Photosynthesis and crop productivity are enhanced by glucose-functionalised carbon dots

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Summary

• From global food security to textile production and biofuels, the demands currently made on plant photosynthetic productivity will continue to increase. Enhancing photosynthesis using designer, green and sustainable materials offers an attractive alternative to current genetic-based strategies and promising work with nanomaterials has recently started to emerge.

• Here we describe the in planta use of carbon-based nanoparticles produced by low-cost renewable routes that are bioavailable to mature plants. Uptake of these functionalised nanoparticles directly from the soil improves photosynthesis and also increases crop production.

• We show for the first time that glucose functionalisation enhances nanoparticle uptake, photoprotection and pigment production, unlocking enhanced yields. This was demonstrated in *Triticum aestivum* ‘Apogee’ (dwarf bread wheat) and resulted in an 18% increase in grain yield.

• This establishes the viability of a functional nanomaterial to augment photosynthesis as a route to increased crop productivity.

Introduction

Plants greatly underperform compared with the theoretical maximum photosynthetic efficiency (Anten, 2005; Ort et al., 2015). Even under moderate solar light intensities, plants often absorb light in excess of what can be safely harnessed due to downstream rate-limiting electron transport. Consequentially, plants take advantage of an inbuilt suite of photoprotective mechanisms, collectively termed nonphotochemical quenching (NPQ), which dissipate excess energy and prevents deleterious photochemical reactions (Melis, 2009). Whilst essential to plant survival, it is widely accepted that NPQ affects the photosynthetic performance of many plant species, with recent studies demonstrating that transgenic modification that targeted reducing specific components of NPQ, or downstream molecular and enzymatic processes, resulted in a 15% increase of crop biomass (Kromdijk et al., 2016; Simkin et al., 2017). Whilst recent work has highlighted that nanoparticles (NPs) offer a promising method for enhancing photosynthesis, these studies have yet to achieve the significant desired increases in crop yield (Giraldo et al., 2014; Sai et al., 2018; Kah et al., 2019; Swift et al., 2019; Li et al., 2020; Xu et al., 2020).

The work presented here achieved a dramatic increase in crop productivity by augmenting plants with specially engineered NPs. Here, the use of functionalised carbon dots (CDs) is explored to overcome the limitations, such as toxicity, poor bioavailability or complex and inefficient syntheses, of previously used nanomaterials, whilst retaining desirable properties such as being able to exchange electrons when photoexcited (Giraldo et al., 2014; Swift et al., 2018, 2019). CDs are a carbon-based, fluorescent, small (less than 10 nm) class of nanoparticle (Xu et al., 2004; Baker & Baker, 2010; Hill & Galan, 2017; Swift et al., 2018). Our team and others have demonstrated previously that glycan-surface functionalisation of NPs mitigates acute toxicity and enhances internalisation in mammalian cells (Barras et al., 2013; Reichardt et al., 2013; Benito-Alifonso et al., 2014; Hill et al., 2016, 2018; Swift et al., 2018).

Here we report the first example of glycan-functionalised CDs used to significantly increase grain yields of elite bread wheat by 18%. Our mechanistic investigations revealed that CD–plant interactions affected many facets of photosynthesis, and that glycan functionalisation of CDs was essential to realise improved yields by altering NPQ and pigment production, whilst also reducing reactive oxygen species generation.

Materials and Methods

Statistics and significance

Data were initially analysed for significance using analysis of variance (ANOVAs) and *P*-values were calculated using the
appropriate T-tests. One, two or three asterisks are used throughout the manuscript to indicate a significant increase above the control of \( P \leq 0.05 \), \( P \leq 0.01 \) or \( P \leq 0.001 \), respectively. Additional details are given in the Supporting Information.

**CD synthesis and characterisation**

Amine-coated CDs (core-CDs) were synthesised following a modified procedure from our group (Hill et al., 2016; Swift et al., 2018). The glucose-functionalised CDs were prepared via a two-step procedure starting from core-CDs. Briefly, treatment of core-CDs with succinic anhydride generated acid-coated CDs, that were then conjugated with 1-aminoglucose, and subsequently purified through a 200-µm syringe filter and size-exclusion chromatography (Sephadex G-10; Sigma Aldrich, Gillingham, UK) to yield the functionalised CDs (see Supporting Information for full experimental details). Glycan conjugation was performed with an excess of 1-aminoglucose to ensure all the acid groups reacted. This solution was then stirred vigorously overnight. For storage, the glucose-CDs were dissolved in methanol and kept at 4°C to prevent aggregation. See Supporting Information Figs S1–S7 for full details of the synthesis and characterisation by NMR, Fourier transformed infrared spectroscopy, dynamic light scattering; absorbance spectroscopy and fluorescence spectroscopy.

**Plant growth conditions and treatments**

The plants were grown in compost (Levington, F2) in individual 10 cm × 10 cm plots, in trays of 15 pots, and in a glasshouse at a constant 20.0°C temperature. Natural sunlight (dynamically ranging from 100 to 800 µmol m\(^{-2}\) s\(^{-1}\)) was supplemented with light-emitting diode (LED) lighting (PhytoLux, Attis-7) 80–120 µmol m\(^{-2}\) s\(^{-1}\) to provide a 16-h photoperiod. From 3 wk post germination until harvesting the plants were treated three times per week on Monday, Wednesday and Friday with 3.3 mg of treatment per plant (by adding 1 l of 50 mg l\(^{-1}\) treatment solution to each tray of 15 plants to be soaked up by the soil) totalling 10 mg of treatment per plant per week. The trays of pots were randomly rearranged and rotated three times per week on Monday, Wednesday and Friday to compensate for any undetected differences in light or air quality across the glasshouse. Productivity measurements were made 10 wk post germination at Zadoks stages 9.0–9.2 and after 7 wk of treatment.

**Fluorescence microscopy**

Samples were imaged with a Leica DM200 and Leica MC120HD detector. Chlorophyll fluorescence was imaged using 470 nm excitation and the emission between 650–700 nm was collected, the CDs were imaged with a 365 nm excitation and 430–470 nm emission. Plants were selected for imaging from those available at random. Leaf tissues were dissected from the middle of flag leaves 7 wk post germination and after 4 wk of treatment. To demonstrate the CD fluorescence, each treatment was imaged using the same excitation, microscope and detector settings. Histograms of the fluorescence observed across three images for each treatment across are given in Fig. S9. CDs were observed throughout the leaves but not in the grain of the plants.

**Chlorophyll fluorescence and gas exchange measurements**

A GFS-3000 (Waltz) and a MAXI-PAM (Waltz) were used for infrared gas analysis (IRGA) and chlorophyll fluorescence measurements respectively. Measurements were made at Zadoks 7.3–7.7 on flag leaves at 7 wk post germination. Plants were selected for measurements from those available at random. Plants were dark adapted for 40 min before light curve measurements. For chlorophyll fluorescence, the average across 10 equally sized areas evenly distributed across the leaf were recorded for each value. For the light curves the actinic-light step length was 30 s. A carbon dioxide concentration [CO\(_2\)] of 400 µmol mol\(^{-1}\), [H\(_2\)O] of 17 mmol mol\(^{-1}\), leaf temperature of 22°C and vapour pressure deficit of 1.0 ± 0.2 kPa were all maintained for IRGA experiments.

**Pigment measurements**

Pigments were extracted from flag leaves and analysed by high-performance liquid chromatography (HPLC) using previously developed methods (Van Heukelem & Thomas, 2001). All solvents used were HPLC-grade. Samples were taken at the middle of the photoperiod on the same day, 0.1 g of flag leaf was used for each sample, \( n = 5 \). Plants were selected for measurements from those available at random. A stationary phase 3.5 µm spherical silica particle with an 80 Å pore size was used (Agilent, Eclipse XDB C8, 4.6 mm × 150 mm). Each of the peaks in the chromatogram were assigned by comparing their absorption spectrum and elution time to known standards. Absorption wavelengths of 430 nm, 470 nm and 450 nm were used to quantify the concentrations of Chl\(_a\), Chl\(_b\) and the carotenoids respectively.

Due to the dark adaptation of the leaves no zeaxanthin or antheraxanthin were observed in the samples. It was not possible to separate \( \alpha \) and \( \beta \) carotene, so peaks are simply labelled carotene however it is expected to be predominantly \( \beta \)-carotene.

**Results**

**Synthesis of functionalised fluorescent CDs**

Unfunctionalised amine-coated CDs (core-CDs) were synthesised in one-pot after 3 min microwave heating of glucosamine-HCl and 4,7,10-trioxo-1,13-tridecanediame following a modified reported procedure (Hill et al., 2016; Swift et al., 2018). Glucose functionalisation of the CDs was carried out in a two-step process by reaction of core-CDs with succinic anhydride to yield carboxylic acid bearing CDs, followed by N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC)-mediated conjugation with 1-aminoglucose to generate glucose-functionalised CDs (glucose-CDs) (Figs S1–S7).
CDs uptake in plants

The mechanisms by which the CDs interact with plants were investigated in *Triticum aestivum* treated with core-CDs or glucose-CDs and compared with untreated controls. Each of these CDs were applied directly to the soil from 3 wk post germination. CD uptake from the soil was observed by fluorescence microscopy for both CD treatments (Figs 1, S8; Table S1), which have a peak fluorescence intensity at 455 nm (Swift *et al.*, 2018) and are spectrally isolated from chlorophyll emission. The CD uptake was quantified, yielding 29 ± 1 µg and 32 ± 1 µg per g of leaf tissue for core-CDs and glucose-CDs respectively. This measurement demonstrated that glucose functionalisation slightly enhanced plant CD uptake (*N* = 15; *P* < 0.05; Supporting Information).

Changes to *Triticum aestivum* biomass production

The effect of uptake of CDs into the leaves on the physiology and yield was investigated, and no toxic effects or responses were initially observed after treatment. The total ear mass per plant for glucose-CD treatments was 18% greater than that of the control (*P* = 0.008; Fig. 2b), whereas the core-CD treatment was not significantly different from the control (*P* = 0.4; Fig. 2b). Glucose-CD treatment also increased the number of seeds produced by 12% (*P* = 0.02; Fig. 2c) and shoot biomass by 17% (*P* = 0.02; Fig. S10). In all measurements, no significant effect was observed with treatment with glucose alone at the same concentrations (see Supporting Information).

Effect of CDs on photosynthetic performance in plants

To investigate the origin of the observed yield increases, the effect of the CD treatments on the photosynthetic performance of *Triticum aestivum* was investigated using standard protocols that measured chlorophyll fluorescence and IRGA as a function of photosynthetic photon fluence rate (PPFR). At high actinic-light intensities, a small but significant enhancement of the operating efficiency of photosystem II (*Fq/Fm*) was observed for both CD treatments (Fig. 3a) compared with the control. Furthermore, we observed a significant decrease in NPQ for plants treated with glucose-CDs compared with the other samples (Fig. 3b). Moreover, both CD treatments resulted in a significant increase in carbon assimilation (*A*) and the stomatal conductance (rate of CO₂ passage through stomata, *gs*) compared with the control (*P* < 0.05) for a wide range of PPFR (Fig. 3c, d). The *A* under saturating light conditions (*A_{SAT}*), and the maximum *A* (*A_{MAX}*), were extracted from exponential fits to data in Fig. 3(c) and Fig. S11, respectively. The values for *A_{SAT}* and *A_{MAX}* for each treatment are given in Table 1, with *A_{MAX}* invariant to CD treatment, but *A_{SAT}* enhanced by CD treatment.
Fig. 2 Physiology and yield measurements in *Triticum aestivum*. (a) Image of the dried, threshed seed yield after treatments. For each box the red central line indicates the median, the blue top edges indicate the 25th percentile, the blue bottom edge indicates the 75th percentile, the whiskers indicate the range excluding outliers, red squares indicate outliers. Black asterisks indicate a difference from the control, blue asterisks indicate a difference between the core and glucose-carbon dot (CD) treatments. *, *P* ≤ 0.05; **, *P* ≤ 0.01; ***, *P* ≤ 0.001. (a) *n* = 32 (for b, c, f) *n* = 36, 32 and 36 for the control, core-CD and glucose-CD treatments, respectively; (d) *n* = 35, 32 and 35 for the control, core-CD and glucose-CD treatments, respectively and (e) *n* = 9. Additional tables of statistical parameters are given in Supporting Information Tables S2, S3.

Fig. 3 Irradiance-dependent photosynthetic performance of *Triticum aestivum*. Light curves for (a) photosystem II operating efficiency (*F*_{qa}/*F*_{m}). (b) Nonphotochemical quenching. (c) Carbon assimilation (*A*). (d) Stomatal conductance (*g*_{s}). Values were obtained from chlorophyll fluorescence (a, b) and infrared gas analysis (c, d) measurements of the three different treatments. The light curve was fitted with an exponential model. The values of *A* under saturating light conditions (*A*_{SAT}) are given in Table 1. *R*^2 ≥ 0.9945 for all fits. Red or blue asterisks are used to indicate a difference between the core-carbon dot (CD) treatment and the control or the glucose-CD treatment and control respectively. *, *P* ≤ 0.05; **, *P* ≤ 0.01; ***, *P* ≤ 0.001. For scatter or column plots, error bars shown correspond to the SE.
The greater enhancement of carbon assimilation compared with photosystem II activity ($F_{q}/F_{m}$) by treatment of plants with CDs indicated that they alter processes downstream from the initial light harvesting, such as electron transport chain and stomatal efficiency, to enhance photosynthesis (Lawson et al., 2002). This hypothesis is supported by the increase in $A_{\text{SAT}}$ upon addition of CDs, suggesting that the treated plants were able to perform more photochemistry with the light that they absorb, for example an increased operational efficiency, but did not enhance the maximum photosynthetic capacity of plants, as $A_{\text{MAX}}$ is unaffected.

### Kinetics of photosynthetic performance

We then focused on the response of the treated plants to changes in light conditions by measuring the induction and relaxation of $A$, $g_{s}$, $F_{q}^{a}/F_{m}^{a}$ and NPQ. These measurements were performed as dark–light–dark cycles as displayed in Fig. 4. The induction of stomatal conductance was fitted using a previously developed model (McAusland et al., 2016) to quantify the maximum rate of stomatal conductance ($S_{\text{MAX}}$) upon exposure to light. These experiments revealed that $S_{\text{MAX}}$ increased for both the CD treatments (Fig. 4b).

To quantify the relaxation of NPQ upon re-adaptation to dark conditions, the curves for 70–130 min in Fig. 4(d) were fitted to bi-exponential decays, as per previous studies (Kromdijk et al., 2016) and tabulated in Table 1. Relative to the control, the rapid component of NPQ relaxation (NPQ1) was sped up by the core-CD treatment, whereas NPQ2 was accelerated by the glucose-CD treatment compared with the untreated control implied a greater photoinhibition that resulted in increased photodamage by the core-CD treatment, whereas NPQ2 was accelerated by the glucose-CD treatment, indicative of less photodamage (see Table 1).

### Table 1 Calculated photosynthetic parameters in *Triticum aestivum*.

| Parameter               | Control       | Core-carbon dots | Glucose-carbon dots |
|-------------------------|---------------|------------------|---------------------|
| $A_{\text{SAT}}$ (μmol m$^{-2}$ s$^{-1}$) | 22.0 ± 0.8    | 24.0 ± 0.9       | 24.0 ± 0.9          |
| $A_{\text{MAX}}$ (μmol m$^{-2}$ s$^{-1}$) | 31 ± 2        | 31 ± 1           | 30 ± 1              |
| $S_{\text{MAX}}$ (μmol m$^{-2}$ s$^{-2}$) | 222 ± 6       | 336 ± 4          | 244 ± 7             |
| Amplitude of NPQ1       | 0.574 ± 0.008 | 0.50 ± 0.01      | 0.71 ± 0.01         |
| $\tau_{1}$ (s)          | 85 ± 3        | 72 ± 5           | 94 ± 3              |
| Amplitude of NPQ2       | 0.42 ± 0.04   | 0.502 ± 0.004    | 0.29 ± 0.03         |
| $\tau_{2}$ ($\times 10^{3}$ s) | 7 ± 2        | 10.3 ± 0.4       | 3.0 ± 0.6           |

Values obtained from fits to infrared gas analysis and chlorophyll fluorescence measurements. $S_{\text{MAX}}$ is the maximum rate of stomatal opening calculated from Fig. 4(b). The amplitudes of NPQ1 and NPQ2 are the proportions of fast and slow nonphotochemical quenching relaxation respectively with their associated lifetimes $\tau_{1}$ and $\tau_{2}$ as calculated from Fig. 4(d). Errors given are the 95% confidence bounds of fitting, $r^{2} \geq 0.9737$ for all fits. NPQ, nonphotochemical quenching; $S_{\text{MAX}}$, maximum rate of stomatal conductance.

Fig. 4 Kinetics of photosynthetic performance in *Triticum aestivum*. Adaptation kinetics for (a) carbon assimilation ($A$); (b) stomatal conductance ($g_{s}$), the maximum rate of $g_{s}$ ($S_{\text{MAX}}$) values were fitted using a previously developed model (McAusland et al., 2016). (c) Photosystem II operating efficiency ($F_{q}^{a}/F_{m}^{a}$). (d) Non-photochemical quenching (NPQ) kinetics in which the relaxation of NPQ was fitted with a bi-exponential decay (Kromdijk et al., 2016). The periods of dark and light are indicated with black and white boxes, respectively. Light corresponds to a photosynthetic-photon fluence rate (PPFR) of 1000 μmol m$^{-2}$ s$^{-1}$ and dark corresponds to a PPFR of 0 μmol m$^{-2}$ s$^{-1}$ for (c, d) and 100 μmol m$^{-2}$ s$^{-1}$ for (a, b). $n = 5$ except for the control in (a, b), where $n = 4$. Calculated values are given in Table 1 and additional details are given in the Materials and Methods section.
The effect of CD treatment on pigment composition was further investigated by the extraction and quantification of light harvesting and photoprotective pigments by HPLC (Figs 5, S12). No significant differences in pigment ratios were observed between the untreated control and core-CD treatments. Conversely, for the glucose-CD treatment, an increase in the ratio of chlorophylls to carotenoids, Chl\textsubscript{a} to Chl\textsubscript{b} and Chl\textsubscript{a} to lutein were observed compared with untreated controls or core-CD treatments. The increase in the ratio of Chl\textsubscript{a} to lutein supported the earlier conclusion that a reduced carotenoid pool in glucose-CD-treated plants slows NPQ\textsubscript{1}, however there are other processes that could affect NPQ\textsubscript{1} (Nilkens \textit{et al.}, 2010; Demmig-Adams \textit{et al.}, 2014). By contrast no effect was observed on the ratio of Chl\textsubscript{a} to violaxanthin for either CD treatment. In general, the glucose-CD treatment results in an increased production of light harvesting pigments compared with photoprotective pigments and suggests that glucose-CD treatment enabled the plants to maintain reduced levels of photoprotection compared with the control or core-CD treatment.

The effects of the reduced photoprotection for the glucose-CD treatment were further investigated by measuring reactive oxygen species (ROS) production by illumination of chloroplasts isolated from untreated plants that were then treated with CDs. Core-CDs were observed to increase ROS production compared with the untreated control (Fig.S13), however little effect was observed for the glucose-CD-treated chloroplasts, despite the fact that glucose-CD-treated plants produced fewer photoprotective pigments and performed less NPQ (see Figs 4, 5). This suggests that the core-CD treatment resulted in increased photodamage compared with the control, but this was prevented by the glucose functionalisation of the CDs.

**Discussion**

Our results demonstrated that by utilising biomolecule functionalised CDs it is possible to realise increased crop yields. Whilst previous studies with CDs might have provided a route to the beneficial effect of enhancing photosynthesis, none have resulted in significant increased crop productivity: in agreement with observations on unfunctionalised core-CD treatment in this study (Chandra \textit{et al.}, 2014; Li \textit{et al.}, 2016; Xu \textit{et al.}, 2020). This accentuated the need to design a functional NP surface for biological applications. The greater increase of A compared with F\textsubscript{m}/F\textsubscript{0} observed in this paper agreed with the hypothesis previously proposed by Chandra and colleagues that CDs primarily enhanced electron transport in the thylakoid membranes, resulting in augmented photosynthesis rather than acting as optical amplifiers, as recently reported by Xu and colleagues (Chandra \textit{et al.}, 2014; Xu \textit{et al.}, 2020). CDs are most likely able to enhance electron transport because of their ability to easily exchange electrons when photoexcited (Swift \textit{et al.}, 2018). Enhanced electron transport between the photosystems also explained the comparative reduction in NPQ for the glucose-CD treatment at high irradiance, as more electrons can be directed to photochemistry. This effect is present in both the core-CD and glucose-CD treatments however it is mitigated in the core-CD treatment by an increase in ROS production, explaining the observed difference in crop yield between the core-CD and glucose-CD treatments.
CD treatment was able to capitalise on the increased electron transport and chlorophyll production and decrease lutein production, which resulted in an enlarged photosynthetic capacity, whilst core-CD treatment was limited by ROS production. These disparities between the core-CD and glucose-CD treatments were exacerbated by enhanced uptake and internalisation into the mesophyll cells for glucose-CD treatment.

The methods demonstrated here provide a renewable, low-cost and facile route to an increase in *Triticum aestivum* productivity of 18% using functional nanomaterials. If we are to address the increase in crop productivity to meet forecasted global food demand in the future (Zhu et al., 2010; Ray et al., 2013; Tilman & Clark, 2015; Kromdijk & Long, 2016) one potential route could be to embrace the use of nanomaterials to enhance yields. The use of designer NPs has the potential to be combined with previously demonstrated transgenic techniques to bring crop productivity towards a theoretical maximum. These new techniques could also be applied to biofuels, biomass and bio-photovoltaics with the potential to enhance green-energy production.

We have demonstrated the use of glycan-functionalised nanomaterials to obtain an increase in the yield of crop that has eluded previous studies (Giraldo et al., 2014; Xu et al., 2020). These materials also exhibited high bioavailability enabling simple treatment on a larger scale and providing a significant advantage over previous methods. The synthetic routes used to access the nanomaterials were simple and sustainable. As a consequence, we believe that this work represents a step towards the use of designer nanomaterials for agricultural applications.

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Author contributions

TAS, MLY, TAAO, MCG, TL and HMW conceived the experiments. TAS carried out the microscopy, chlorophyll fluorescence, CD–pigment interactions, IRGA and physiology experiments. TAS and DF performed the HPLC. DBA developed the modified synthesis. SAH created the original synthesis. TAS synthesised and characterised the CDs; and TAS, TAAO, HMW and MCG co-wrote the manuscript, which all authors commented on and edited.

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**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.