argon gas. An orange aqueous dispersion was obtained, which exhibited yellow PL under the excitation of UV light (365 nm). Subsequently, a series of purification procedures were followed that included the filtration of unreacted OPD with the help of an ice bath and SEC to remove small fluorophores. After 2 days of lyophilization, o-CDs were eventually acquired as an orange powder. Characterizations of the obtained o-CDs were described in our previous studies.[20,21]

2.3. Treatment of Maize with o-CDs. Maize seeds were germinated in DI water (sown on and covered with filter paper moistened with 10 mL of DI water) in Petri dishes. The o-CDs were applied in two ways: by adding to the hydroponic medium (solution treatment) and spraying on the leaves (foliar treatment). In solution treatment, after germination, 10 seedlings per each treatment (control, 1, 5, or 10 mg/L o-CDs) were transferred to the plastic vessels (17.5 cm height) containing 2.5 L of KNOP/2 medium[21] with 1, 5, or 10 mg/L o-CDs and were grown under 16/8 h photoperiod. The nutrient solution was aerated by bubbling and renewed weekly. After 9 and 14 days of growth in hydroponics, photosynthetic parameters (TR, WUE, and PR) were measured. On the 14th day of growth in hydroponics, pH was measured, the leaves and roots of 10 plants (two plants for one replicate) were collected, frozen in liquid nitrogen, and kept at −80 °C until determination of TPC, TAA, and pigments. Before freezing, the roots, stems, and leaves of a certain number of plants were cut into sections for fluorescence microscopy.

In the foliar treatment, seedlings were germinated and grown in a hydroponic medium without o-CDs as in the solution treatment. After 7 days of growth in hydroponics, one leaf from each treatment was sprayed with 1, 5, or 10 mg/L o-CDs. After 9 and 14 days of seedlings' transfer to hydroponics, photosynthetic parameters were measured. On the 14th day of growth in hydroponics, pH was measured, and the plants were collected for further analyses (TAA, TPC, and pigments) in the same way as in solution treatment.

2.4. Fluorescent Microscopy. Fluorescent microscopic images of plant sections were obtained using Axio Observer Z1 Mikroskop, with an AxioCamMR3 camera (8 bit per channel). The fresh sections of the roots, stems and leaves were deposited on glass plates during measurements. The same optics was used to record all the images to avoid chromatic aberrations. The excitation/emission wavelengths were 558/580 nm, respectively (DsRED filter combination). A series of images were captured for each sample.

2.5. Measurements of Photosynthetic Parameters. PR (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and TR (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) were measured using the LC pro + portable photosynthesis system (ADC Bioscientific Ltd.), whereas the WUE (μmol CO_2 $mmol^{-1}$ H_2O) was calculated as a ratio of PR/TR. Measurements were conducted between 9 and 13 hours, with four replicates on five different plants per each treatment. Light was set using the LCpro + light unit, which emitted photosynthetically active radiation at 1000 μmol photons$\cdot m^{-2} \cdot s^{-1}$. The air supply unit provided a constant rate of ambient air flow of 100 μmol/s. Temperature, humidity, and CO_2 concentrations were at ambient levels.

2.6. Determination of the Chlorophyll and Carotenoid Content. Extraction of frozen leaves was performed with 80% acetone (1:10 w/v). The obtained extracts were centrifuged at 10,000 rpm at room temperature, and then, the precipitates were re-extracted with acetone. Supernatants were pooled together and diluted using 80% acetone. The absorbance was measured at 663, 646, and 470 nm. Concentrations of chlorophylls *a* and *b* (Chl a and Chl b) and carotenoids (Car) were determined spectrophotometrically (2501 PC spectrophotometer "Shimadzu", Japan),[22] and the values were expressed in μg of pigment per g of fresh weight.

2.7. Extraction of Phenolic Compounds and TPC Determination. Phenolic extracts were obtained from roots and leaves of 10 plants (two per sample in five replicates) after foliar and solution treatments, which were separately homogenized in a mortar with liquid nitrogen. Homogenates were resuspended in 80% methanol in 1:10 (m/V) ratio and stirred at room temperature for 60 min. Extracts were centrifuged for 5 min at 10,000 rpm, and phenolics were obtained in the supernatant.

Folin−Ciocalteu's spectrophotometric procedure[23] was used for the determination of TPC in the samples. Phenolic extracts were mixed with Folin−Ciocalteu reagent in 1 mL total volume. After 3

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min, sodium carbonate solution was added, and the mixture was incubated at 25 °C for 60 min. The standard curve (0.1−2.0 mM) was constructed using gallic acid. Absorbance was read at 724 nm (2501 PC spectrophotometer, "Shimadzu", Japan), and the results were expressed as micromoles of gallic acid equivalents per gram of fresh weight.

2.8. Determination of TAA. TAA of the samples was measured using the ABTS/HRP end point method, according to a modified procedure described in the literature.[24] In brief, the reaction mixture contained 2 mM ABTS, 15 μM H_2O_2, 0.25 μM horse radish peroxidase (HRP) type II, and 20 μL of 80% methanol extract of the samples in 50 mM potassium phosphate buffer, pH 7.5, in 1 mL total volume. The assay was performed in five replicates per treatment at 25 °C. The reaction was monitored at 730 nm (2501 PC spectrophotometer "Shimadzu", Japan) until a steady absorbance was achieved, due to ABTS radical ($ABTS^{\bullet+}$) formation in the reaction with HRP. After adding methanolic plant extracts, the decrease in absorbance due to $ABTS^{\bullet+}$ depletion was used for the calculation of TAA from the standard curve obtained with ascorbic acid (0.1−1 mM) as a universal antioxidant. The TAA was expressed as micromoles of ascorbic acid equivalents per gram of fresh weight.

2.9. Statistical Analysis. Exploratory and data analyses were performed using IBM SPSS Statistics 20 software (IBM, USA). The raw data (TPC, TAA) and pigments' concentration in maize roots and leaves after foliar and solution treatments were used as input variables. The number of observations was $n = 5$ for each group. The two-way analysis of variance (ANOVA) for independent samples was applied to test the differences in TPC, TAA, and pigments' concentration measured in maize roots and leaves under different treatments. Post hoc inter-group comparisons of variables (between different treatments and control) were performed by the Bonferonni or Tamhane post hoc tests at the level of the significance $p < 0.05$. The three-way mixed ANOVA test was applied to evaluate the effect of o-CD concentration on three measured properties (PR, TR, and WUE) under two different modes of application at two time points. This means that between-subject factors were the o-CD concentration (four levels: control, 1, 5, and 10 mg/L) and application method (two levels: foliar or solution), and the within-subject factor was time (two time points: T_1 and T_2). The number of observations was $n = 20$ for each group. ANOVA tests were followed by an additional post hoc Tamhane test with a significance level of $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

There were no visible signs of damage to the plants induced by o-CDs during their applications in hydroponics.

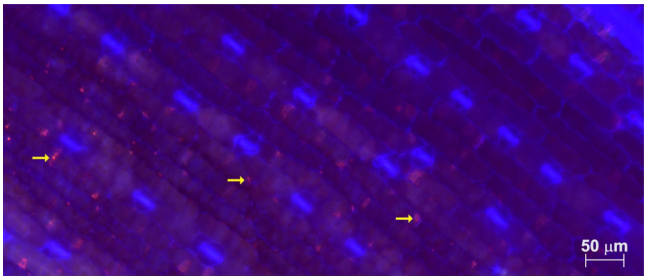

Figure 1. Localization of o-CDs (1 mg/L) in a part of the abaxial maize leaf surface after solution treatment, observed using fluorescence microscopy with the DsRed filter combination. Arrows show o-CDs.

3.1. Localization of o-CDs in Plants and Their Effect on Photosynthetic Parameters. The absorption, emission spectra (Supporting Information, Figure S1) and fluorescence quantum yield data (Supporting Information) are in accordance with the previously published data for o-CDs (Zhou et al. 2018)[21].

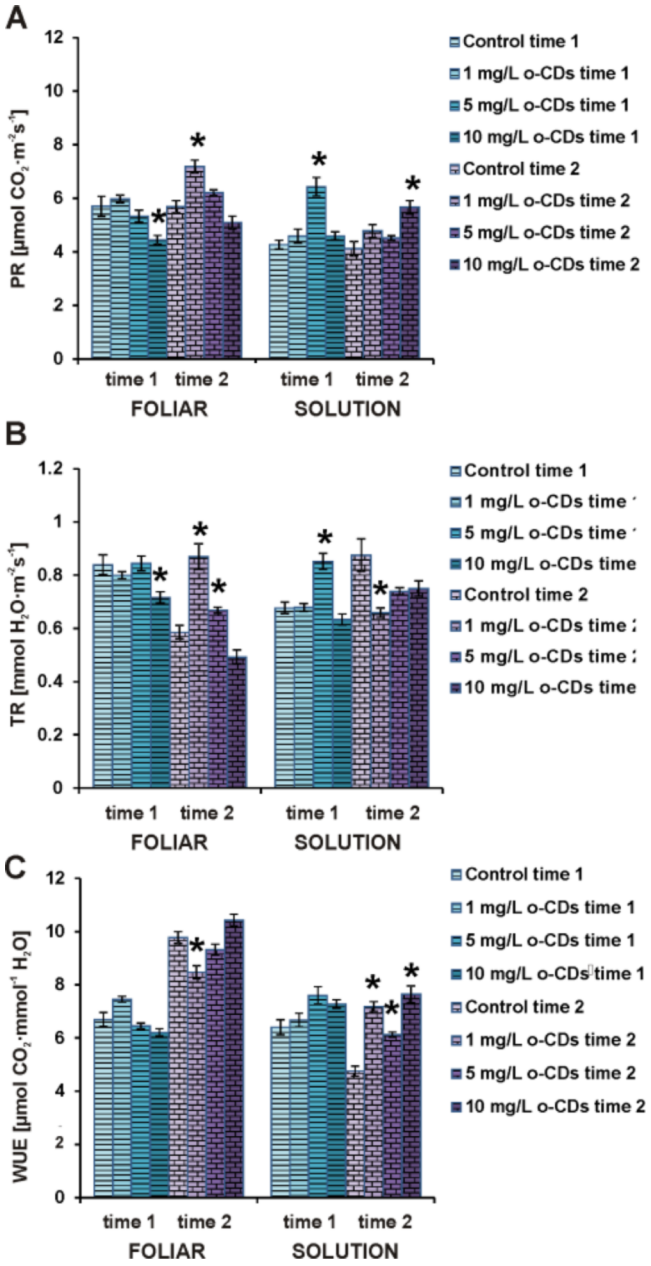

Figure 2. (A−C) PR, TR, and WUE, respectively, in the maize leaves after foliar and solution o-CD treatments, at two time points: 9 days (time 1—blue bars) and 14 days (time 2—purple bars) after transferring seedlings to the hydroponic solution. *—statistically significant difference in relation to the corresponding control.

The o-CDs have yellow fluorescence emission (Supporting Information, Figure S1), whose spectral region is distant from the range of plant autofluorescence. Thus, they could be observed in the maize sections using DsRed filter combination on the fluorescence microscope (Figure 1). The images show that in solution treatment, the o-CDs are visible in the leaves, showing that they are able to pass through the whole plant.

The effects of o-CD concentration on three measured properties (PR, TR, and WUE) under different modes of application (solution and foliar) are shown in Figure 2. Generally, the PR and WUE values are higher for foliar than for solution application. The effect of o-CD concentration and the application method on the time course significantly ($p < 10^{-3}$) influenced PR, TR, and WUE changes throughout the

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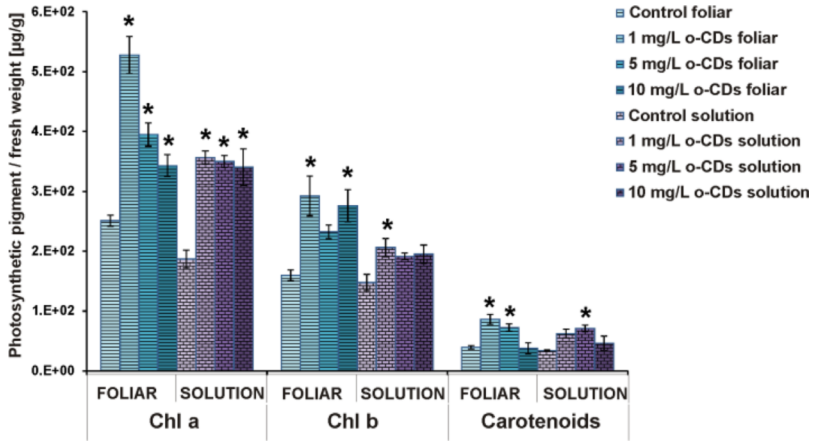

Figure 3. Content of photosynthetic pigments—chlorophyll *a* and *b* (Chl a and b, μg/gFW) and carotenoids (Car, μg/gFW) in the maize leaves after foliar (blue bars) and solution (purple bars) treatments with o-CDs *—statistically significant difference in relation to the corresponding control (e.g., under the same conditions) at the level of the significance $\alpha = 0.05$.

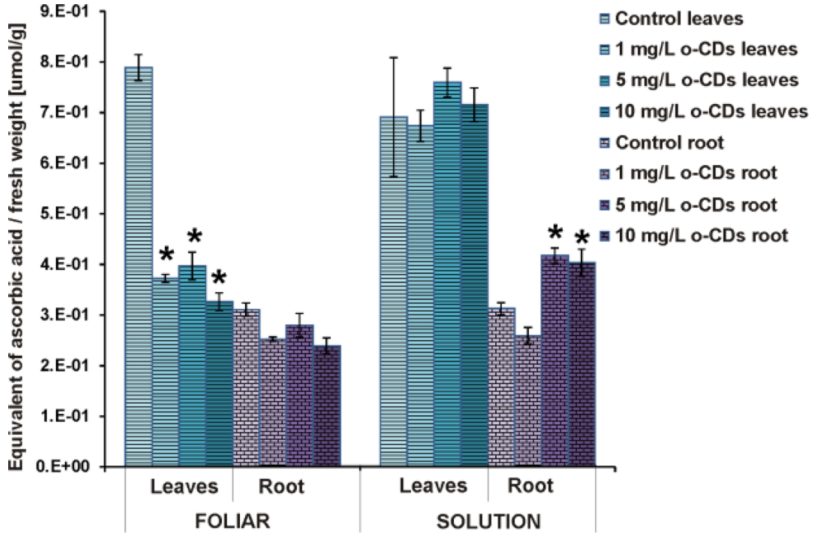

Figure 4. Total antioxidative activity in the maize leaves and roots after foliar (blue bars) and solution (purple bars) treatments with o-CDs *—statistically significant difference in relation to the corresponding control (e.g., under the same conditions) at the level of the significance $\alpha = 0.05$.

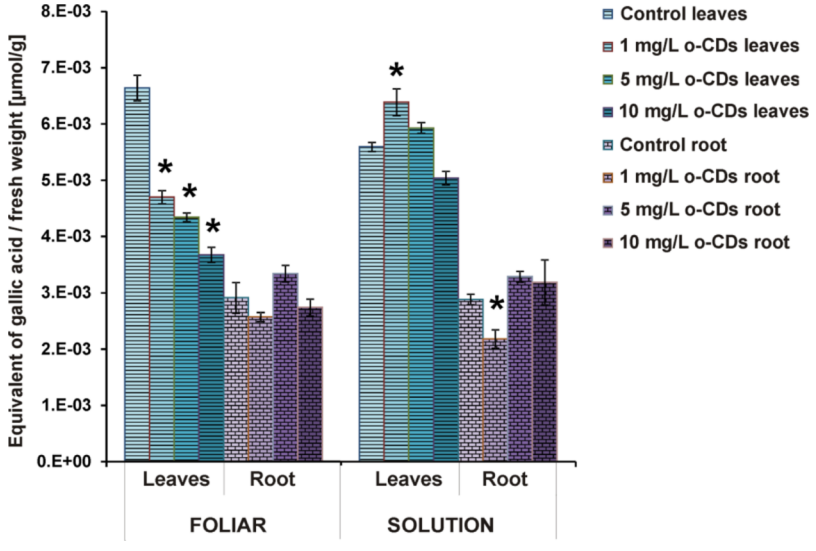

Figure 5. TPC in the maize leaves and roots after foliar (blue bars) and solution (purple bars) treatments with o-CDs *—statistically significant difference in relation to the corresponding control (e.g., under the same conditions) at the level of the significance $\alpha = 0.05$.

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interaction of all three factors. The time point of measurement had a significant effect on PR and TR ($p < 10^{-3}$), as opposed to the case of WUE ($p = 0.523$). The between-subject test revealed that the application method had a significant effect on the TR ($p < 0.05$), whereas it did not apply to PR ($p = 0.841$) and WUE ($p = 0.456$). Application of o-CDs in different concentrations caused significant changes in PR ($p < 0.01$). On the contrary, o-CD concentration did not affect TR and WUE changes ($p = 0.337$ and 0.061, respectively). Detailed pairwise comparisons of PR, TR, and WUE are shown in Figure 2. It is obvious that in foliar application, at time 1 point (T_1), there is no change in any of the measured parameters for 1 and 5 mg/L o-CDs compared to the control; however, PR and TR decreased at the highest concentration of 10 mg/L. At time 2 point (T_2), PR and TR were enhanced, while WUE was reduced with 1 mg/L o-CDs, while only TR was increased with 5 mg/L o-CDs. Meanwhile, in solution treatment, at T_1, 5 mg/L o-CDs increased both PR and TR without changing WUE. It indicates that in solution treatment, higher o-CD concentration had a similar effect on photosynthetic parameters as lower o-CD concentration in foliar application. This may be due to a different way of o-CD application in the plants. These results indicate that the lowest concentration of 1 mg/L applied foliarly increased photosynthetic parameters in maize leaves. At T_2 of solution treatment, 10 mg/L o-CDs increased PR and WUE, without changing TR, and 1 mg/L o-CDs decreased TR, while all tested concentrations increased WUE. As the goal of application of these NPs is to increase PR without compromising WUE, these results indicate that this goal is best achieved with foliar application of NPs at a concentration of 1 mg/L.

3.2. Positive Effect of o-CD Treatments on Photosynthetic Pigments. The content of photosynthetic pigments, Chl a, Chl b, and Car, increased in the leaves after foliar o-CD treatment at all concentrations except for Car at 10 mg/L o-CDs (Figure 3). As for the solution treatment with o-CDs, Chl a increased at all o-CD concentrations, while Chl b increased only for 1 mg/L and Car for 5 mg/L. These results indicate the positive o-CD effect on photosynthetic pigments, which may be related to the increase in PR. The highest increase in PR after foliar application of 1 mg/L o-CDs may be due to the highest increase in photosynthetic pigments at this o-CD concentration. It has been reported that treatments with certain types of CDs promoted the synthesis of chlorophyll in the leaves of treated lettuce, wheat, and lupin,[18,25] which is beneficial for providing more light energy for photosynthesis. The absence of such an effect for the higher o-CD concentrations may be due to the higher oxidative stress induced by these o-CD concentrations, reflected by decreased TAA and TPC. The obtained results also indicate that 5 mg/L o-CDs supplied in hydroponics leads to an increase in both photosynthetic parameters and pigments, without negative effects on biochemical parameters, TAA and TPC. This makes the o-CD NPs a good candidate for possible applications in the improvement of agricultural plants' yield through an increase in photosynthetic parameters.

3.3. Plants' Antioxidative Response to o-CD Treatment. The biochemical tests in the present study comprised screening TAA and TPC. TAA includes the contribution of different nonenzymatic components with antioxidant capacity (low-molecular weight antioxidants such as vitamins, phenolic acids, sugars, etc.),[26,27] while TPC reflects the contribution of phenolics as a group of secondary metabolites participating in the regulation of plant growth and in defense responses.[26,28] Both parameters may be an indicator of metabolic disorder in plants. In our research, foliar applications with all concentrations of o-CDs induced decreases in TAA and TPC in maize leaves but did not affect these parameters in the roots (Figures 4 and 5), indicating that the o-CDs induced a reduction in antioxidant activity in the leaves but not in the roots. In solution treatment, o-CDs did not induce any change in TAA in the leaves, while 1 mg/L even increased TPC (Figures 4 and 5). In the roots, TAA increased after application of 5 and 10 mg/L o-CDs, while TPC decreased after 1 mg/L o-CD treatment. These results indicate an increase in oxidative stress in roots with higher o-CD concentrations (increase in TAA) and in leaves with 1 mg/L o-CDs (increase in TPC). The results indicate that o-CDs induce higher oxidative stress in the plant organs more exposed to the NPs, depending on the way of application.

It is shown that o-CDs in water solution do not have a high quantum yield, 1.02% (Supporting Information), which was also found previously (Zhou et al. 2018)[21]. One could propose that o-CDs, besides increasing photosynthetic pigments, may increase electron-transfer efficiencies in the photosystem, due to the high light harvest capability of o-CDs based on their optical properties, that is, the absorption peaks and absorption edge in the UV/vis spectrum of o-CDs. It was previously found that certain types of CDs and SWNTs increase photosynthetic activity in extracted plant chloroplasts compared to that of controls, by enhancing electron transport rates through a mechanism consistent with augmented photoabsorption.[18,29] Growth induction by CDs in rice has been shown to be related to enhance electron transfer or the efficiency of enzymes such as rubisco in the photosynthesis reaction.[30] It has been reported that due to the strong conjugation over the surface of chloroplasts, carbogenic QDs can easily transfer electrons to the chloroplasts using absorbed light.[19]

If one proposes an increase in plant productivity following the increase in photosynthetic parameters, both ways of application of o-CDs may be efficient; however, the higher efficiency achieved with the lowest o-CD concentration (1 mg/L) in foliar treatment makes this way of application advantageous compared with the solution counterpart.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c01094.

Excitation and emission spectra and fluorescence quantum yield of o-CDs (PDF)

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Zhou, Y.; Sharma, S.; Peng, Z.; Leblanc, R. Polymers in carbon dots: a review. *Polymers* **2017**, *9*, 67.

(2) Sun, Y.-P.; Zhou, B.; Lin, Y.; Wang, W.; Fernando, K. A. S.; Pathak, P.; Meziani, M. J.; Harruff, B. A.; Wang, X.; Wang, H.; Luo, P. G.; Yang, H.; Kose, M. E.; Chen, B.; Veca, L. M.; Xie, S.-Y. Quantum-sized carbon dots for bright and colorful photoluminescence. *J. Am. Chem. Soc.* **2006**, *128*, 7756−7757.

(3) Wang, Y.; Hu, A. Carbon quantum dots: synthesis, properties and applications. *J. Mater. Chem. C* **2014**, *2*, 6921−6939.

(4) Liyanage, P. Y.; Graham, R. M.; Pandey, R. R.; Chusuei, C. C.; Mintz, K. J.; Zhou, Y.; Harper, J. K.; Wu, W.; Wikramanayake, A. H.; Vanni, S.; Leblanc, R. M. Carbon nitride dots: A selective bioimaging nanomaterial. *Bioconjugate Chem.* **2018**, *30*, 111−123.

(5) Hettiarachchi, S. D.; Graham, R. M.; Mintz, K. J.; Zhou, Y.; Vanni, S.; Peng, Z.; Leblanc, R. M. Triple conjugated carbon dots as a nano-drug delivery model for glioblastoma brain tumors. *Nanoscale* **2019**, *11*, 6192−6205.

(6) Liu, M. L.; Chen, B. B.; Li, C. M.; Huang, C. Z. Carbon dots: synthesis, formation mechanism, fluorescence origin and sensing applications. *Green Chem.* **2019**, *21*, 449−471.

(7) Zhou, Y.; Zahran, E. M.; Quiroga, B. A.; Perez, J.; Mintz, K. J.; Peng, Z.; Liyanage, P. Y.; Pandey, R. R.; Chusuei, C. C.; Leblanc, R. M. Size-dependent photocatalytic activity of carbon dots with surface-state determined photoluminescence. *Appl. Catal., B* **2019**, *248*, 157−166.

(8) Jiang, K.; Sun, S.; Zhang, L.; Lu, Y.; Wu, A.; Cai, C.; Lin, H. Red, green, and blue luminescence by carbon dots: full-color emission tuning and multicolor cellular imaging. *Angew. Chem.* **2015**, *127*, 5450−5453.

(9) Zhao, D.; Liu, X.; Wei, C.; Qu, Y.; Xiao, X.; Cheng, H. One-step synthesis of red-emitting carbon dots via a solvothermal method and its application in the detection of methylene blue. *RSC Adv.* **2019**, *9*, 29533−29540.

(10) Shi, X.; Meng, H.; Sun, Y.; Qu, L.; Lin, Y.; Li, Z.; Du, D. Far-Red to Near-Infrared Carbon Dots: Preparation and Applications in Biotechnology. *Small* **2019**, *15*, 1901507.

(11) Ruban, D. A. Geotourism-A geographical review of the literature. *Tourism Manag. Perspect.* **2015**, *15*, 1−15.

(12) Long, S. P.; ZHU, X.-G.; Naidu, S. L.; Ort, D. R. Can improvement in photosynthesis increase crop yields? *Plant, Cell Environ.* **2006**, *29*, 315−330.

(13) Simkin, A. J.; López-Calcagno, P. E.; Raines, C. A. Feeding the world: improving photosynthetic efficiency for sustainable crop production. *J. Exp. Bot.* **2019**, *70*, 1119−1140.

(14) Nabiev, I.; Rakovich, A.; Sukhanova, A.; Lukashev, E.; Zagidullin, V.; Pachenko, V.; Rakovich, Y. P.; Donegan, J. F.; Rubin, A. B.; Govorov, A. O. Fluorescent quantum dots as artificial antennas for enhanced light harvesting and energy transfer to photosynthetic reaction centers. *Angew. Chem., Int. Ed.* **2010**, *49*, 7217−7221.

(15) Su, M.; Liu, H.; Liu, C.; Qu, C.; Zheng, L.; Hong, F. Promotion of nano-anatase TiO2 on the spectral responses and photochemical activities of D1/D2/Cyt b559 complex of spinach. *Spectrochim. Acta, Part A* **2009**, *72*, 1112−1116.

(16) Khatri, K.; Rathore, M. S. Plant nanobionics and its applications for developing plants with improved photosynthetic capacity. *Photosynthesis: From Its Evolution to Future Improvements in Photosynthetic Efficiency Using Nanomaterials*; IntechOpen, 2018; Vol. *95*.

(17) Swift, T. A.; Oliver, T. A. A.; Galan, M. C.; Whitney, H. M. Functional nanomaterials to augment photosynthesis: evidence and considerations for their responsible use in agricultural applications. *J. R. Soc. Interface Focus* **2019**, *9*, 20180048.

(18) Li, W.; Wu, S.; Zhang, H.; Zhang, X.; Zhuang, J.; Hu, C.; Liu, Y.; Lei, B.; Ma, L.; Wang, X. Enhanced biological photosynthetic efficiency using light-harvesting engineering with dual-emissive carbon dots. *Adv. Funct. Mater.* **2018**, *28*, 1804004.

(19) Chandra, S.; Pradhan, S.; Mitra, S.; Patra, P.; Bhattacharya, A.; Pramanik, P.; Goswami, A. High throughput electron transfer from carbon dots to chloroplast: a rationale of enhanced photosynthesis. *Nanoscale* **2014**, *6*, 3647−3655.

(20) Zhou, Y.; Liyanage, P. Y.; Devadoss, D.; Rios Guevara, L. R.; Cheng, L.; Graham, R. M.; Chand, H. S.; Al-Youbi, A. O.; Bashammakh, A. S.; El-Shahawi, M. S.; Leblanc, R. M. Nontoxic amphiphilic carbon dots as promising drug nanocarriers across the blood−brain barrier and inhibitors of β-amyloid. *Nanoscale* **2019**, *11*, 22387−22397.

(21) Zhou, Y.; Mintz, K.; Oztan, C.; Hettiarachchi, S.; Peng, Z.; Seven, E.; Liyanage, P.; De La Torre, S.; Celik, E.; Leblanc, R. Embedding carbon dots in superabsorbent polymers for additive manufacturing. *Polymers* **2018**, *10*, 921.

(22) Lichtenthaler, H. K.; Wellburn, A. R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591−592.

(23) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(24) Cano, A.; Hernández-Ruíz, J.; García-Cánovas, F.; Acosta, M.; Arnao, M. B. An end-point method for estimation of the total antioxidant activity in plant material. *Phytochem. Anal.* **1998**, *9*, 196−202.

(25) Sun, D.; Hussain, H. I.; Yi, Z.; Rookes, J. E.; Kong, L.; Cahill, D. M. Mesoporous silica nanoparticles enhance seedling growth and photosynthesis in wheat and lupin. *Chemosphere* **2016**, *152*, 81−91.

(26) Kasote, D. M.; Katyare, S. S.; Hegde, M. V.; Bae, H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int. J. Biol. Sci.* **2015**, *11*, 982.

(27) Gill, S. S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909−930.

(28) Kubalt, K. The role of phenolic compounds in plant resistance. *Biotechnol. Food Sci.* **2016**, *80*, 97−108.

(29) Giraldo, J. P.; Landry, M. P.; Faltermeier, S. M.; McNicholas, T. P.; Iverson, N. M.; Boghossian, A. A.; Reuel, N. F.; Hilmer, A. J.; Sen, F.; Brew, J. A.; Strano, M. S. Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nat. Mater.* **2014**, *13*, 400−408.

(30) Li, H.; Huang, J.; Lu, F.; Liu, Y.; Song, Y.; Sun, Y.; Zhong, J.; Huang, H.; Wang, Y.; Li, S.; Lifshitz, Y.; Lee, S.-T.; Kang, Z. Impacts of carbon dots on rice plants: boosting the growth and improving the disease resistance. *ACS Appl. Bio Mater.* **2018**, *1*, 663−672.