Effects of plastic-derived carbon dots on germination and growth of pea (*Pisum sativum*) via seed nano-priming

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1. Introduction

As an indispensable component in human society, agriculture is still facing severe challenges in the 21st century. Innovative technologies must be integrated into traditional farming to meet the goal of sustainable agriculture where "zero hunger" is achieved (Usman et al., 2020). Seed nano-priming is a promising technology employed in the agronomic field to promote seed germination and plant growth by utilizing nanomaterials as priming agents prior to sowing (Nile et al., 2022). Recently, carbon dots (CDs) have demonstrated great potential in agricultural applications, owning to their attractive characteristics such as ultrasmall and uniform size, tunable functionalization, high hydrophilicity, facile cell permeation, and benign biocompatibility (Li et al., 2020). However, the use of CDs as a nano-priming agent is still scarce, and the investigation of CD-plant interaction mechanisms regarding different types of CDs and plant species is insufficient.

In the present study, CDs were fabricated using waste polyethylene terephthalate (PET) bottles as the carbon source. Considering the long-lasting negative environmental impact imposed by non-biodegradable plastics, valorization of exhausted plastics into functional CDs offers a sustainable manner for waste volume reduction and environmental pollution alleviation.

2. Material and methods:

Green pea seeds (brand name: Malika Dry Green Peas) were produced in India and purchased from local manufacturer Dashmesh Singapore Pte Ltd in Singapore. All the chemicals were of analytical grade, used as received without further purification, and purchased from commercial suppliers.

CDs were obtained by H_2O_2 -assisted hydrothermal method using waste PET bottles as a carbon precursor. In a typical procedure, waste PET bottles were manually cut into small pieces and placed inside a horizontal tube furnace. Pyrolytic char was obtained after thermal treatment at 400 °C for 2 h under N₂ atmosphere. Then, 50 mg of char was transferred to a Teflon-lined autoclave containing 30 mL 2% H_2O_2 solution and reacted at 110 °C for 15 h to prepare CDs.

Green pea seeds were selected with nearly the same size and plumpness. After sterilization with 5% NaClO solution, the seeds were immersed in Milli-Q water or CD solutions with different concentrations (0.25, 0.5, 0.75, 1, 2 mg/mL) in 50 mL beakers. The beakers were placed on a mechanical shaker (150 rpm) under dark condition at room temperature to ensure the seeds were in full contact with priming solution. After 9 h priming treatment, the seeds were gently rinsed with water, loosely wrapped in a paper towel moisturized with 10 mL Milli-Q water inside a sterile Petri dish for germination. The germination rate was recorded every 6 h until 36 h when all the seeds were germinated. Seed coat surface morphology, wettability as well as seed water uptake capability were assessed after priming.

For post-germination growth, the seeds were then transferred to a 1 mL pipette tip box filled with Milli-Q water, where each seed was placed above the hole and the root was vertically immersed in water. The boxes were exposed to 12h light (mixed red and blue LED light, 3000 lux)/12 h dark, and pea seedlings were harvested after 10 d. Phenotypes of pea seedlings, including root and shoot length, diameter, biomass, and moisture level, were measured after harvest. Biochemical indices such as root vigor, chlorophyll content, carbohydrates content, and antioxidant enzyme activity were also assessed.

3. Results and discussion

As shown in Fig. 1, the water primed seeds held the lowest germination rate while CD solutions of all concentrations, especially the group of 0.75 mg/mL, were more efficient in promoting seed germination. Acidic environment created by CDs induced partial damage to the waxy layer of seed testa, contributing to the enhancement of surface wettability. Moisture content of pea seeds primed with CD solution (65.6%) was significantly higher than that of the control group (61.9%), demonstrating the favorable effect of CDs on enhancing

the water uptake capability of pea seeds. It is expected that the improved seed imbibition observed in the current study would facilitate the seed germination process and promote seedling development.

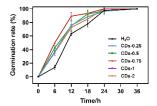


Fig.1. Germination rate of pea seeds primed with CDs of different concentrations (0, 0.25, 0.5, 0.75, 1, and 2 mg/mL)

Phenotypes of pea seedlings were measured after harvest and summarized in Fig. 2. In general, the results demonstrated that CDs have a dose-dependent effect on seedling growth and all the groups treated with CDs (0.25-2 mg/mL) exerted eminent performance when compared with the control group, including increased shoot and root elongation, biomass accumulation, and root moisture level. According to the results obtained so far, the optimal concentration of CDs for promoting seed germination and seedling growth is considered to be 0.75 mg/mL.

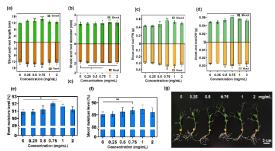


Fig. 2. Effects of CD priming on pea seedling growth. Shoot and root (a) length, (b) diameter, (c) fresh weight (FW), (d) dry weight (DW), (e) root moisture level, and (f) shoot moisture level of pea seedlings after seed priming with water and CD solution. (g) Photograph of pea seedling morphology after CD treatment at different concentrations (0-2 mg/mL)

Besides, the CD-plant interaction during priming may stimulate multiple metabolic pathways, resulting in enhanced antioxidant enzyme system, increased root vigor, chlorophyll content, and carbohydrate content. The presence of CDs inside pea seeds and seedlings was identified using transmission electron microscopy, which may be partially responsible for the regulation of biochemical parameters and the ultimate quality of mature plants.

4. Conclusions

The present study adopted a low-cost, eco-friendly, and facile approach to synthesize CDs using PET bottles through thermochemical conversion followed with H_2O_2 -assisted hydrothermal method. For the first time, plastic-derived CDs were employed as a seed nano-priming agent for the cultivation of green pea, showing beneficial effects on promoting seed germination and seedling development. Seed nano-priming with CDs could effectively avoid excess NMs exposure in the environment. The synthesis of plastic-derived CDs is another sustainable strategy to alleviate environmental pollution caused by non-biodegradable waste. This study provides new insights into the construction of sustainable agriculture and circular economy via nanotechnology.

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