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Review

Structural Features of Carbon Dots and Their Agricultural Potential

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Cite This: *ACS Omega* 2024, 9, 4166–4185

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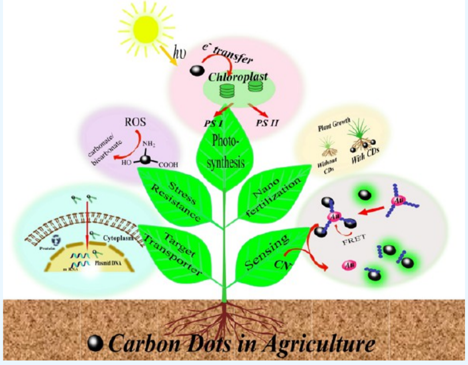
ABSTRACT: Carbon dots (CDs) have drawn attention due to their enticing physical, chemical, and surface properties. Besides, good conductivity, low toxicity, environmental friendliness, simple synthetic routes, and comparable optical properties are advantageous features of CDs. Further, recently, CDs have been explored for biological systems, including plants. Among biological systems, only plants form the basis for sustainability and life on Earth. In this Review, we reviewed suitable properties and applications of CDs, such as promoting the growth of agricultural plants, disease resistance, stress tolerance, and target transportation. Summing up the available studies, we believe that the applications of CDs are yet to be explored significantly for innovation and technology-based agriculture.

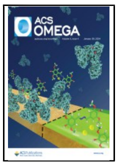


1. INTRODUCTION

It is a well-known fact that the main role of agriculture is to cultivate plants and crops, poultry, dairy farming, forestry, and shelter for all living forms directly or indirectly, which makes it an indispensable part of the ecosystem.[1] Agriculture is a fundamental basis for the sustenance of life on Earth, as plants are the source of food. The Global Hunger Index (GHI) report points to a dire hunger situation fueled by a toxic combination of the climate crisis, the COVID-19 pandemic, the continuously increasing population, and severe protracted violent conflicts.[2] Millions of people are suffering from acute hunger, as per the World Food Programme Report 2020, and without resolving food insecurity it will be challenging to build a sustainable egalitarian society.[3] Many improvisations have been done so far in this area; however, data shared by GHI shows that progress is too slow to reach our goal of zero hunger by 2030, and the COVID crisis deeply affects the process.[4] It is the need of the hour to boost the agriculture system, addressing the challenges to increase overall agricultural production via sustainable pathways providing both high yields and environmentally accepted agricultural practices (efficient use of water, maintaining soil fertility, and minimal agrochemical pollution).[5−7] The conventional methods to improve plant growth include the use of agrochemicals, but the inefficient and ubiquitous use of these has become a threat to the ecosystem and has also resulted in the development of resistance. Another way of improving plant productivity and its features is via genetic engineering, but there is a high level of public concern about the safety of transgenic crops. Using modern technology seems to be an alternative to fulfill the sustainable pathway to enhance crop production.[8,9]

Nanotechnology and nanomaterials have proven to be a boon in science and technology; with all the studies and research going on to improve plant growth, whether it is the monitoring of plant health or improving the overall efficacy of fertilizers, nanomaterials seem to have covered a large part of the problem.[10−13] The advancements of nanomaterials in agriculture are evident from the extent of research performed in the last two decades in the field of nanomaterials for soil remediation, fertilizers, and pesticides.[14,15] Nanoparticles possess unique properties such as a high surface-to-volume ratio and high adsorption, and they are good candidates as fertilizers.[14,16] The coating of nanoparticles on bulk fertilizers, called nanofertilizers, results in controlled release, increases the plant adsorption for a longer time, and decreases the soil pollution caused by traditional chemical fertilizers.[17] Many critical reviews have focused on discussing the applications of these metallic nanoparticles in agriculture and their limitations.[14−16] These applications include using nanoparticles in plant growth,[18] seed germination,[19] herbicides,[20] biosensing,[21] and to aid biotic[22] and abiotic plant stress.[23] Using metal-based nanomaterials comes with major drawbacks, including high toxicity, low solubility, size, less surface area, and biocompatibility.[24] However, the discovery of carbon dots (CDs), which include carbon quantum dots (CQDs), graphene quantum dots (GQDs), and polymeric carbon dots (CPDs), newly

Received: June 28, 2023
Revised: November 22, 2023
Accepted: November 23, 2023
Published: January 13, 2024



https://doi.org/10.1021/acsomega.3c04638

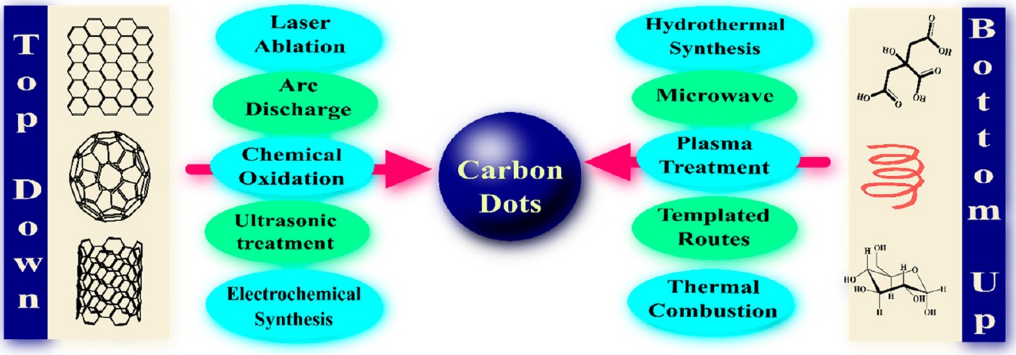


Figure 1. Fabrication techniques used to prepare CDs via the top-down and bottom-up approaches.

found variants in carbon nanomaterials, has eliminated or minimized the above-mentioned shortcomings. They were accidentally discovered in 2004 by Xu et al. while synthesizing carbon nanotubes. Since their discovery in 2004,[25] scientists worldwide have been attempting to synthesize carbon quantum dots using different carbon sources and methods.[26−29] They are widely used in all fields of science, including chemistry, biology, energy, and medicine.[27,28,30−34] CDs can achieve different surface passivation by functionalization or modification.[30] They can be crystalline or amorphous with carbon hybridization as sp^2 generally, but in some cases sp^3 hybridization has also been reported.[35] Restricted in size from 2 to 10 nm, they are categorized into 0D nanostructures, and the crystal lattice parameter for CDs was calculated to be about 0.34 nm.[35] Carbon dots are composed of a nontoxic carbon core functionalized with different surface groups like −COOH, −NH, −COOR, −CO, and −CN. The presence of different surface groups and modifications imparts distinctive electron donor−acceptor and fluorescence properties. The electron donor−acceptor properties assist in enhancing photosynthesis in plants; similarly, the fluorescence properties aid in the sensing of pesticides and plant metabolites as well.[36−38] CDs have oxygen-containing functional groups on their surface, which contribute to their water solubility and make plant uptake of CDs through roots accessible. Therefore, applications of CDs in plant systems have been widely explored, including sensing, nanofertilization, photosynthesis, target delivery, and stress management. The overall applications of CDs have been well documented and reviewed. However, there is only one review article that discusses plant-based applications.[39] Therefore, there is a need for a comprehensive and up-to-date review that focuses on the advantages of using CDs in urban agriculture. The present review summarizes the use and benefits of CDs over other conventional methods in the fields of agricultural growth and productivity. The following aspects of CDs are examined and discussed in detail: synthesis strategies, structural features, CDs in plant applications, and, last, conclusions of the review along with future aspects.

2. SYNTHETIC STRATEGIES

Since the discovery of CDs in 2004, scientists all over the world have been making attempts to synthesize carbon quantum dots in different processes to obtain CDs with desirable size, chemical activity, and photophysical properties. The name carbon dot was given in 2006 when Sun and coworkers synthesized them via laser ablation, but the quantum yield was very low and just 10% surface passivation was achieved.[40] This was followed by a vast amount of literature and work using different methods and precursors to formulate carbon dots with simple fabrication and functionalization, high yield, low cost, less pollution, better properties, large-scale production, and uniform size distribution. The approaches for fabrication can be classified into two categories: the "top-down" and "bottom-up" approaches. The bottom-up approach refers to the synthesis of larger molecules from their small basic units and is achieved using several methods, such as electrochemical carbonization, thermal decomposition, microwave irradiation synthesis, and hydrothermal treatment, whereas the top-down approach refers to the breakdown of larger components into smaller units and can be achieved via laser ablation, chemical ablation, oxidation, and ultrasonication, as shown in Figure 1.

The raw materials used for carbon quantum dot synthesis are readily available, and many of them can also undergo green synthesis procedures, such as tamarind leaves,[41] lime juice,[42] fennel seeds,[43] chitosan,[44] glucose,[45] banana juice,[46] beetroot,[38] fenugreek seeds,[47] neem leaves,[48] and many more. Thus, CDs are eco-friendly not only due to their nature but also due to their environmentally friendly synthesis procedures. During the fabrication of CDs, aggregation must be avoided while ensuring uniform size and functionalization, which can be done as per the requirements.

2.1. Top-Down Strategy.

The top-down strategy includes the synthesis of CDs by cutting large carbon materials into smaller nanosized particles.

2.1.1. Laser Ablation.

The laser ablation method for fabricating CDs is among the latest techniques in the top-down approach.[26,40,48−52] This recent technique is preferred over others because one can regulate and control the size and morphology of CDs in the solution. Sun et al. projected a laser beam of 1064 nm and 10 Hz frequency (Q-switched Nd:YAG laser unit) on the carbon source passing through the argon flow at high temperature (900 °C) and 75 kPA pressure.[40] They further treated the prepared sample with acid and passivated it with polyethylene glycol to obtain carbon dots having highly colorful luminescence. Cao et al. prepared CDs using laser ablation and passivation with poly(propionylethylenimine-*co*-ethylenimine) and utilized the obtained CDs for multiphoton bioimaging of breast cancer MCF-7 cells.[53] Further, Hu et al. could fabricate carbon dots from the graphite flakes of the desired size. The size was controlled by varying the pulse width of the laser used for the fabrication.[52] Interaction of the laser beam with a surface of graphite flakes leads to the formation and expansion of a bubble in the liquid medium. Once the particular pulse width finishes, the contraction of the bubble takes place owing to the external pressure of the liquid medium, causing cooling and the formation of the nuclei/cluster of CDs. Since nucleation depends on pulse width, CDs of different sizes can be fabricated from the same laser source by varying the pulse width. The analysis confirms that a laser having a longer pulse width affects the nucleation and growth of CDs and was thus found to be more effective for tailoring the size and morphology of the quantum-sized dots.

2.1.2. Arc Discharge. This technique refers to arc generation between two electrodes that results in gas plasma formation, which vaporizes the bulk carbon. This vaporized carbon reorganizes and assembles in the form of CDs. The first time CDs were synthesized via the arc discharge method was in 2004.[25] The authors used the arc discharge method to prepare single-walled carbon nanotubes and obtained three kinds of fluorescent particles of different sizes, which were later called CDs. The arc discharge method results in the formation of water-soluble CDs, but they have a large particle size distribution, which limits the use of this method. Chao-Mujica et al. used the submerged arc discharge in water (SADW) method to fabricate CDs.[54] The SADW method has resulted in the formation of multiple kinds of carbon-based materials, such as nanotubes, nanoonions, and CDs, which need to be further separated.

2.1.3. Electrochemical Synthesis. The advantages of using electrochemical synthesis are high yield, exceptional purity, and good reproducibility.[55−61] Additionally, the size can be controlled using the electrochemical oxidation method. This electrochemical synthesis of CDs was started by Zhau et al. in 2007 from multiwalled carbon nanotubes (MWCNTs).[61] As the MWCNTs are composed of graphene sheets layered on each other, tetrabutylammonium cations intercalate in those layers during electrochemical cycling. Under the applied pressure, the cations can chop the nanotubes at the defects of the CNTs, resulting in the formation of nanocrystals. Li et al. have reported the fabrication of size-controllable CDs by using graphite rods as electrodes and sodium hydroxide and ethyl alcohol as electrolytes.[62] The obtained CDs showed size-dependent photoluminescence and possessed potential in photocatalyst design. Liu et al. also fabricated highly crystalline CDs using graphite as an anode and cathode in the alkaline alcoholic electrolytic solution. The observed CDs from the graphite electrodes were colorless. However, they turned yellowish over time due to oxidation of the functional groups present on the surface at room temperature.[60]

2.1.4. Ultrasonic Synthesis. The ultrasonication process includes forming low- and high-pressure shock waves, which cause vicious collision vacuum bubbles and thus produce a high-temperature microenvironment in the solution. Ultrasonication has been used in various applications, such as the fabrication of nanomaterials, hydrodynamic shear forces, sonoporation, and high-speed liquid jets.[28,63−66] These ultrasonic waves thus have enough energy to break significant carbon precursors into nanosized materials. Water-soluble and highly stable CDs were fabricated using ultrasonication of the activated carbon and hydrogen peroxide.[64] Park et al. performed the green synthesis of CDs from waste food under ultrasonication. The obtained CDs had identical spherical shapes and a size of 4−6 nm.[66]

2.1.5. Chemical Oxidation. This method is preferred over the others for fabricating CDs at a large scale, as it does not need complicated experimental setups.[67−69] Qiao et al. first introduced the concept of synthesizing CDs in bulk using chemical etching.[69] The CDs were fabricated by treating the activated carbon source (coal activated carbon (CAC), wood activated carbon (WAC), and coconut activated carbon (CNAC)) with nitric acid. After etching with nitric acid, the surface passivation of the CDs was carried out using 4,7,10-trioxa-1,13-tridecanediamine (TTDDA). CDs observed from this process were found to be amorphous with sizes of 2−6 nm. CDs were monodispersed and did not show any lattice structure.

2.2. Bottom-Up Strategy. The bottom-up synthesis routes are being used more frequently due to their ability to control the shape and size of CDs, but they have some limitations. They strongly tend to aggregate, which could be resolved by adding surfactants.

2.2.1. Hydrothermal/Solvothermal Treatment. Hydrothermal treatment is the most frequently used facile, eco-friendly, one-step process with a low synthesis cost for preparing CDs using a variety of readily available and biocompatible precursors.[29,70−73] It results in the formation of monodisperse CDs, which are uniform in size. It involves heating the carbon precursor in water/solvent at high temperature and pressure conditions in a closed Teflon vessel. Pan et al. introduced this method for the fabrication of graphene quantum dots, which belong to the family of CDs, in 2010 from graphene sheets.[74] Later, Zhao et al. and Halder et al. also used a similar method with slight modification to fabricate graphene quantum dots.[75,76] Mehta et al. used *Saccharum officinarum* juice instead of a traditional chemical-based carbon source to prepare CDs with the hydrothermal method and obtained 3 nm fluorescent CDs.[51] Aschalew and his team synthesized CDs via hydrothermal treatment of lemon juice.[42] Pomelo peel was used as a carbon precursor by Lu et al. to prepare CDs via the hydrothermal technique.[77] Sahu et al. used 1.5−4.5 nm CDs from orange juice, while Huang et al. used strawberry juice as a precursor.[72] Multiple publications reported the usage of food/plant-based precursors, such as bamboo leaves,[78] strawberry juice,[72,79] lemon juice,[42] *Tridax procumbens* leaves[80] *Plectranthus amboinicus*,[81] etc., to fabricate CDs via the hydrothermal technique.

2.2.2. Microwave Irradiation. Microwave irradiation is a rapid, scalable, cost-effective, and eco-friendly process that sometimes lacks size control.[82] Kumar and his co-workers synthesized CDs using microwave irradiation and observed that the optical properties were enhanced up to a specific exposure to heat, but heating beyond that condition led to degradation of the band gap and absorption, whereas the photoluminescence seemed to become saturated.[83] Apart from being used as a synthesis method, microwave irradiation also increased the photoluminescence properties of already synthesized CDs.[84]

2.2.3. Plasma Treatment. Denes et al. fabricated CDs via a distinctive approach using plasma for the first time in 2010.[85] This approach resulted in fabrication and surface functionalization in a one-step reaction. A submerged arc plasma reactor provided a free radical on the surface of carbon dots, supporting surface functionalization with EDTA in benzene solution. The technique was successful in generating monodisperse carbon dots with a uniform size distribution.

2.2.4. Templated Routes. In this method, a templated route, namely, a pre-existing scaffold, is utilized to prepare CDs. The carbon source is deposited or injected into the template and processed to obtain CDs. Yang et al. used a soft and hard template to fabricate CDs from four different carbon sources, including 1,3,5-trimethylbenzene (TMB), diaminebenzene (DAB), pyrene (PY) and phenanthroline (PHA).[54] As a soft acid, uniform morphologies of the copolymer Pluronic P123 were used; as a rigid template, ordered mesoporous silica (OMS) SBA-15 was used. Liu et al. used a SiO_2 composite as the template for synthesizing CDs.[86] Zong et al. prepared CDs using mesoporous silica spheres as templated nanoreactors by impregnating the carbon precursors in the template.[87] This route results in uniformly sized CDs, which are monodisperse in nature.

2.2.5. Thermal Combustion. As the name suggests, thermal decomposition refers to the combustion of carbon precursors to form CDs. This method is convenient and can be used in large-scale manufacturing. Wang et al. used citric acid and *N*-(β-aminoethyl)-γ-aminopropyl methyl dimethoxysilane with thermal combustion for 1 min at 240 °C and obtained CDs of 0.9 nm size.[88] The Wang group also used citric acid decomposition to fabricate CDs at 200 °C for 30 min and obtained CDs of 0.7−1 nm size.[89] Another study by Wan et al.[90] carried out the thermal decomposition of cysteine amino acid in combination with ionic liquid 1-butyl 3-methyl imidazolium bromide as a precursor to fabricate CDs, which were used in photovoltaic cell devices.

3. STRUCTURE OF CDS

3.1. Chemical Structure. CDs, as portrayed by the documented literature, are spherical orbs with a size less than 10 nm.[26,91,92] They are best explained when divided into two portions: the first is the carbogenic core and the second is the surface. A few reports describe the CDs' core as carbogenic, comprising crystalline sp^2 hybridized carbons. However, others say the CDs' core is somewhat more amorphous than that of graphene quantum dots (GQDs). GQDs have portions of graphene layers mounted on each other, which explains the more crystalline structure. CDs and GQDs are considered members of the same family, despite possessing different core structures. The surface portions of CDs and GQDs are, to some extent, analogous. At the terminal positions, both have oxygen-terminating functional groups. The core structure of CDs has been interrelated to amorphous carbon, diamond-like, and graphite−graphite oxide structures. Hu et al. synthesized polyethylene glycol-coated CDs, which show a diamond-like refraction pattern. The lattice structure of CDs observed by HRTEM images illustrates that the lattice spacing lies in the range of 0.2−0.23 nm.[49] CDs fabricated from carbon fibers as the precursor using the chemical etching method were found to be 0.325 nm, resembling a graphite-like core structure.[93] CDs prepared from citric acid and ethylene diamine via single-step hydrothermal processing did not show proper lattice fringes in the HRTEM images, suggesting the amorphous carbon core of the CDs.[94] All the above discussion suggests that the core of CDs varies depending on external factors such as the methodology used for synthesis, reactants, and the processing time.

The surface of CDs has also been exclusively studied, as it can be modified to make CDs bearing the required properties and characteristics. CDs obtained from organic acids have carboxylic acid functionalities at the terminals. CDs with multicolor fluorescence derived from candle soot via oxidation with nitric acid were terminated by carboxylic and hydroxyl groups.[95] The elemental analysis of candle soot identified a chemical composition of 91.69% C, 1.75% H, 0.12% N, and 4.4% O for the CDs; after oxidation, the chemical composition changes to 36.79% C, 5.91% H, 9.6% N, and 44.66% O, showing that the extent of oxygen is considerably high for the CDs after oxidation. This shows the introduction of oxygen-related functional groups. ^{13}C NMR results showed three types of carbon peaks at 114, 138, and 174 ppm. Out of these, the first two belong to C═C at the terminal and internal position, and the 174 ppm peak is ascribed to the carbonyl carbon. Fourier transform infrared (FTIR) spectroscopy showed a peak at 1721 cm^{-1}, which belongs to the C═O stretching vibrational mode, confirming the existence of carbonyl groups at the surface of CDs.

3.2. Electronic Structure of CDs. It is possible to explain the CD electronic structure using molecular orbital theory, as suggested by various researchers.[96−98] Generally, the CDs show two types of electronic transitions, namely, $\pi-\pi$ * and n−π *. They both are the result of the structural patterns of the CDs. As discussed in the chemical structure of the CDs, the core is carbogenic, consisting of sp^2 carbons. The $\pi-\pi$ * transitions belong to the sp^2-hybridized carbons of the core. Similar to the case for standard organic compounds, the energy gap between the electronic states of CDs depends on the number of aromatic rings present in the core of CDs. The n−π * transitions arise from the surfaces of the CDs. The surface of the CDs consists of oxygen-containing functional groups, which have plenty of nonbonding molecular orbitals, thus resulting in n−π * electronic transitions.

3.3. Defects in CDs. Defects in the structure of CDs are quite common; they may vary depending upon the fabrication technique and reactants used to prepare CDs. Hai et al. studied the CDs fabricated using graphite as a carbon source.[99] They recorded the XRD pattern of CDs and compared it with the XRD pattern of pure graphite, showing evident defects in the CDs. The CDs show features of both amorphous carbons as well as sp^2 carbons. CDs show a peak at 26° belonging to the (002) plane of graphite; in addition to this, CDs also have shown peaks at 18.2°, which refers to hexagonal carbon having 103 planes, and 22.59° belonging to amorphous carbon. A thorough assessment of CDs and their graphite XRD pattern reveals that electrochemical synthesis results in the broadening and decrease in strength of graphite peaks in the spectra. This suggests that the oxidation process leads to surface defects in the CD structure in the form of oxygenated moieties such as carbonyl, hydroxyl, and carboxylic groups.

4. EFFECT OF STRUCTURE ON PROPERTIES OF CDS

The structural features have a vast impact on the properties of CDs; even slight variations in size or surface functionalities can powerfully affect their behavior. Thus, the properties of CDs can be tailored for required applications by manipulating their structural features.

4.1. Size. The size of CDs impacts their optical properties, which is the result of quantum confinement effects. The larger CDs tend to emit in the red or far IR range and smaller-sized CD emissions are blue-shifted toward the visible and UV range. In addition to optical properties, the size plays a crucial role in the quantum yield and photostability of the CDs. Larger-size CDs are more photostable but have lower quantum yields. Li et al. prepared CDs via alkali-assisted electrochemical oxidation and separated three different sizes (1.2, 1.5, and 3.8 nm) with column chromatography.[100] The smaller-sized CDs (1.2 nm) showed emission at 350 nm in the UV range, the medium-sized CDs (1.5 nm) showed a fluorescence profile ranging from 400 to 700 nm in the visible range, and the larger-sized CDs showed emission around 800 nm in the near-infrared range. These studies show that the emission of CDs is red-shifted as the size of the CDs increases. Theoretical studies also confirmed that an increase in the size of CDs results in a reduction of the HOMO − LUMO gap, and thus a red shift is observed.[101] Different sizes of CDs can be prepared for the required emission wavelength of cell imaging by using CDs.

4.2. Surface Functionalization. The surface functionalization of CDs significantly affects their physical and chemical properties, including their water solubility. Hydroxyl groups, amino groups, and carboxyl groups are the usual functional groups on the surface of the CDs, impacting their dispersity in different solvents and chemical reactions.[24,27,33] The surface groups also impact the optical properties of the CDs. A study by Kwon et al. used different *para*-substituted anilines and was able to control the photoluminescence behavior of the prepared CDs.[102] Yuan et al. prepared CDs from the same precursor urea under different reaction conditions, and the prepared CDs had different surface functional groups; one type had a carbonyl surface group, and the other had an amine group.[103] CDs rich in the carbonyl functional group showed phosphorescence in addition to fluorescence, whereas CDs rich in the amino functional group showed only fluorescence, suggesting the vital impact of the surface groups on the optical properties of the CDs.

4.3. Crystallinity. Crystallinity is another important structural feature of CDs that affects their properties. Crystalline CDs are more photostable than the amorphous ones and have narrow emission, and amorphous CDs have broader emission profiles. CDs with graphitic cores are more crystalline and exhibit superior electrical conductivity, making them suitable for electronic and optoelectronic applications. Zhu et al. performed density functional theory-based calculations to explain the photoluminescence of graphitic and amorphous cores of CDs.[101] The report suggests that if CDs have an amorphous core, the larger the size of the CDs, the higher the photoluminescence, and a reversible trend was observed for CDs having a crystalline core.

4.4. Heteroatom Doping. It has been shown by multiple reports that the introduction of a heteroatom amends the electronic structure of the CDs and thus significantly affects the photophysical properties. The introduction of nitrogen, sulfur, or boron enhances the electrocatalytic activity, alters the band gap of CDs, and thus influences the absorption and emission profiles.[104−107] The introduction of a heteroatom thus makes CDs appropriate candidates for applications in photocatalysis and sensors. The co-doping of two heteroatoms show dual emission, which makes them good candidates for bioimaging.[105,107]

5. TOXICITY OF CDS

CDs are generally classified as nontoxic and biocompatible. There are numerous applications of CDs; however, during their usage, they are bound to enter the environment directly or indirectly and therefore have an effect on the environment. In low concentrations, they do not harm human cells and may help cell growth.[108] At high concentrations, CDs tend to show toxic effects. As per studies by Ray et al., CDs at a concentration of 0.5 mg/mL led to a 90% survival rate of human hepatoma cells when incubated for 24 h, but at 1 mg/mL the survival rate for human hepatoma cells incubated for the same period was found to be 75%.[109] Yang et al. incubated CDs with MCF-7 cells for 24 h and observed that at a 20 μg/mL concentration of CDs, the survival rate of MCF-7 cells was 100%; at a higher concentration of 200 μg/mL, the survival rate was 40%.[110] Arul et al. investigated MCF-7 and L-929 cells with CDs for 24 h and observed the CDs were more toxic to MCF cells at the same concentration than L-929 cells.[111] Wang et al. studied the toxicity of CDs in BALB/c mice and Wistar rats by tail vein injection.[112] In both male and female BALB/c mice, the authors did not observe any acute toxicity but did observe a noticeable effect in the subacute toxicity experiment after 14 days of exposure to CDs. No changes were observed in Wistar rats with CD exposure in subacute toxicity experiments. In zebrafish (*Danio rerio*), the toxicity of CDs was studied by Kang et al. in the fertilized egg of the fish via microinjecting or immersing it with CDs.[113] For microinjection and immersion tests, the survival rate of the embryo was more than 80% at 1.5 mg/mL CDs. At 2.5 mg/mL, the survival rates for microinjection and immersion tests were 55% and 60%, respectively. Another study by Chousidis et al. compared CDs upon heteroatom doping on the zebrafish (*Danio rerio*).[114] As per this study, the neurobehavior of the zebrafish was disturbed in the presence of CDs, and doping with a N or S heteroatom enhanced the effect. The mechanism of action on the locomotive behaviors of the fish has yet to be explored. All of these studies suggest that CDs have minor effects in low concentrations. However, they can be toxic at higher concentrations.

6. CDS IN PLANT APPLICATIONS/FEATURES BASED ON CDS ESSENTIAL FOR PLANT GROWTH

CDs possess some very interesting and universal properties over traditional quantum dots and carbon nanomaterials, such as intrinsic photoluminescence, biocompatibility, low or no toxicity, good water solubility, good conductivity, chemical inertness, resistance to photobleaching, high quantum yield, strong absorption, abundant low-cost sources, and small size, making them suitable to be used efficiently in many agricultural applications, as shown in Figure 2.

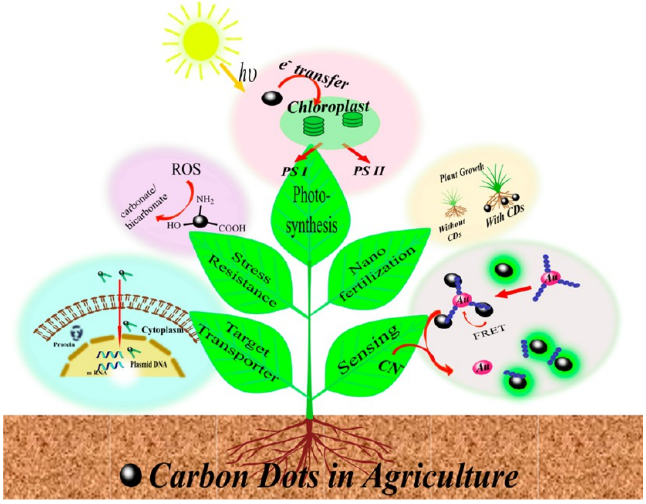


Figure 2. Applications of carbon dots in agriculture.

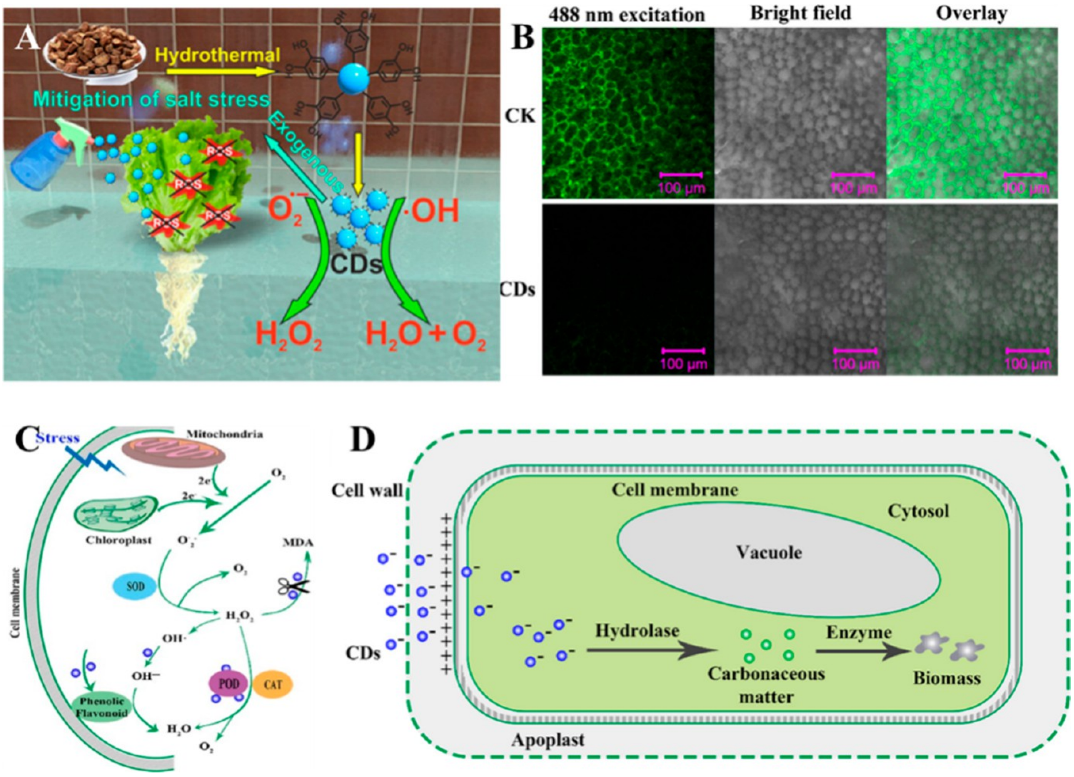


Figure 3. (A) Schematic diagram of the effect of *S. Miltiorrhuza*-derived CDs on reducing the salt stress in plants. (B) Reactive oxygen species (ROS) generated by salt in mesophyll cells of Italian lettuce leaves in TES buffer (control, CK) and in the presence of CDs (2 mg/mL), suggesting effective quenching of the ROS in the presence of CDs. (C) Mechanism of CDs for scavenging the free radicals in plant cells via abiotic stress. (D) Cellular uptake and the fate of CDs in plant cells. Panels A and B reprinted with permission from ref 115. Copyright 2021 American Chemical Society. Panels C and D reprinted with permission from ref 116. Copyright 2020 American Chemical Society.

6.1. Plant Stress Resistance. Plants are affected by two kinds of stress and require resistance to overcome them in order to survive. The first one is abiotic stress, which is caused by environmental factors and climate change. The second one is biotic stress, which is the result of infections caused by pathogens or herbivores.

6.1.1. Abiotic Stress. Increased salinity of the soil, drought, cold, strong solar radiation, and abuse of pesticides are a few common causes of poor crop yield. They disturb the balance between reactive oxygen species (ROS) and plants by increasing the amount of ROS, consequently causing abiotic stress on plants. Excessive ROS such as $O_2^{•-}$, H_2O_2, and $^•OH$ have a strong oxidation ability, which results in DNA damage, cell membrane damage, and protein denaturation. Plants have their own defense mechanisms to deal with such conditions, but the natural enzymes assigned by plants for the purpose face problems due to the microenvironment and pH changes.

To resolve this issue, the use of artificial enzymes was considered; however, due to their specificity for ROS, single-component nanoenzymes can not efficiently deal with the multiple ROS produced by plants. Owing to their superior properties, CDs, as a newly developed class of nanomaterials, are being studied and used to reduce plant stress. In 2020, Li and co-workers synthesized multifunctional CDs using *Salvia miltiorrhiza* via a simple one-step hydrothermal method.[115] The obtained CDs exhibited superior antioxidant activity to eliminate ROS as compared to that of the *S. miltiorrhiza* extract itself (Figure 3A). Apart from this, the prepared CDs can also effectively alleviate the oxidative damage of Italian lettuce under salt stress (Figure 3B). A significant increase was observed in the root and leaf biomass of the Italian lettuce plant (52.2% and 58.1%, respectively), and the water content increased by 48.2%. In 2020, Yadong Li and his co-workers synthesized heteroatom-free CDs and studied their effect on rice (*Oryza sativa L.*) for the first time.[116] It was observed that, along with showing antioxidant properties, these CDs also had a radical scavenging ability, which was studied by investigating the contents of malondialdehyde (MDA), a biomarker of oxidative cleavage by ROS in the plant cells, as shown in Figure 3C and D.

Therefore, CDs reduce the abiotic stress on plants along with increasing the biomass of plants. CDs can overcome abiotic stress in plants without even using their antioxidant and radical scavenging ability, as demonstrated by Xiao and co-workers.[117] They studied the ability of CDs to protect wheat plants from the abiotic stress caused by heavy toxic metal pollution. An adsorption experiment they performed revealed CDs' ability to adsorb and remove Cd^{2+} from the plant system and its surroundings. Similarly, Li and co-workers also demonstrated the ability of CDs to adsorb Cd^{2+} ions and decrease the phytotoxicity in grapefruit (*Citrus maxima*) seedlings.[36] Their study also indicates the importance of controlled loading of CDs, as the reverse impact was observed in leaf. The mechanism for the CDs protecting against abiotic stress and Cd(II) in the plant cells is shown in Figure 4A. CDs facilitate a defensive system toward metal-based stress, which causes ROS production, as shown in Figure 4B. A recent study by Chanderker et al. demonstrated the role of CDs in alleviating arsenic's toxic effects.[118] The presence of arsenic resulted in enhanced accumulation of ROS, effects of

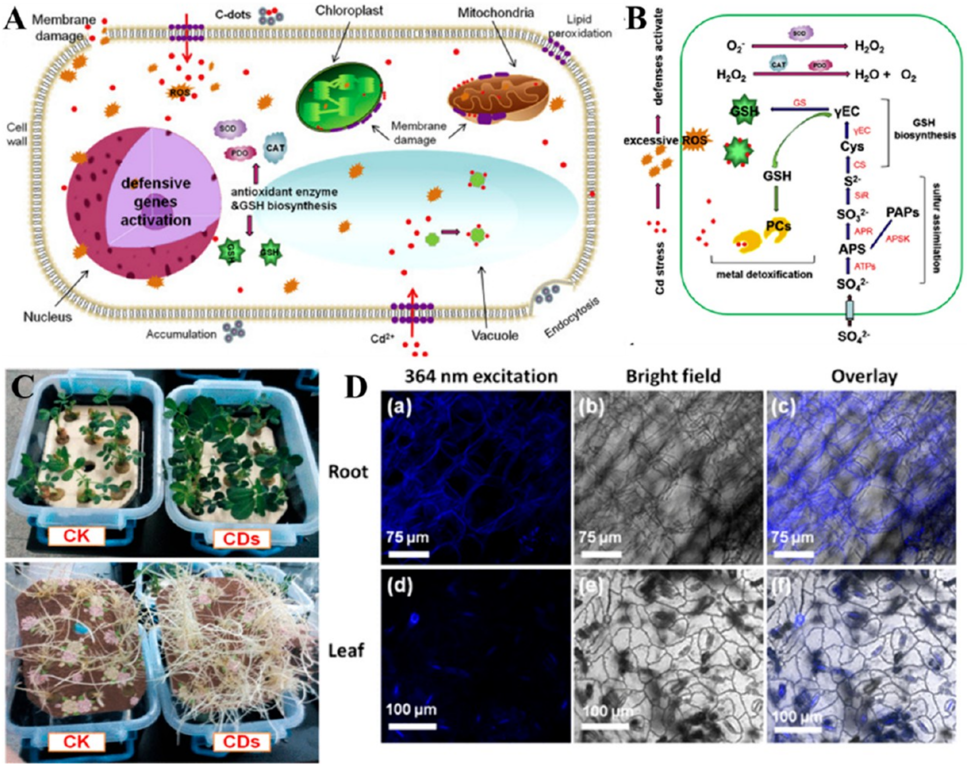


Figure 4. (A) Mechanism of action of CDs in defending the cell wall against Cd(II) toxicity. (B) CD inhibition of reactive oxygen species generated by abiotic stress. (C) Pictures of the stem and root of a peanut plant in the control and CD groups. (D) LSM images of the root and leaf of a peanut plant cultured for 15 days with CDs: cross sections of the root under (a) λ_{ex} = 364 nm and (b) bright light, (c) overlay of images in subpanels a and b, cross sectional area of the leaf under (d) λ_{ex} = 364 nm and (e) bright light, and (f) overlay of subpanels d and e. Panels A and B reprinted with permission from ref 36. Copyright 2019 Science Direct. Panels C and D reprinted with permission from ref 119. Copyright 2018 American Chemical Society.

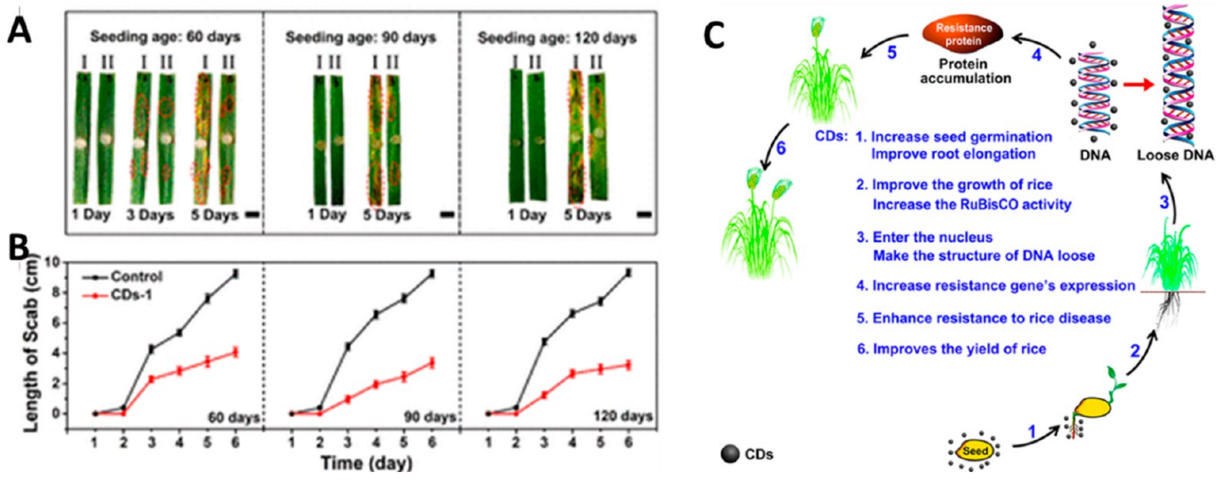


Figure 5. CDs as an efficient biocompatible nanocomposite to increase the disease resistance in rice plants. Reprinted with permission from ref 120. Copyright 2018 American Chemical Society.

malondialdehyde, which is a stress marker, and defensive enzymes pyrroline-5-carboxylate synthetase and glutathionate. Arsenic decreases the plant yields by reducing the germination rate, biomass, and stability of the cell membrane, which then resulted in cell death in *Cicer arietinum* L. Scavenging of free radicals was studied using dye-stained radicals in the presence of As and As + CDs or (As complexed with CDs). Results show that the application of CDs resulted in the scavenging of the free radicals in *Cicer arietinum* L. Stress caused by drought is another reason for lower crop yields in a rural area that depends on rainwater and does not have irrigation facilities. Su et al. studied the drought impact on peanut plants in the presence of carbon nanodots (Figure 4C and D).[119] As per their observations, the CDs were able to provide a stress resistance and an antidrought effect on the plants and increased the yield by up to 9%. This was caused by the hydrophilic functional groups of the CDs, which can retain water and nutrients for a longer duration. CDs then gradually

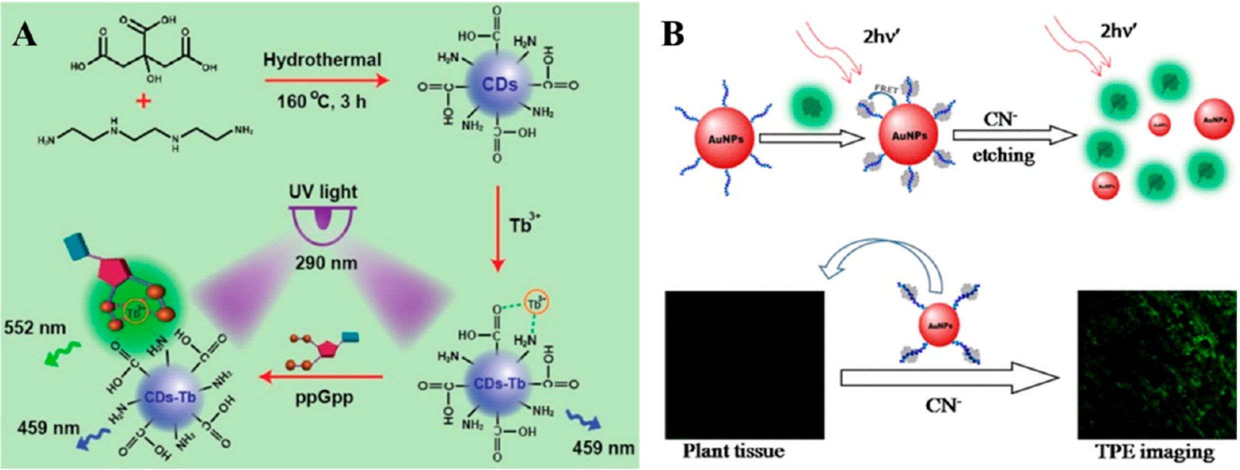


Figure 6. (A) Hydrothermally synthesized CDs in association with the Tb^{3+} ion were able to detect ppGpp, a plant stringent released during environmental stress in plants. (B) Detection of biological cyanide using gold nanoparticle-functionalized CDs in plant tissue. Panel A reprinted with permission from ref 123. Copyright 2018 American Chemical Society. Panel B reprinted with permission from ref 124. Copyright 2015 American Chemical Society.

release these nutrients, which are transferred from CDs to the xylem vessel, thus hindering the drought effects and inducing promising growth of the peanut plants. They also studied the effect of carbon nanodots on the stress resistance of peanuts. It was found that the stress-resistance ability of peanut plants can be enhanced significantly when they are cultivated in the optimal concentration of an aqueous solution of CDs. Because of the increased stress-resistance ability, the growth of peanut plants can be boosted, and the output of the peanuts can be increased by about 9%. Confocal fluorescence images showed that CDs can be transferred from the roots to the leaves through the xylem vessels. The existence of hydrophilic radical groups on the surface of CDs may result in large retention and slow release of micronutrients inside the xylem vessels, further guaranteeing the supply of essential nutrients and boosting the stress-resistance ability of the peanuts.

6.1.2. Biotic Stress. Herbivore attack and pathogenic infection are the main reasons behind biotic stress on crop plants. The most commonly used method to deal with such situations is the use of pesticides, but traditional pesticides are mainly toxic, causing damage to health and environmental risks. Moving forward, to reduce the health risks, nanopesticides were considered due to their reduced runoff, small size, bioavailability, and easy uptake. Still, there were some limitations in using nanopesticides due to their small size and toxicity. With the discovery of CDs, these limitations seemed to be resolved due to their sufficiently small size, bioavailability, and low toxicity.[72] In 2018, Li and co-workers synthesized a series of ∼5 nm carbon dots having different oxygen contents by a facile electrochemical approach;[99] among them, CDs-1 resulted in increased disease resistance in the rice plant along with a 14.8% increase in yield (Figure. 5).[120] The CDs were found to play a significant role in the life cycle of the rice plant and also showed therapeutic properties. In the presence of CDs, the resistance to sheath blight (fungal infection in rice plants caused by *Rhizoctonia solani*) of the rice leaves was investigated. After 5 days, leaves with CDs were ultimately blasted; however, the length of the scab declined significantly, as shown in Figure 5A and B. The length of the scab can be decreased by up to 60% in seedlings aged 60−90 days.

There is still a lot to explore the role of CDs in plant disease resistance, but they are an excellent candidate to act as antibacterial[121] and antifungal agents, which can be applied for controlling and managing plant diseases. In 2017, Liu et al. synthesized CDs using metronidazole via a hydrothermal synthesis procedure, which exhibited selective antibacterial activity against obligate anaerobes for the first time.[122] CDs synthesized from metronidazole are highly photoluminescent, with an average size of 2.9 nm, and consist of a highly carbon crystalline core. The functions and fluorescence mechanisms of CDs were further investigated by adjusting the reaction time and reaction temperature, respectively. Experimental data revealed that CD-250 inhibits the growth of anaerobes, such as *Porphyromonas gingivalis*, without any functionalization.

6.2. Sensors.

Sensors are the most prominent tool to keep an eye on plant growth and health by detecting the metabolites released under various environmental conditions. In 2018, Chen, along with his co-workers, synthesized an excellent fluorescence probe in which CDs, obtained via the hydrothermal method followed by surface binding with Tb^{3+}, were able to sense guanosine-3′-diphosphate-5′-diphosphate (ppGpp), a molecule released as a result of plant stringent response to environmental stress (Figure 6A).[123] The Tb^{3+}-coordinated CDs were able to preserve their intrinsic fluorescence. The CD-Tb^{3+} nanohybrid sensor could selectively detect the ppGpp stringent due to the antenna effect from Tb^{3+} and the specific recognition capacity of CDs. The phosphate groups present in ppGpp can efficiently coordinate with rare earth metals to easily produce the antenna effect via energy transfer, which also led to an increased fluorescence of Tb^{3+} ions. The narrow emission peak of the CDs in an aqueous solution remains unchanged, serving as a reference, whereas the Tb^{3+} ions exhibit an enhanced sharp fluorescence emission signal upon binding with ppGpp and serve as the response signal, resulting in the selective detection of ppGpp.

Excessive usage of pesticides for enhancing agricultural yields has contaminated soils and affected the quality of the products. These toxic components enter the food chain via the consumption of contaminated produce. It is therefore essential to detect these analytes even in trace amounts, and carbon dots, owing to their fluorescence properties, act as excellent

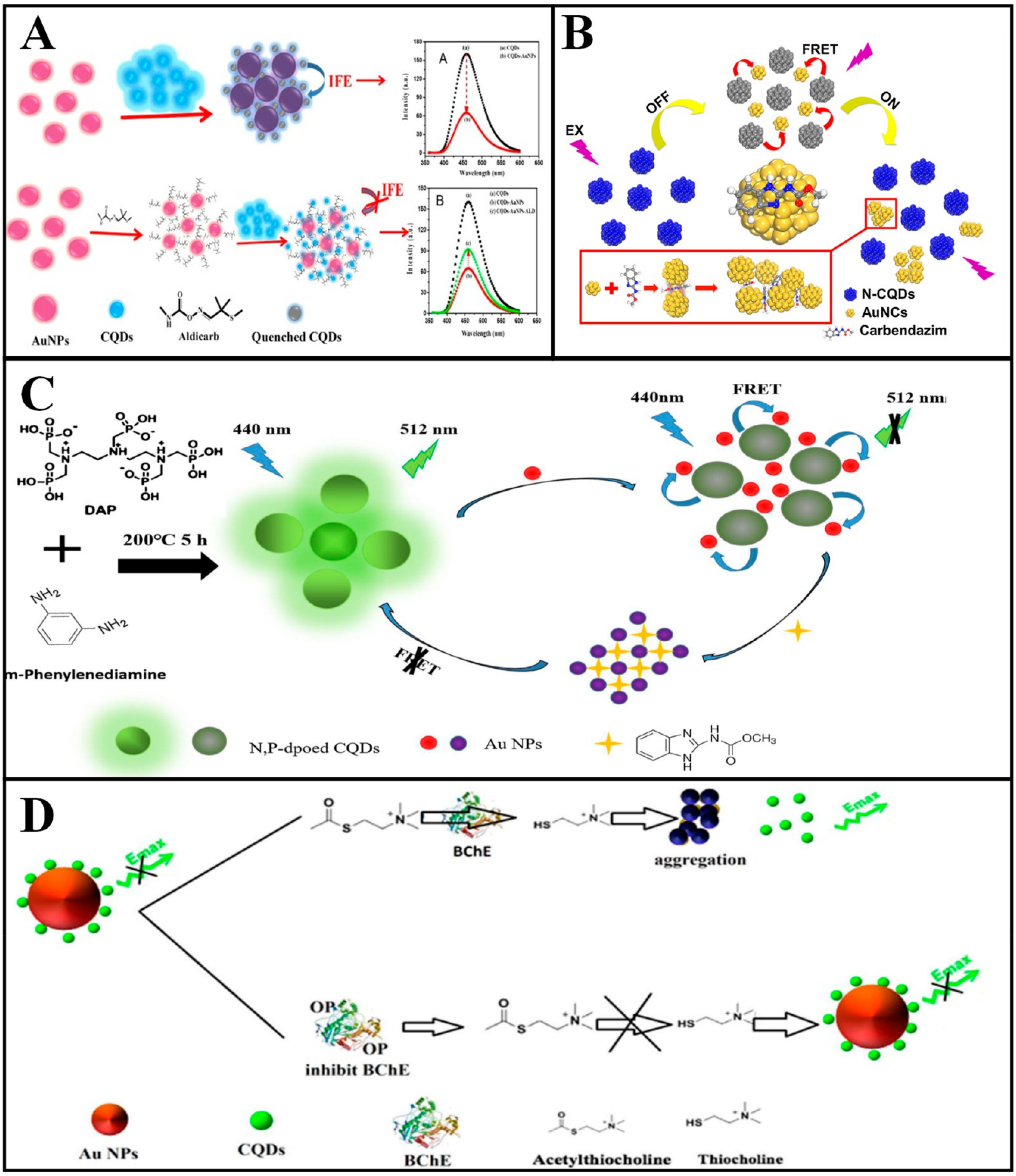


Figure 7. Gold nanoparticle−carbon dots-based detection of pesticides. (A) Aldicarb sensing utilizing an inner filter effect quenching-based strategy. (B) Carbenidzam detection utilizing the FRET pairing between the N-doped CDs and gold nanoparticles. (C) Carbenidzam sensing using the turn-on FRET strategy between gold nanoparticle and carbon dots. (D) Paraoxon sensing with the CD-coated gold nanoparticles utilizing butyrylcholinesterase (BChE). Panel A reprinted with permission from ref 37. Copyright 2021 Science Direct. Panel B reprinted with permission from ref 125. Copyright 2020 Science Direct. Panel C reprinted with permission from ref 126. Copyright 2018 Science Direct. Panel D reprinted with permission from ref 127. Copyright 2017 Science Direct.

optical sensors. A study by Wang et al. utilized gold nanoparticle-conjugated graphene quantum dots for the detection of the biological cyanide (CN^-) in plant tissue using two-photon systems, as shown in Figure 6B.[124] Plant tissues have considerable spectral background noise and a large thickness, which hinder the conventional fluorescence process. Therefore, a two-photon fluorescence process was utilized for the effective and sensitive detection of biological cyanide. They achieved low detection limits (0.52 μM) and a high penetration depth of 400 μm in plant tissue and thus have potential in food safety analysis.

Organic farming is a new zone in urban agriculture; however, the disproportionate usage of pesticides has already compromised soil quality and produce. Thus, it is crucial to

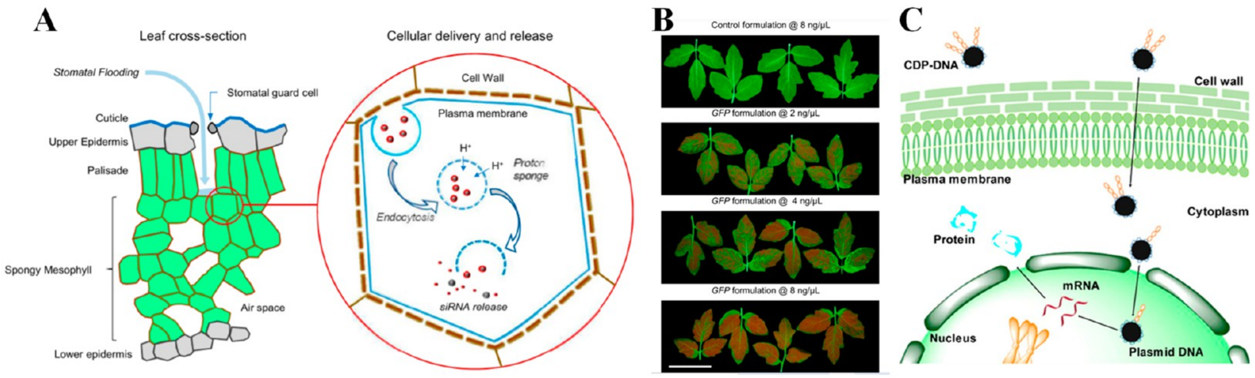


Figure 8. (A) Strategy for CD-based nanocomplexes for the transport of small interfering RNA (siRNA) in a plant cell. (B) GFP trangene silencing with siRNA transported by CDs. (C) Mechanism of carbon dots in combination with polyethylenimine for the transfer of the genetic material inside plant cell wall. Panels A and B reprinted with permission from ref 137. Copyright 2020 Oxford University Press. Panel C reprinted with permission from ref 138. Copyright 2020 American Chemical Society.

screen the quality of soil and groundwater and monitor the concentrations of pesticides. Various research groups have worked on CD-based sensors for detection purposes. In one such strategy, researchers used gold nanoparticles in combination with CDs to prepare FRET pairing or the inner filter effect. An aldicarb insecticide sensor was fabricated using CDs and gold nanoparticles.[37] A simple inner filter effect quenching-based strategy was employed for detection purposes. The fluorescence of the CDs is quenched when combined with AuNPs due to aggregation caused by the electrostatic interaction between them. However, the addition of aldicarb inhibits the aggregation of the addition of CDs due to the formation of an intercalation layer between the insecticide and the AuNPs, as shown in Figure 7A. The intercalation is caused by the interactions of the N and S of the aldicarb with the metal nanoparticles, which consequently hinder the aggregation and inhibit the inner filter effect. The nanosensor worked up to the 3.02 μg L^{-1} detection limit and showed efficiency in fruits, vegetables, and soft drinks. Yang et al. fabricated a ratiometric sensor for detecting carbendazim, a broad-spectrum benzimidazole fungicide, using CDs/gold nanoclusters.[125] FRET pairing between the nitrogen-doped CDs and gold nanoparticles enabled a turn-on sensing mechanism for the detection process, as shown in Figure 7B, with limits of detection of 0.83 and 37.25 μM in two linear response ranges (1−100 and 50−1000 μM, respectively). Similarly, carbazenium nanosensors based on N,P-carbon dots in combination with gold nanoparticles were used, as shown in Figure 7C.[126] The nanosensor provides a quite low detection limit of 0.002 μM for carbazenium, no interference from competitive analytes, and the potential for actual sample measurements.

Wu et al. could also carry out the sensing of paraoxon organophosphate using CD-coated gold nanoparticles utilizing butyrylcholinesterase (BChE) in the sensing process.[127] BChE can hydrolyze acetylcholine to thiocholine, which consequently causes the aggregation of gold nanoparticles and recovers the FRET-quenched emission profile. However, the organophosphates can inhibit the catalytic activity of BChE and hinder the recovery of fluorescence inhibited by the FRET process, as shown in Figure 7D. Using the same strategy, Korram et al. fabricated gold nanoparticles in combination with CDs, which detected four different pesticides: paraoxon, malathion, carbaryl, and methamidophos.[128] The detection limits were found to be in the nanomolar range for each (0.05 nM for paraoxon, 0.10 nM for malathion, 0.13 nM for carbaryl, and 0.12 nM for methamidophos). Various other scientific groups have published similar works for detecting pesticides, including chlorpyrifos,[129] malathion,[130] and other pesticides,[131] using acetylcholine in combination with CDs. Scientists have also explored silver nanoparticles[132−134] and vitamin B12[135] in place of gold nanoparticles for the ratiometric approach in combination with CDs for the detection of pesticides.

6.3. Targeted Transporter. Genetic engineering is widely used in biological sciences; however, due to certain limitations such as complicated processes and inhibition of the cell-wall structure while using a gene gun or *Agrobacterium*-mediated gene transfer, there is a need to shift to more acceptable methods. The thickness of the plant cell wall is about 0.2 μm (200 nm), and many of the commonly used transfer agents produce nanocomplexes that are too large to enter the cell wall. As carbon dots are small-sized nanoparticles (<10 nm), they can easily penetrate the cell wall and can be used to transfer the genetic material for target delivery. They can be transported to almost every part of a plant depending on their size and functionalization and are thus considered a superior candidate for the targeted delivery of required molecules in plants.[136] Recently, in 2020, Schwartz and co-workers used CDs to deliver an interfering RNA into model plants *Nicotiana benthamiana* and tomato using a low-pressure spray method of application,[137] which seemed like an impossible task with other nanomaterials due to their large size. In plants, leaves have stomatal pores that facilitate gas exchange. These pores may provide access to nanodelivery of these genetic materials. As shown in Figure 8A, siRNA-loaded CDs enter the apoplast, enter the cell wall, and then cross the plasma membrane. Endocytosis was followed by the release of siRNA via the proton sponge effect, including nanocomplex disintegration. siRNA delivery by CDs was analyzed by monitoring the gene-silencing effect of the green fluorescent protein (GFP) transgene. Results indicated intense silencing of the GFP, as shown in Figure 8B, at different concentrations of the nanocomplex. This study presents the pathway for nanomaterial-based delivery agents. Further, Wang and co-workers prepared a nanocomposite of CDs with polyethylenimine that was able to perform gene transfer in both plants and animals. Experimental studies were performed on rice, wheat, and mung beans, where efficient gene delivery and strong gene expressions were observed (Figure 8C). Additionally, the

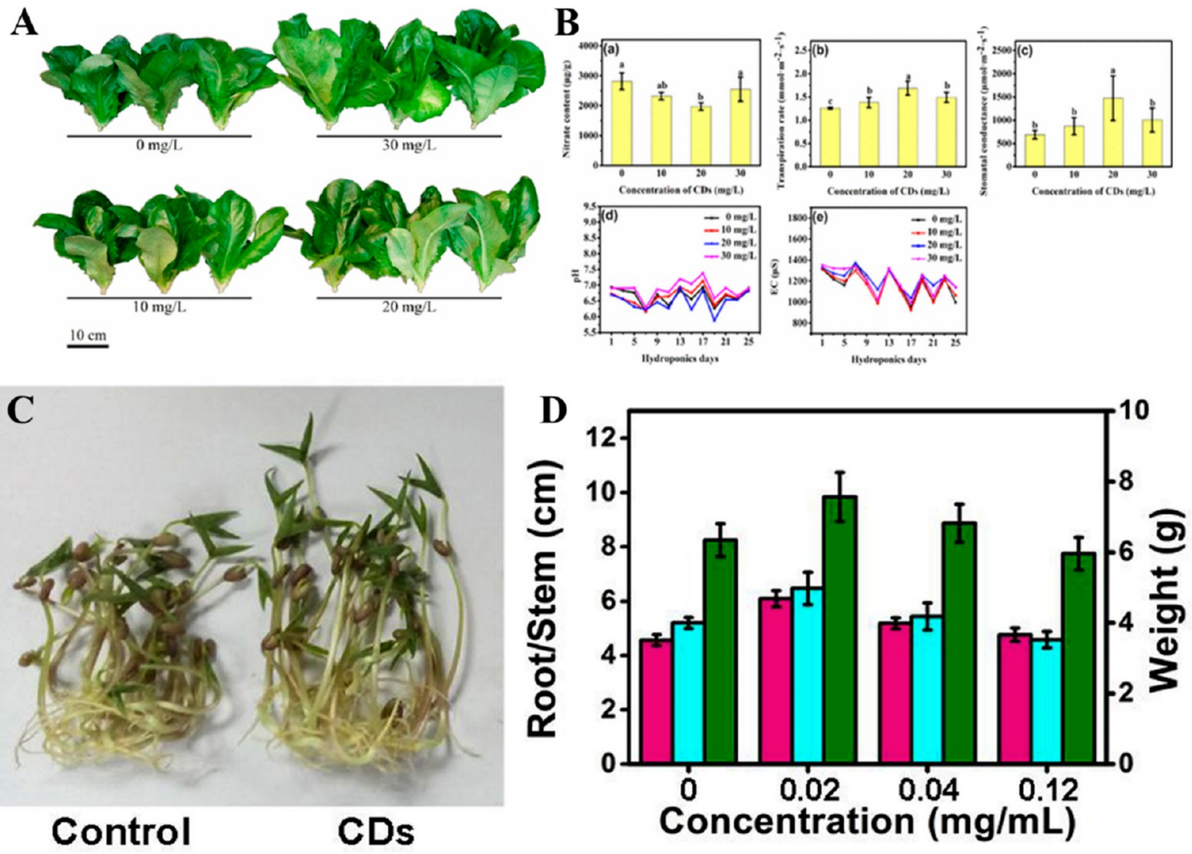


Figure 9. (A) Increase in the leaf biomass and yield upon CD exposure. (B) Effect of CDs on (a) nitrate content, (b) transpiration rate, (c) stomatal conductance, (d) pH, and (e) electrical conductivity of a nutrient solution in a lettuce plant. (C) Picture of mung beans in the presence and absence of CDs. (D) Growth of mung bean plants at different concentrations of CDs in terms of root length (pink), stem length (cyan), and weight (green). Panels A and B reprinted with permission from ref 148. Copyright 2017 American Chemical Society. Panels C and D reprinted with permission from ref 143. Copyright 2018 Science Direct.

prepared CDs are able to not only transfer DNA but also protect it from DNase degradation.[138] In addition to this, a comparison study between CDs, cerium oxide nanoparticles, and silica nanoparticles for the efficiency of foliar delivery was carried out by the Hu group.[139] They utilized high-resolution confocal microscopy to visualize the translocation of the nanoparticles in different parts of cotton and maize plants.

6.4. Nanofertilization. The use of fertilizers is one of the most common and widely accepted methods to enhance plant growth and productivity, but their inefficient use has led to agricultural pollution. In order to resolve these issues, researchers have been doing many studies; in recent studies, it was observed that using CDs as fertilizer leads to low or no agricultural pollution. As already discussed, CDs are widely used in plant applications, such as disease resistance, stress resistance, stress tolerance, sensing, transfer agents, and many others, which directly or indirectly help plant growth. Several advantages of CDs directly enhance plant growth and productivity, such as the ability to absorb and uptake the water and nutrients from soil and photosynthesis enhancement. Various scientific groups have worked and realized the growth impact of CDs in various plants, including wheat,[117,140,141] coriander,[142] mung bean,[143] garlic,[142] lettuce,[144,145] rice,[120] brassica,[146] and chickpea.[147] In 2017, Zheng and co-workers synthesized CDs from rapeseed pollen, which exhibited excellent growth-promoting effects when added directly to the Hoagland nutrient for planting *Lactuca sativa* L. via hydroponic cultivation and was thus referred to as miraculous fertilizer.[146] As shown in Figure 9A, with an increase in the concentration of CDs, the plant's growth increased in terms of biomass and leaf area. At a concentration of 30 mg/mL, the CDs increased the biomass 48% compared to the control. The nutrient content of ascorbic acid, soluble proteins, and soluble sugars was unaffected in the presence of CDs, but they affected other factors that result in the overall growth of plants, such as nitrate content, transpiration rate, and stomatal conductance (Figure 9B). Additionally, the pH and electrical conductance of the nutrient solution were also monitored, and no change upon the addition of CDs was observed. The in vivo transport route of CDs was also evaluated using UV light excitation, thus qualifying as an efficient in vivo bioimaging tool (Figure 9A and B).[148] The Wang group studied the positive effect of CDs on the growth of mung beans, as shown in Figure 9C.[143] There was a significant improvement in the plant's growth regarding root length, stem length, and weight, as depicted by the concentration-based studies of CDs shown in Figure 9D.

Similarly, in 2019, Li and co-workers synthesized CDs via a one-step electrochemical method and studied their plant-growth-promoting ability using *Arabidopsis thaliana* and *Trifolium repens* L. as model plants. The CDs resulted in increased metal ion uptake by plants and enhanced yield by 20%.[149] The main reason behind the ability of CDs to act as plant growth regulators is their easy penetration into all parts

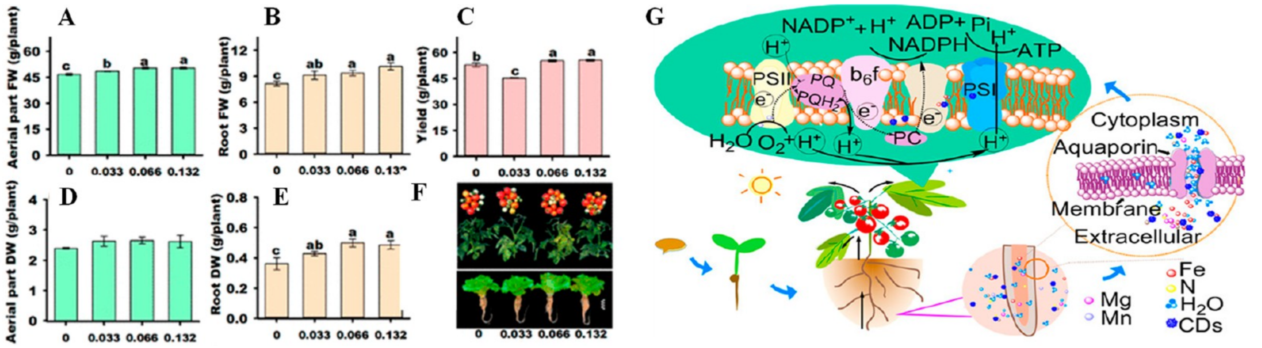


Figure 10. Effect of carbon dots (CDs) on overall development of lettuce and tomato. (A) Aerial part and (B) root of lettuce in the presence of different concentrations of CDs. (C) Yield of tomato fruit, (D) aerial part, and (E) root of tomato plants at different concentrations of CDs. (F) Images of tomato and lettuce plant at different concentrations of CDs. (G) Mechanism of nutrient assimilation with CDs. Reprinted with permission from ref 4. Copyright 2021 American Chemical Society).

of plants and the presence of hydrophilic functional groups that bind efficiently with the minerals and ions. Apart from this, upon degradation, CDs are converted into hormone analogues, which promote plant growth, and CO_2, which is converted into carbohydrates via the Calvin cycle, thus further promoting plant growth and reducing the use of fertilizers. In 2020, Xu et al. reviewed the effects of graphene quantum dots (GQDs), siblings of carbon dots, on plant growth.[150] They showed a direct dependence of plant growth on the sizes of GQDs for the growth of bean sprouts. The studies performed experimentally and the theoretical calculations also indicated that GQDs could influence plant growth. GQDs of size 10 nm synthesized employing a robot embedded in a materials acceleration operation system (MAOS) showed the maximum growth in the plants. This study demonstrated a novel technique for customized fabrication methods for the growth of nanoparticles for nanofertilization.

In 2021, Kou and co-workers synthesized excellent tomato and lettuce plant growth-regulating CDs from L-cysteine and glucose using the hydrothermal method and observed that these CDs were able to activate the gene encoding aquaporin proteins, which led to accelerated seed germination and promoted root and hypocotyl elongation, as shown in Figure 10A−F. These CDs also promoted mineral absorption and enhanced photosynthesis, which led to enhanced yield and nutritional qualities, as shown in Figure 10G.[4] The hydrophilic groups on the surface of CDs could bind with the mineral contents via hydrogen bonds and electrostatic interactions, which resulted in their easy uptake by plants due to the ability of CDs to easily cross the biological barrier. The moisture content in plants also increased upon treatment with CDs, which is a significant factor for the enhanced plant metabolism.

A significant factor that impacts plant growth is the soil's nitrogen content. Nitrogen-fixing bacteria can convert atmospheric nitrogen to urea, which acts as an essential fertilizer in agriculture. A study performed by Wang et al. suggests that CDs are able to enhance the nitrogen-fixing activity of *Azotobacter chroococcum*.[151] The surface groups of the CDs form noncovalent interactions with the iron in the nitrogenase enzyme, which is a critical enzyme in the nitrogen fixation process. In the formation of a hybrid, CDs affect the secondary structure of the nitrogenase by deepening the α-helix structure, which increases the electron transfer rate between F1 and F2 sites of nitrogenase via CDs; this increase results in fast and efficient reduction of nitrogen to ammonia, as shown in Figure 11A and B. The nitrogen fixation with CDs was increased up to 158% in comparison to the control. This offers a cost-effective and eco-friendly method for enhancing biological nitrogen fixation and consequently increasing agricultural yield.

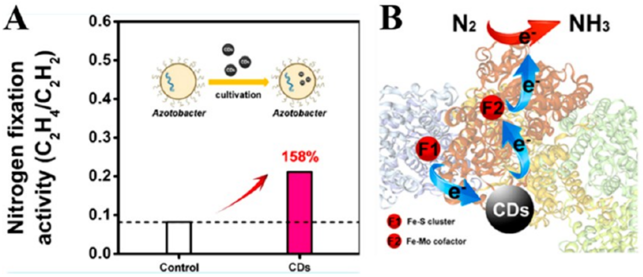


Figure 11. (A) Nitrogen fixation activity in the absence and presence of CDs. (B) Mechanism of biological nitrogen fixation aided by CDs. Reprinted with permission from ref 151. Copyright 2018 American Chemical Society.

6.5. Photosynthesis. Photosynthesis is the most important biochemical process in plants, vital for life on earth, and involves converting light energy into chemical energy. Enhancing photosynthetic efficiency and electron transfer reactions is thought to be an excellent approach for better quality plant growth. The crop yield will increase with the increase in a plant's ability to perform photosynthesis, but due to some limitations plants show less photosynthetic capacity than is possible theoretically.[152] According to a theoretical study done by Long and co-workers in 2006, the primary factor responsible for the low photosynthetic efficiency of plants is their low sink capacity, i.e., the ability to use photosynthate.[152] The theoretically calculated photosynthetic ability (ability to absorb and convert solar light) is only 11% out of 45%, which lies in the photosynthetically active wavelength range, but in actuality the maximum photosynthetic efficiency is only 3−6%. Fluorescent carbon dots have been found to act as an artificial antenna to augment the harvesting ability of solar radiation, which eventually resulted in enhanced photosynthesis in plants. In 2021, Milenković and co-workers synthesized nontoxic orange CDs to increase the photosynthesis efficiency in maize and found that the increase in photosynthesis pigments was higher with foliar application than via a growth solution. They also measured total phenolic content (TPC) and total antioxidant activity (TAA) to monitor the antioxidant activity in plants.[153] In 2020, Xu and co-workers synthesized CD-based nanohybrid $NaYF_4$:Yb,Er@CDs, and

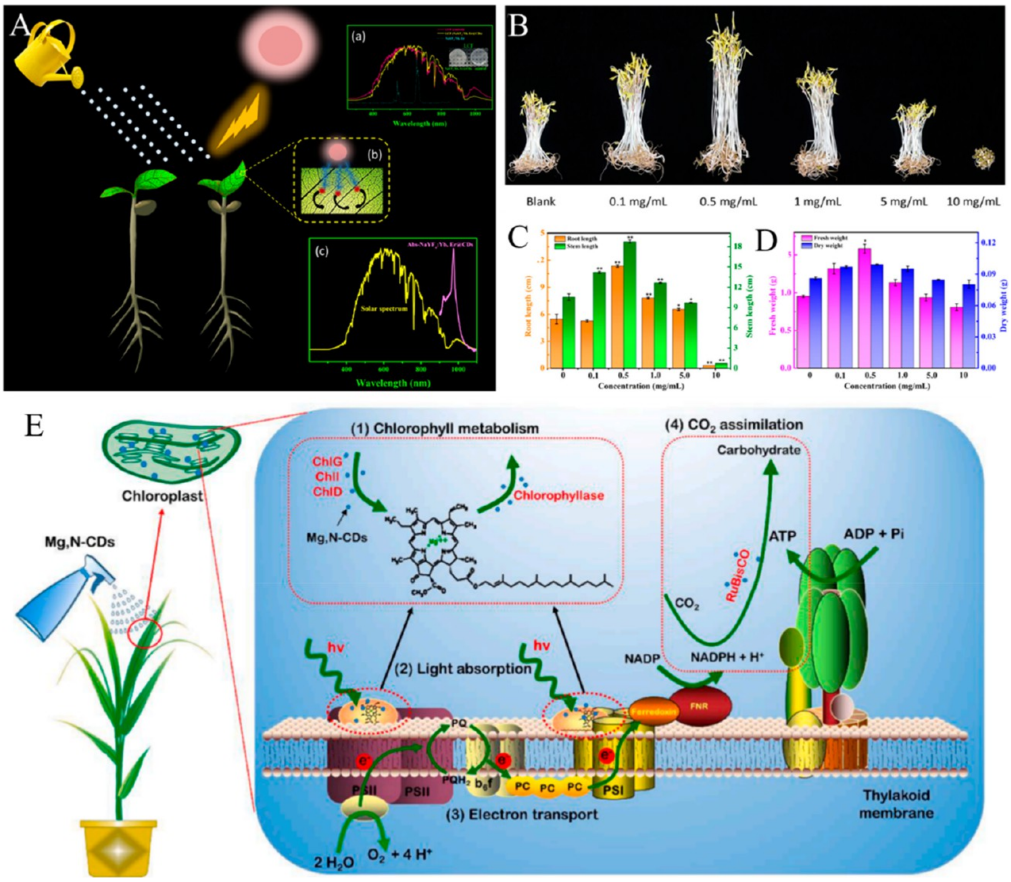


Figure 12. (A) Photosynthesis-enhancing effect of nanohybrid NaYF$_4$:Yb,Er@CDs in a mung bean plant. (B) Concentration-dependent growth of a mung bean plant showing maximum growth at 0.5 mg/mL. (C) Root length and stem length of mung bean plants at different concentrations of nanohybrid. (D) Fresh weight and dry weight of the mung bean at different concentrations of nanohybrid. (E) Schematic illustration of the plant photosynthesis enhancement mechanism in the presence of Mg/N-CDs. Panels A–D reprinted with permission from ref 154. Copyright 2020 American Chemical Society. Panel E reprinted with permission from ref 145. Copyright 2021 Science Direct.

their effects on the growth of mung bean plants were investigated (Figure 12A).[154] It was found that at the concentration of 0.5 mg/mL, these nanohybrids led to increased water absorption and plant growth, as shown in Figure 12B. The growth rate was measured in terms of root length, stem length, fresh weight, and dry weight at different concentrations, which also suggested that a 0.5 mg/mL concentration of the CDs-based nanohybrid enhances the growth, whereas above and below this the growth rate decreases (Figure 12 C and D). It was also observed that these nanohybrids increased the transpiration rate and stomatal conductance by 144% and 148%, respectively. It was concluded that the growth enhancement ability of CDs was due to their ability to be transferred from root to stem and leaf very quickly and their ability to convert near-infrared (NIR) radiations into red light, which is advantageous for photosynthesis. They explained how a significant portion of the light region goes unutilized by plants, as they absorb primarily in blue and red regions, and how upconverting nanoparticles like CDs can help to enhance the solar energy conversion and absorption by plants. Upconverting nanoparticles (UCNPs) were used to absorb NIR range radiations and emissions in the visible range made available to plants for growth promotion and photosynthesis via a multiple photon absorption process. CDs can modulate the emission of NaYF4:Yb,Er and NaYF4:Yb,Tm by efficient energy transfer, as UCNPs show anti-Stokes emission and thus more robust luminescent properties.

Li et al. fabricated magnesium- and nitrogen-doped CDs as a light converter for plant photosynthesis, augmenting light coverage and the quantum yield effect.[145] They observed within 1 h that the CDs could enter the chloroplasts and augment the photosynthetic activity. Moreover, Mg–N:CDs at a concentration of 300 μg mL^{-1} boost the gene expression of enzymes (15.26–115.02%) related to chlorophyll production (chlorophyll a and chlorophyll b by 14.39% and 26.54%, respectively) and metabolic activity in rice plants. The functionalized nanomaterial led to 109.54%, 104.48%, and 127.16% higher photosynthetic activity, electron transport rate, and photosynthetic efficiency, respectively. It also enhanced the rubisco activity of rice plants by 46.62% (as shown in schematic Figure 12E), summarizing outstanding performance of the newly formed nanomaterials and emphasizing their application to increase the productivity of agricultural crops globally. The same scientific group later used CDs synthesized by incorporating graphitic nitrogen and hydroxyl group content.[155] A rice plant was treated with CDs for 2 h, and it was concluded that CDs facilitate the conversion of ultraviolet radiation into photosynthetically active radiations (Figure 13A–D). CDs with a moderate quantum yield and concentration of 0.2 and 300 μg mL^{-1}, respectively, significantly enhanced the photolytic activity of chloroplast (200 μg mL^{-1}) and reduced 2,6-dichlorophenolindophenol,

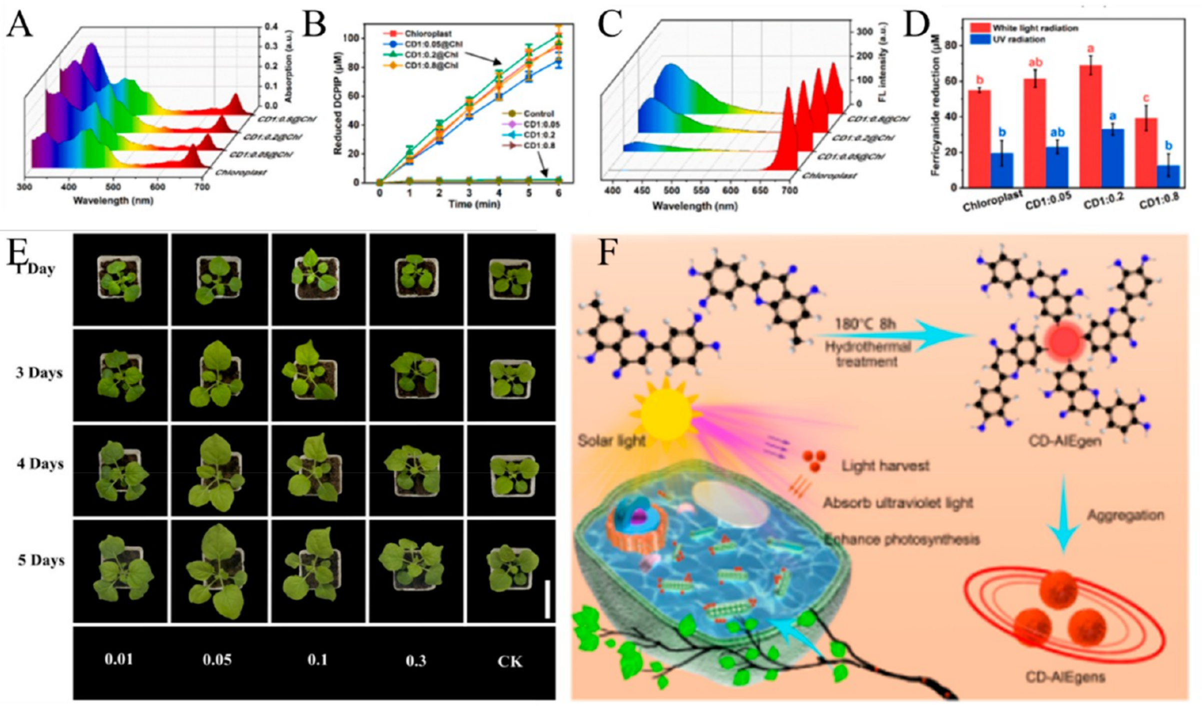


Figure 13. (A) UV−visible absorption spectra, (B) Hill activity, (C) fluorescence spectra, and (D) ferricyanide reduction capability of chloroplast pristine and coated on CDs. (E) Images of *N. banthamiana* depicting the plant growth at different concentrations of NIR CDs. (F) CD-AIEgens supported augmentation in the photosynthesis in plants. Panels A−D reprinted with permission from ref 155. Copyright 2021 Science Direct. Panel E reprinted with permission from ref 156. Copyright 2021 Springer. Panel F reprinted with permission from ref 157. Copyright 2021 American Chemical Society.

which is reflected in hill activity of chloroplast as well as ferricyanide reduction, as measured via the spectrophotometric method. In conclusion, the carboxyl group of CDs recombined with the graphitic nitrogen group, which might have introduced new energy levels to CDs to form π-bonds in the plane, thus improving the quantum yield of the newly synthesized CDs. The quantum yield was improved from CD1:0.05 to CD1:0.8 by increasing the absorption of photons and decreasing energy loss by manipulating the molecular vibration.

Similar experiments have been performed by Wang et al., in which photosynthesis and light harvesting properties were observed due to fluorescent carbon dots enhancing the expression of Psbp and Psik genes.[156] They emphasized how NIR-CDs could be observed as more potential nanodots with better fluorescence than shorter wavelength CDs. As explained above, a major part of solar absorption cannot be directly utilized by the chloroplast; Wang and co-workers synthesized NIR-CDs via the microwave-assisted carbonization method using glutathione and formamide as the starting materials. *Nicotiana banthamiana* plants treated with synthesized CDs at different concentrations exhibited an improved chlorophyll content and rate of photosynthesis. Moreover, the better plant growth rate might be due to the positive effect of CDs on seedlings. To investigate the expression of photosynthetic genes of *N. banthamiana*, eight major genes, including Psi-K, PsbP, PsbS1, PsbY, HCF 136, Psb Q1, Psb Q2, and Psb O4, were examined for 5 days, as shown in Figure 13E. Upon incubation with 0.05 mg/mL NIR-CDs, five genes showed enhanced expression compared to the control group; however, PsbP and Psi-K genes were most sensitive to the stimulation effect of NIR-CDs, while the HCF family did not display any noticeable differences. Xiao et al. reported sustainable carbon dots as a promising light-harvesting material for enhancing the photosynthetic process (Figure 13F).[157] They used aggregation-induced emission carbon dots (CDs-AIEgens) synthesized from natural quercetin. Thus, the author has harnessed a similar concept to utilize UV radiation into visible region radiation that can be available to plants for the photosynthetic process to take place. The elevated rate of electron transport in photosystem II and these novel CDs-AIEgens complexes operates as an optical amplifier for enhanced activity. These newly introduced CDs-AIEgens complexes exhibited properties proven to show better results than typical chloroplast.

The role of functionalization has also been examined and has shown to improve the photosynthesis process compared to the core CDs. Swift et al. demonstrated the positive effect of glucose-functionalized CDs on photosynthesis and crop productivity by altering nonphotochemical quenching (NPQ).[144] Penning down on newly synthesized CDs intensifies the photoprotection and pigment production (Figure 14A), increasing the absorption/uptake of CDs by plants and unlocking elevated/quantified yields. They explained that CDs most likely enhance the electron transport by improving the performance of photosystems, which leads to comparative/prominent reduction in NPQs and lutein production, resulting in enhanced photosynthesis. Treatment with unfunctionalized CDs, however, showed limitations in ROS production. Competitive studies were performed between CDs and nitrogen-doped CDs by Wang et al., as shown in Figure 14B. The study demonstrated that the foliar application of N-CDs led to an increase in the net photosynthetic rate by 21.51%, an increase in the carbohydrate content by 66.43% in roots and 42.03% in shoots, the enhancement of the fresh

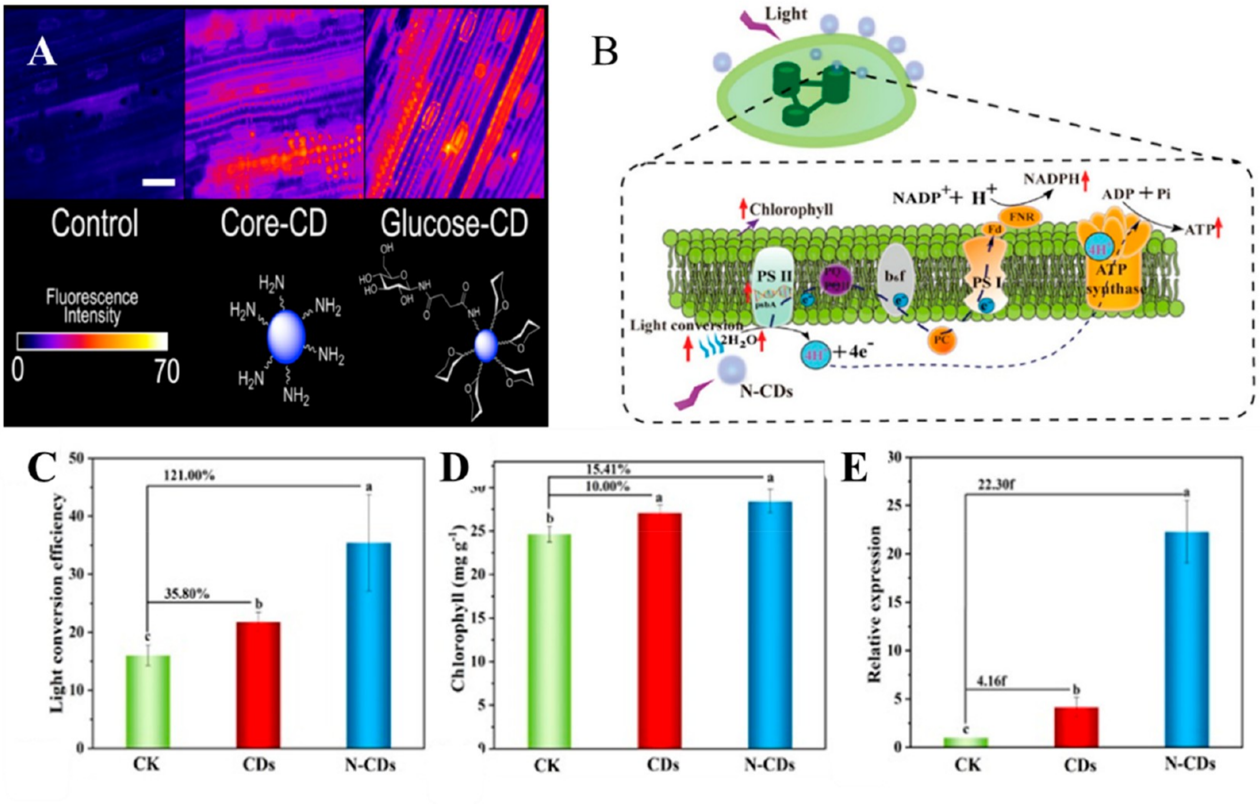


Figure 14. (A) Fluorescence images of plant cells in control, core-CDs, and glucose-functionalized CDs. (B) Mechanism of action of N-CDs in enhancing the photosynthesis process. Comparison between CDs and functionalized CDs in terms of (C) light conversion efficiency, (D) chlorophyll content, and (E) relative expression. Panel A reprinted with permission from ref 144. Copyright 2021 Wiley Online Library. Panels B–E reprinted with permission from ref 158. Copyright 2021 American Chemical Society.

weight by 24.03% in roots and 34.56% in shoots, and an increase in the dry weight by about 72.30% in roots and 55.75% in shoots, which were significantly higher than those of CDs.[158] N-CDs showed enhanced electron transport and light conversion compared to undoped CDs. Furthermore, a better yield was reported in N-CDs than in normal ones (Figure 14C, D, and E). Yankai Liu et al. developed fluorescence polymeric coatings (FPC) containing N-doped carbon dots with tannic acid.[159] The application of FPCs on tomato leaves improved the photochemical efficiency and chlorophyll content significantly, as 38.3% and 48.2% enhancements in growth rate were reported in dry and fresh weight, respectively.

7. CONCLUSION, DRAWBACKS, AND FUTURE ASPECTS

Owing to their excellent properties such as photoluminescence, biocompatibility, low toxicity, water solubility, small size, cell wall permeability, and chemical inertness, CDs have been a research topic of immense interest and high impact for several applications. Adding to this, the facile, environmentally friendly, cost-effective, and abundant synthetic schemes with a wide variety of possible carbon sources make them even more accessible to all. As a result of this, numerous research studies have been done, and a large variety of synthetic schemes, applications, and mechanistic investigations have been reported. This Review is focused on the improved properties of CDs over other nanomaterials and their applicability in plants. CDs mainly have a positive impact on plant growth when applied in appropriate concentrations using a suitable application method. The effect of CDs also depends on their synthesis procedure, which is responsible for their specific properties. Compared with other nanomaterials, these are still new to many researchers. From these data, one can conclude how versatile these CDs are, and there is much more to do before all of the applications and properties of these are discovered and scaled. As easy and exciting as it sounds, some issues associated with CDs still need to be addressed. (1) Most important is the unavailability of standardized synthesis, which is essential to optimize the nature, shape, and size of CDs. It is also the reason behind the lack of action and formation process mechanisms. Once these issues are resolved, large-scale synthesis of cost-effective CDs with desired properties, can be achieved. (2) Due to their excellent inherent photoluminescence properties, CDs are widely used as sensors in various fields, but the applicability of CDs as sensors for many essential metabolites has yet to be discovered. An example worth mentioning is the application of single-walled carbon nanotubes (SWCNTs) as fluorescence sensors in the near-infrared region, which have been shown to detect plant hormones in vivo and predict plant stress.[160] (3) The application of CDs for crop plants is still in its infancy despite their good cell wall permeability and easy translocation. Thus, more research is needed to utilize CDs for plant growth improvement, including disease resistance and biocompatible agrochemical development. However, before that, a study on the effect of CDs on microorganisms, insects, and aquatic species needs to be done to avoid any harmful side effects. (4) Due to abundant hydrophilic groups on the surface of CDs, they can not exist as solids, which limits their storage and easy transport. Thus, it is necessary to synthesize CDs in a solid state for their real-time use. (5) CDs have also been used as target delivery agents in biomedicine, but only a few delivery applications have been seen in the case of plants, indicating that there is still a need to explore the use of CDs as target delivery agents for some essential and specific molecules into plants, such as pesticides, nutrients, plant growth regulators, etc.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Department of Science and Technology, Government of India (DST/TDT/AGRO-54/2021). We acknowledge funding received from the BRICS Research Project (RFBR 20-53-80002 and DST/INT/BRICS/COVID-19/2020).

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