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Biochar nanoparticles enhance flowering in *Gentiana szechenyii* Kanitz. by modulating source-sink carbon allocation and gene expression

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Abstract

Biochar enhances soil nutrient availability and promotes flowering. Biochar nanoparticles (BCNPs), a key biochar component, migrate into plant tissues to regulate flowering; however, the mechanisms remain unclear. We investigated the impact of biochar on *Gentiana szechenyii* Kanitz. (*G. szechenyii*) flowering under nutrient-rich conditions. Biochar (18 g) application increased flower number by 24.2%, with no significant changes in soil N, P, K, Mg, S, Si, Zn, and Mn. Transmission electron microscopy showed increased BCNPs accumulation in chloroplasts and higher starch concentration in leaves, while sucrose levels remained unchanged. ¹³CO₂ isotope tracing revealed reduced ¹³C in leaves and flower branches. Transcriptomic analysis revealed the upregulation of genes involved in sucrose-starch metabolism, flowering regulation, floral meristem development, and hormone. The study shows that BCNPs, after detaching from biochar, migrate into plant tissues and regulate carbon allocation, as well as the expression of flowering- and hormone-related genes, synergistically promoting flowering. BCNPs enhance source activity and sink capacity, with a preferential increase in sink strength, thereby increasing flower number. This study advances the understanding of biochar nanomaterial applications in agriculture.

Highlights

- Biochar nanoparticles promote flowering in *Gentiana szechenyii*.
- Nanoparticles migrate to chloroplasts and modulate sucrose and starch metabolism.
- Biochar nanoparticles upregulated key genes for sugar metabolism, flowering, and hormone.
- Carbon allocation shifts from source to sink, supporting floral development.

Keywords Biochar nanoparticles, *Gentiana szechenyii* Kanitz., Sucrose, Starch, Photosynthate allocation, Flowering

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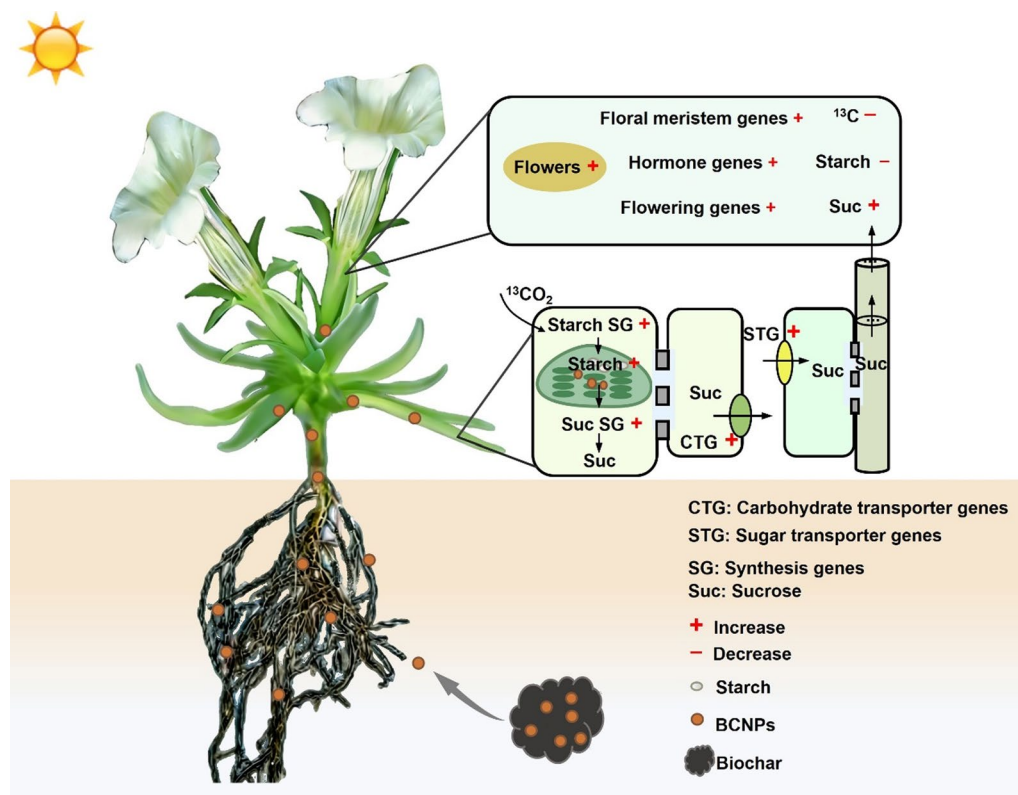
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Graphical Abstract



1 Introduction

Biochar is an emerging, low-carbon, and environmentally sustainable soil amendment that improves soil physicochemical properties, enhances nutrient availability, and promotes root growth, potentially increasing agricultural productivity by up to 20% (Liu et al. 2012; Saini et al. 2025). Its high surface area, cation exchange capacity, and strong adsorption ability (Liu et al. 2012; Thies and Rillig 2009; Woolf and Lehmann 2012) facilitate the adsorption of NH_3 (Spokas et al. 2012) and NH_4^+ (Malińska et al. 2014; Mandal et al. 2016), reducing ammonia volatilization. Biochar also increases soil pH, enhancing alkaline phosphatase activity (Jin et al. 2016; Lopes et al. 2021) and organic acid concentration, thereby improving phosphorus availability (Mendes Gde et al. 2014). The addition of 30% biochar to the growth medium increased nitrogen concentration in *Pelargonium zonale* leaves and significantly boosted flower number (Conversa et al. 2015). Previous studies suggest that biochar promotes plant growth and flowering by enhancing soil nutrient

availability. However, biochar nanoparticles (BCNPs) (diameter < 100 nm), an essential component of biochar, are present at concentrations of 100–1000 ppm. During biochar application, these nanoparticles can easily detach from the biochar matrix, migrate with the solution into plant tissues, and interact with plants (Pereira Lopes and Astruc 2021; Zhang et al. 2020). However, whether BCNPs regulate flowering remains unclear.

Nanoparticles can exert either positive or negative effects on plant flowering. In soil, they are internalized by plant cells via endocytosis, entering the protoplast and, upon reaching the lateral roots, are transported through the xylem to the stem, leaves, and other organs, where they regulate the plant's physiological and molecular balance. Flowering marks the transition from vegetative to reproductive growth, responding to external and internal signals. Silver nanoparticles applied to *Arabidopsis thaliana* downregulated flower organ development genes by 10–40%, delaying flowering by 4–8 days and reducing petal and pollen viability, indicating a negative effect on flower development

(Thakur et al. 2022). In contrast, silica nanoparticles enhanced nutrient uptake, increased photosynthetic pigments, boosted antioxidant capacity, and increased flower production (Attia and Elhawwat 2021).

Application of gold nanoparticles accelerated flowering in *Arabidopsis thaliana* by 5 days (Kumar et al. 2013). This effect was linked to upregulation of leaf ferredoxin-NADP+ oxidoreductase, *PsaA*, and *PsbA* enhancing the photosynthetic rate and sucrose transport to the apical meristem. Additionally, genes involved in gibberellin biosynthesis (*GA20ox2*, *GA20ox3*, *SIGAST*) and the flowering-inducing gene *SFT* were significantly upregulated (Yue et al. 2022). Gold nanoparticles also enhanced the leaf's free radical scavenging potential and antioxidant enzyme activity, reducing the expression of microRNA167 and accelerating flowering. Other studies suggest that the early flowering response is due to nanoparticle accumulation in leaf tissues, which alters the expression of phytochrome B (*PHYB*) and modifies the photoperiod-dependent pathway to induce early flowering (Kumar et al. 2018). Nanoparticles regulate the transcription of flowering-related genes, photosynthetic performance, and hormone synthesis signaling, thereby influencing flowering time and number. However, BCNPs, as a key component of biochar, may also modulate flowering, though the underlying regulatory mechanisms remain unclear.

Gentiana szechenyii Kanitz. (*G. szechenyii*), a perennial, endangered medicinal plant native to high-altitude regions, has its flowers as the medicinally valuable part. This study investigates *G. szechenyii* while ensuring sufficient soil nutrient availability through repeated fertilizations, thus eliminating the potential influence of biochar on flowering via nutrient utilization. We hypothesize that nanoparticles adsorbed onto biochar migrate into the plant, modulating photosynthate synthesis and distribution, and consequently, affecting flowering. Nanoparticle accumulation in roots and leaves was observed, and transcriptomic analysis was conducted with a focus on carbohydrate metabolism pathways. The transcriptional levels of genes related to photosynthate distribution, sugar synthesis, and transport, along with flower traits, were analyzed. This study uncovers the potential mechanism by which BCNPs regulate flowering. From a novel perspective, it further elucidates an important pathway by which biochar promotes flowering, providing theoretical support for the advancement of biochar nanotechnology, the development of biochar nanomaterials, and the progress of sustainable agriculture.

2 Materials and methods

2.1 Experimental materials

Biochar was prepared from maize stalks via pyrolytic carbonization. The maize stalks were crushed, dried, and pyrolyzed at 550 °C for 4 h (purchased from a company). The biochar had the following composition: C 603.21 g kg⁻¹, N 10.52 g kg⁻¹, P 1.47 g kg⁻¹, K 2.09 g kg⁻¹, Mg 1.98 g kg⁻¹. *G. szechenyii* seeds were collected from Aba County, Sichuan Province, China, and grown in seed trays for two years before being used in the experiment.

2.2 Isolation and characterization of BCNPs

Biochar powder was spread onto weighing paper, then removed, leaving fine biochar particles adsorbed on the paper surface. These particles were rinsed off with deionized water, and the suspension was collected. This process was repeated several times to obtain sufficient solution. The collected suspension was divided into two portions: one was used for transmission electron microscopy (TEM) observation, and the other was left to stand for 24 h before centrifugation at 3500 × g for 30 min. The supernatant was freeze-dried for 72 h (SCIENTZ-10N/A, China) to obtain BCNPs powder for physicochemical analysis. BCNPs morphology and size were visualized using a transmission electron microscope (HITACHI H-600, Tokyo, Japan). Samples (0.2 µL) were deposited on 200-mesh copper grids, air-dried, and imaged under TEM.

Dynamic light scattering (DLS) and zeta potential measurements were performed to characterize the physicochemical properties of BCNPs. DLS analysis (Wyatt Technology) was conducted using a polystyrene cuvette with a 633-nm laser and a scattering angle of 90° at 25 °C. BCNP powder was dispersed in deionized water (0.1% w/v) and sonicated for 3 min before measurement. The average particle size distribution and zeta potential were determined using a Zetasizer (ZS-90, Malvern Instruments Ltd., UK), with three replicates per sample.

Raman spectroscopy (HORIBA Scientific LabRAM HR Evolution) was employed to examine the chemical structure of BCNPs, using a 532-nm excitation laser and a spectral range of 100–4000 cm⁻¹ (Yan et al. 2021). Fourier-transform infrared spectroscopy (FTIR; iS 50 Nicolet, Thermo Scientific Inc., Madison, WI, USA) was used to identify functional groups on BCNPs. The specific surface area and pore size distribution were analyzed using a TriStar II Plus 3030 system (Micromeritics, Norcross, GA, USA), based on the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively.

2.3 Experimental design

The experiment was conducted in a solar greenhouse located in Hongyuan County, Aba Tibetan and Qiang Autonomous Prefecture, Sichuan Province (102°34′3.20″E, 32°49′8.40″N, altitude 3497 m). The region has an annual average temperature of approximately 3 °C, with a maximum temperature of 25 °C and a minimum temperature of −24 °C. A single-factor design was employed, with the factor being the biochar application rate. Four treatments were tested: 6 g (T6), 12 g (T12), and 18 g (T18) of biochar per plant, with a control (CK) treatment receiving no biochar. Each treatment was applied to a planting area of 8 m², with row and plant spacing set at 20 cm. Two-year-old seedlings were transplanted into the greenhouse, and fertilization was applied at 20, 50, 80, and 110 days after transplanting. Each plant received 20 g of water-soluble compound fertilizer (N 30%, P₂O₅ 30%, K₂O 10%, B 0.1%, S < 3%, EDTA-Zn 0.1%) at each fertilization interval. Irrigation was provided as necessary to maintain sufficient water and nutrient availability, ensuring that any effects observed on flowering were not due to biochar's impact on nutrient utilization.

2.4 Soil nutrient analysis

Soil samples were collected from the 0–15 cm depth both before transplanting and 142 days (142 d) after transplanting (flowering period). The samples were air-dried, ground, and passed through a 1-mm sieve, then stored in self-sealing bags. pH was measured using a pH meter. Available nitrogen was determined using the alkali diffusion method (Zhao et al. 2020). Available phosphorus was extracted with 0.5 M sodium bicarbonate, and its concentration was measured using the molybdenum-antimony colorimetric method. Available potassium was determined using the ammonium acetate-flame atomic absorption spectrophotometric method (Lamlom et al. 2023). Magnesium and available sulfur were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES). Available silicon was measured by the citric acid extraction–silicomolybdenum blue colorimetric method. Available iron, manganese, and zinc were quantified using atomic absorption spectrophotometry (Zhang et al. 2021). Organic carbon concentration was determined by the potassium dichromate oxidation titration method, and soil organic matter was calculated by multiplying organic carbon by 1.724 (Wang et al. 2020).

2.5 Observation of BCNPs in roots and leaves

During the flowering period, the main root and a 1-cm section of the leaf were harvested. These samples were fixed overnight in 3.5% glutaraldehyde, followed by a

graded ethanol dehydration process. After dehydration, the samples were embedded in LR white resin and cured overnight at 40 °C. Thin sections, approximately 200 nm in thickness, were then prepared using an Ultracut E microtome. To prevent the introduction of artifacts, osmium tetroxide was excluded. Transmission electron microscopy (TEM; HITACHI H-600, Tokyo, Japan) was used to observe and capture images of the samples (Chen et al. 2010).

2.6 Flower traits, root morphology, and leaf SPAD

At 90 and 142 days after transplanting, the number of flowering branches and flowers in each plot was recorded. For leaf SPAD measurements, nine plants were randomly selected from each plot for assessment. Afterward, the roots were excavated and thoroughly washed to remove soil. The cleaned roots were placed in sealable bags and transported back to the laboratory. Root length, surface area, and volume were measured using a root scanner (STD4800 Scanner) and WinRHIZO Pro software. Both roots and aerial parts were dried at 85 °C to determine the dry weights of flowers, leaves, and roots.

2.7 ¹³C Isotope tracing

During the flowering period, a clear, transparent plastic bag (20 cm × 10 cm, with 99.6% light transmittance) was placed over the plant and sealed at the opening. Using a syringe, 10 mL of ¹³CO₂ (99% ¹³CO₂) was injected into the bag, and the injection site was sealed with transparent tape. After one hour of photosynthesis, the remaining air inside the bag was removed using a syringe, and the residual ¹³CO₂ was recovered by injecting NaOH solution.

The bag was then removed, and the plant was separated into leaves and flower branches, which were placed in paper bags and dried at 80 °C to a constant weight. The ¹³C content in each organ was determined using isotope ratio mass spectrometry (Delta VIRMS, Thermo Fisher Scientific, MA, USA) (Chen et al. 2024).

2.8 Sucrose and starch concentration

Sucrose and starch concentrations were measured using the method described by Chen et al. (2024). During the flowering period, approximately 0.1 g of leaf tissue was ground in liquid nitrogen and mixed with 80% ethanol. The mixture was stirred and centrifuged at 3000 × g for 5 min at 25 °C. After removing the supernatant, the residue was mixed with distilled water and placed in a 95 °C water bath for 15 min to completely gelatinize the starch. Subsequently, 18% HCl was added to the extract, and the starch concentration was measured using a spectrophotometer (BioTek Instruments, Inc.) at 620-nm absorbance.

For sucrose concentration, about 0.1 g of leaf tissue was ground in 0.5 mL of 80% ethanol, heated in a 80 °C water bath for 10 min, and then centrifuged at 4000 g for 10 min at 25 °C. After decolorization with 2 mg activated carbon, the sample was centrifuged again at 4000 × g for 10 min at 25 °C, and the supernatant was collected. The absorbance of the supernatant was measured at 480 nm to determine the sucrose concentration.

2.9 Determination of active compounds

For each treatment, six biological replicates were analyzed. Powdered samples (0.5 g) were extracted with 25 mL methanol, weighed, and subjected to ultrasonic treatment (250 W, 40 kHz) for 30 min. After replenishing any lost methanol, the extracts were filtered. Chromatographic analysis was performed on an Inertsil ODS-3 column (50 mm × 4.6 mm, 5 μm; GL Science, Tokyo, Japan). The mobile phase consisted of 0.2% phosphoric acid in water (solvent A) and methanol (solvent B) with the following gradient program: 0–5 min, 20–27% B; 5–12 min, 27% B; 12–55 min, 27–45% B; 55–62 min, 45–48% B; 62–82 min, 48–56% B; 82–85 min, 56% B; 85–90 min, 56–20% B. The flow rate was 1.0 mL min⁻¹, the injection volume was 10 μL, the detection wavelength was 254 nm, and the column temperature was maintained at 35 °C. Standard and sample solutions (10 μL each) were injected into a high-performance liquid chromatography system (Shimadzu, Japan) to obtain chromatograms. Compounds were identified based on the relative retention time (tR) of loganic acid, sweroside, gentiournoside D, and depressine with reference to szechenyin A, and of isoscoparin-2''-β-D-glucopyranoside and isoscoparin with reference to isoorientin. The concentration of the active compounds was then calculated.

2.10 Transcriptomic analysis and real-time PCR

During the flowering period, three plants from each treatment were selected, and leaf samples were collected. Total RNA extraction was carried out using the Trizol reagent kit, following the protocol described by Baute et al. (2015) with modifications. cDNA libraries were constructed, and sequencing was performed using the Illumina HiSeq platform to obtain raw data (Raw reads). The quality of RNA-Seq initial data was assessed using FastQC (Version 0.11.4) and Trimmomatic (Version 0.33), with low-quality reads and sequencing adapters removed. Gene quantification and differential expression analysis were conducted using Cuffdiff software and FPKM values to identify differentially expressed genes. Primers for qRT-PCR were designed using the NCBI Primer Design Tool (Table S1). Reverse transcription products were amplified, and the expression differences

of key genes involved in sugar metabolism were calculated using the $2^{-\Delta\Delta C_t}$ method. The expression levels of genes identified in the transcriptomic analysis were validated by qRT-PCR (Zhou et al. 2019).

2.11 Statistical analysis

Gene Ontology (GO) enrichment analysis and KEGG pathway enrichment analysis of transcriptomic data were performed using Goatools (<https://github.com/tangh-aibao/GOatools>) and KOBAS software (<http://kobas.cbi.pku.edu.cn/home.do>), respectively. Other data were analyzed using Origin software (Origin 2024b, OriginLab Corp., Northampton, MA, USA). For differences between treatments, one-way analysis of variance (ANOVA) was conducted, followed by LSD (Least Significant Difference) test. Statistical significance was set at $p < 0.05$. Error bars and statistical tests are reported in the figure legends.

3 Results

3.1 Characterization of biochar

TEM revealed abundant nanoparticles smaller than 100 nm within the biochar powder (Fig. 1b, c). After dispersion in deionized water and centrifugation, the mean particle size in the supernatant was 122.42 nm, with 19.85% of particles below 100 nm (Fig. 1d, e). Raman spectroscopy showed distinct D (≈ 1344 cm⁻¹) and G (≈ 1586 cm⁻¹) bands, with an I_D/I_G ratio of 1.36, indicating a high degree of structural disorder and defect presence in the carbon matrix (Fig. 1f).

FTIR analysis revealed a broad peak at 3414 cm⁻¹, indicating abundant surface hydroxyl groups (-OH). The absorption at 2914 cm⁻¹ suggests residual aliphatic structures. A pronounced C=C stretching vibration at 1561 cm⁻¹ indicates a highly aromatic structure. The peak at 1076 cm⁻¹ corresponds to C-O bonds in cellulose, hemicellulose, or lignin. The band at 467 cm⁻¹ reflects the presence of inorganic minerals, consistent with high ash content (Fig. 1g). The biochar exhibited a specific surface area of 152.32 m² g⁻¹ and an average pore diameter of 18.04 nm (Fig. 1h). A portion of the biochar powder consists of nanoparticles smaller than 100 nm.

3.2 Soil nutrients and pH

Available N, P, K, and pH in the 0–15 cm soil layer were measured at 0 and 142 days after transplanting. No significant differences were observed among CK, T6, T12, and T18 at 0 days (Fig. 2a, c, e, g). After 142 days, N, P, and K levels remained similar across treatments, while soil pH in T6 was significantly higher than in CK, with no differences among T6, T12, and T18 (Fig. 2b, d, f, h). T18 and T12 exhibited significantly higher organic matter and organic carbon concentrations than CK (Fig. 2i, j). No

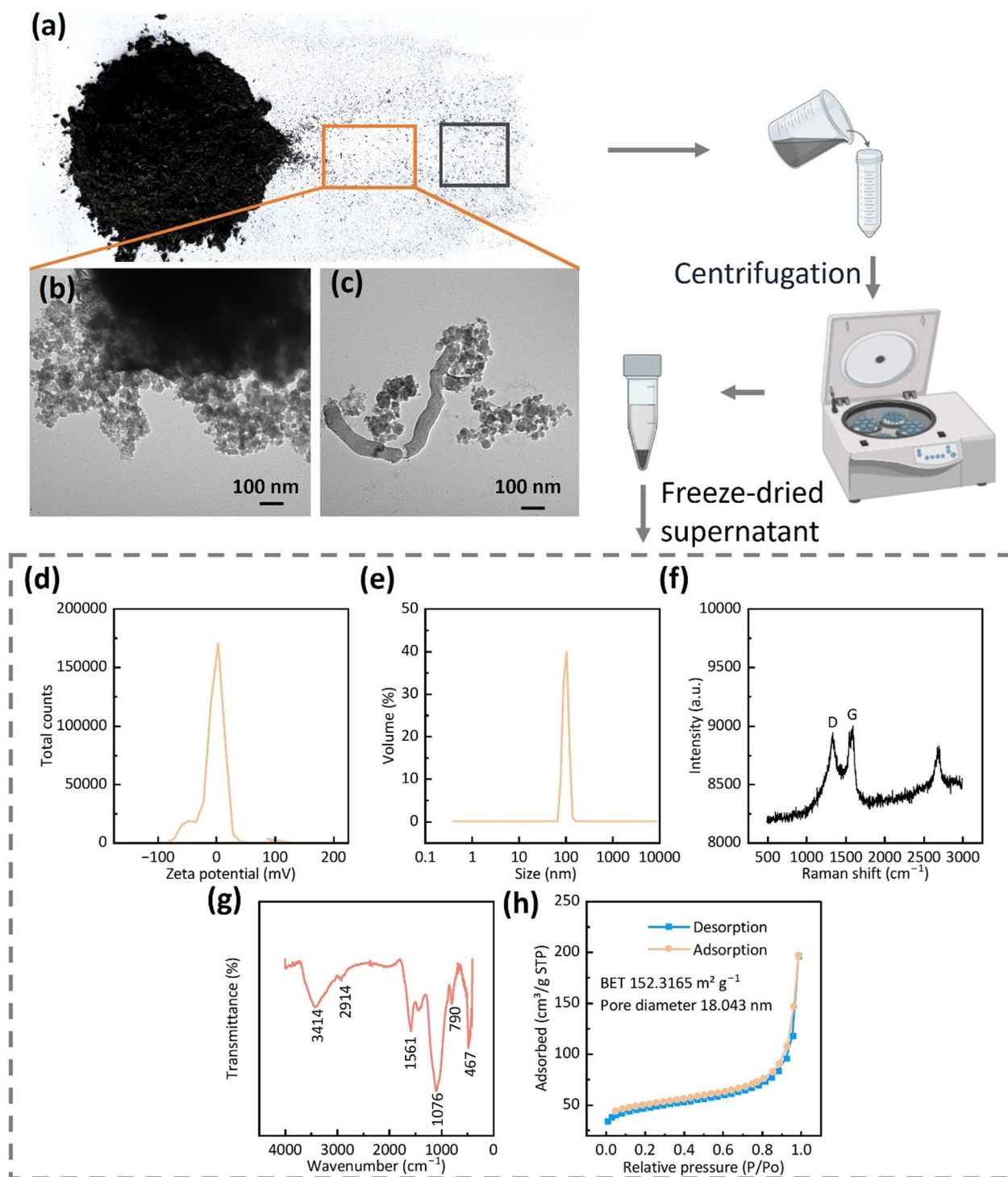


Fig. 1 Biochar image, transmission electron microscope (TEM) images and characterization of biochar microparticles. **a** Biochar and biochar microparticles. The microparticles are adsorbed on the background plate; **b, c** Biochar microparticles magnified 120,000 times by TEM, containing a large number of particles with a particle size of less than 100 nm (also known as biochar nanoparticles); **d** Zeta potential measurements of biochar; **e** DLS of biochar; **f** Raman spectrum of biochar; **g** FTIR spectra of biochar; **h** N₂ adsorption–desorption curve of biochar

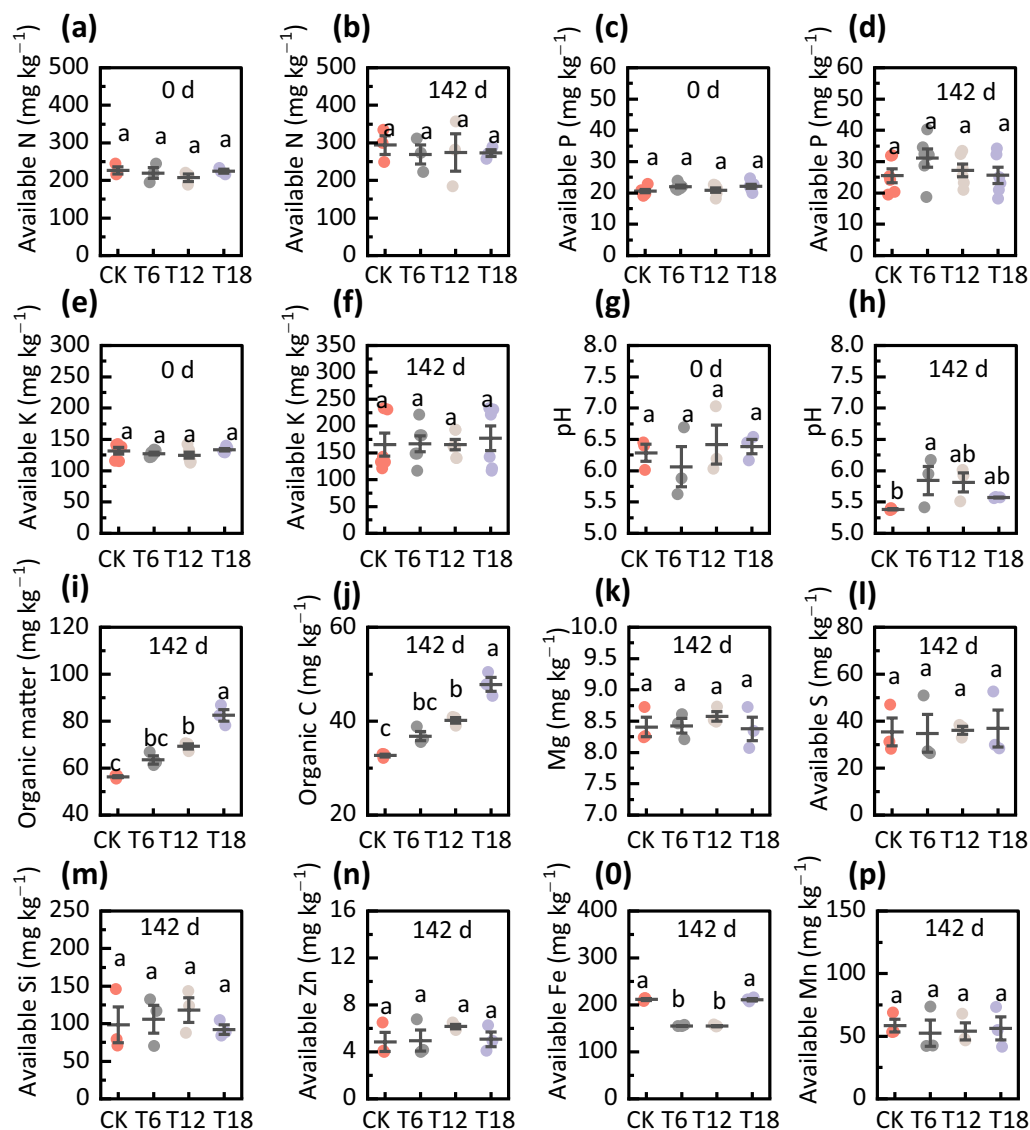


Fig. 2 Soil nutrient concentrations and pH. **a** Available nitrogen, **c** available phosphorus, **e** available potassium, and **g** pH of the soil before transplanting. **b** Available nitrogen, **d** available phosphorus, **f** available potassium, and **h** pH of the soil 142 days after transplanting. **i–p** Organic matter, organic carbon, available magnesium, available sulfur, available silicon, available zinc, available iron, and available manganese, respectively. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK). Different lowercase letters mean significant differences ($p < 0.05$) and error bars show the standard deviation of the mean. Short cross lines represent the mean

significant differences were observed among treatments for Mg, available S, available Si, available Zn, or available Mn (Fig. 2k–p). These results indicate that biochar application did not significantly alter major nutrients under adequate fertilization.

3.3 BCNPs in leaf and root cells

No nanoparticles were observed in CK leaf cells, while T6, T12, and T18 showed clear nanoparticle presence (Fig. 3a–d). Nanoparticle abundance in all treatments

was significantly higher than in CK, though differences among T6, T12, and T18 were not significant (Fig. 3f). No nanoparticles were detected in T18 root cells (Fig. 3e). T6 had significantly more chloroplast starch granules than CK; T12 and T18 also showed increases, but not significantly (Fig. 3g).

3.4 Flower traits and dry weight

At 90 days post-transplanting, flowering branch numbers did not differ significantly among CK, T6, T12, and

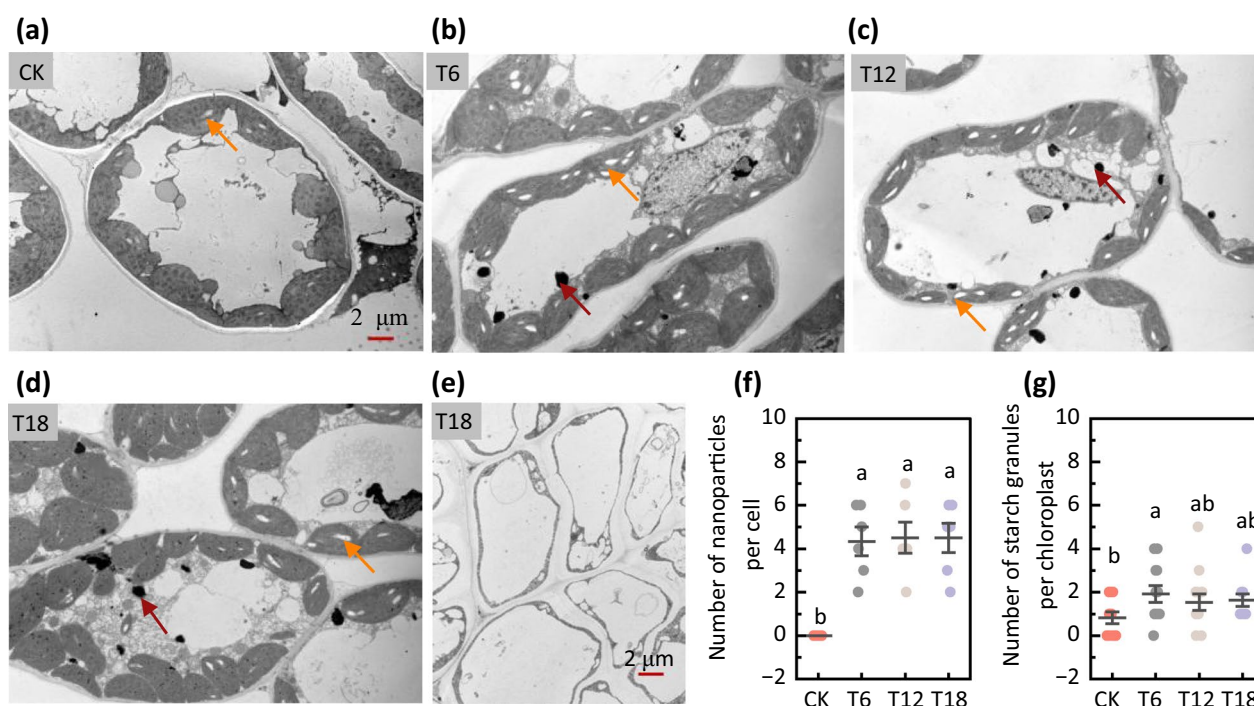


Fig. 3 Transmission electron microscopy (TEM) images of leaf and root tissues. **a–d** TEM images of leaves from biochar-applied treatments and the control; **e** TEM image of root tissues from the T18 treatment; **f** The number of nanoparticles per cell; **g** The number of granules per chloroplast. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK). The red arrows indicate the nanoparticles, and the orange arrows indicate the starch granules. Different lowercase letters mean significant differences ($p < 0.05$) and error bars show the standard deviation of the mean. Short cross lines represent the mean

T18 (Fig. 4e). By 142 days, flower number in T18 was significantly higher than in CK and T6, but not different from T12 (Fig. 4g). Flower dry weight in T18 was similar to CK but significantly lower than in T6 (Fig. 4h). Leaf dry weight in T18 was significantly lower than in CK and T6, with no difference from T12 (Fig. 4i). SPAD values showed no significant differences among treatments (Fig. 4j). These results suggest that 18 g biochar (T18) increased flower number but reduced leaf biomass.

3.5 Root morphology

Root dry weight and length in T18 showed no significant differences compared to CK and T12, while root dry weight in T6 was significantly higher than in CK (Fig. 5e, f). Root surface area and volume did not differ significantly among treatments (Fig. 5g, h). These results suggest that biochar at the T6 level promoted root dry weight, but higher application rates had no further effect on root morphology.

3.6 ^{13}C and sugar concentrations in leaves and flowering branches

After $^{13}\text{CO}_2$ labeling, ^{13}C content in T18 leaves was significantly lower than in T6 and T12 leaves, but not different

from CK (Fig. 6b). In flowering branches, ^{13}C content in T18 was significantly lower than in all other treatments (Fig. 6c). Leaf sucrose levels were similar across treatments (Fig. 6d), while flowering branch sucrose was significantly higher in T18 than in CK (Fig. 6e). T18 leaves had significantly more starch than CK and T6 (Fig. 6f), but flowering branch starch was significantly lower than in CK and T6 (Fig. 6g). These results indicate that T18 reduced ^{13}C allocation and starch accumulation in flowering branches, while increasing sucrose levels.

3.7 Concentrations of active compounds in flowers

Compared with CK, loganic acid concentration was significantly higher in T6, while T12 and T18 showed no significant differences from CK; T18 was significantly lower than T6 and T12 (Fig. 7a). No significant differences in sweroside and gentiouranoside D concentrations were observed among CK, T6, T12, and T18 (Fig. 7b, f). The concentration of isoscorparin-2"- β -D-glucopyranoside did not differ between CK and the treatments, but was significantly lower in T18 than in T6 and T12 (Fig. 7d). Depressine levels in T6, T12, and T18 were significantly lower than in CK, with no

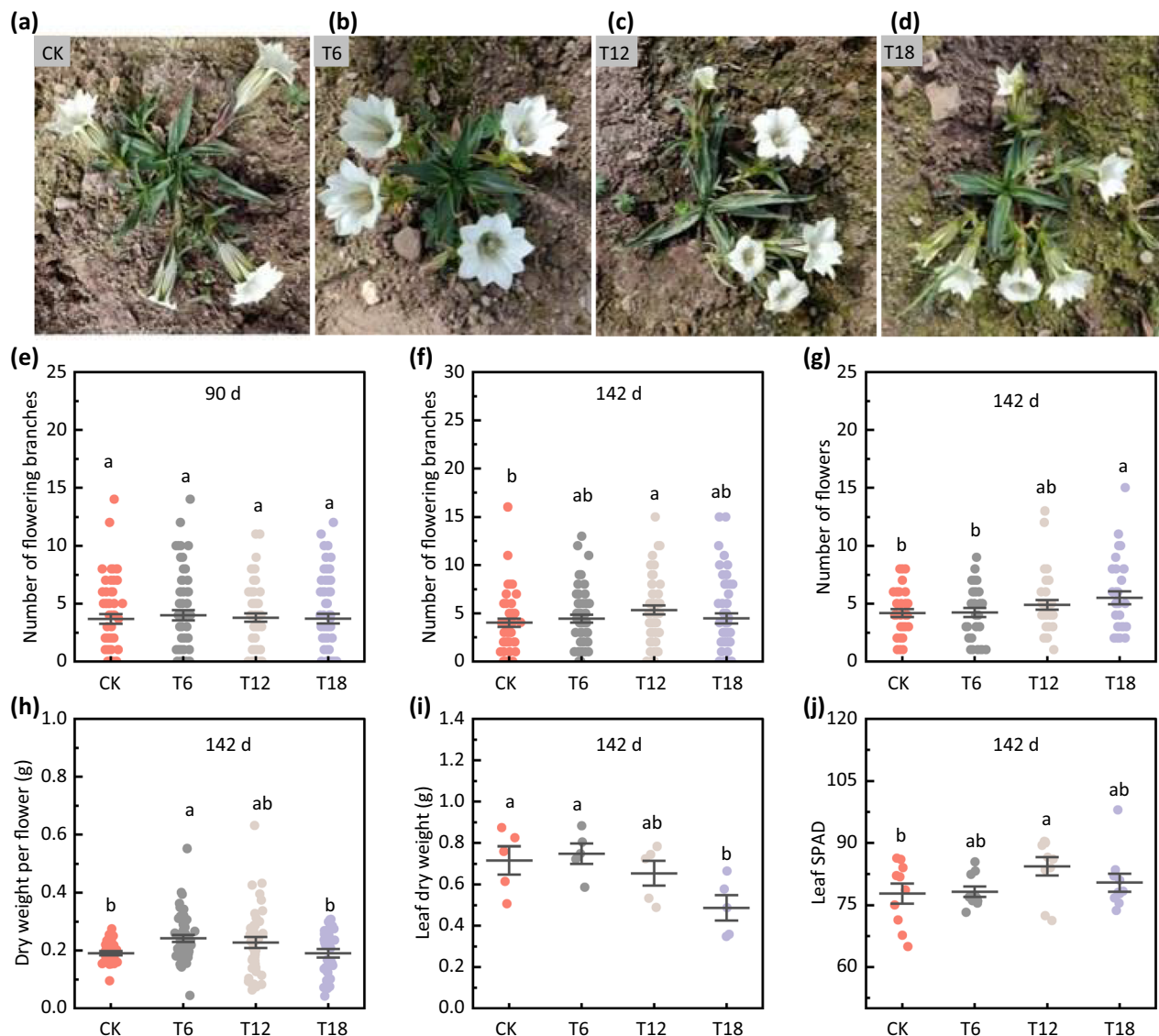


Fig. 4 Flower traits and dry matter weight. **a–d** Photos taken 142 days after biochar application; **e, f** Number of flower branches, **g** number of flowers, **h** dry weight of flowers, **i** dry weight of leaves, and **j** SPAD value of leaves. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK). Different lowercase letters mean significant differences ($p < 0.05$) and error bars show the standard deviation of the mean. Short cross lines represent the mean

differences among the three treatments (Fig. 7g). Szechenyin A concentration in T18 was significantly higher than in CK and T6 (Fig. 7h). Total active compounds in T18 were significantly higher than in CK, T6, and T12 (Fig. 7i). At the highest biochar application level (T18), loganic acid, isoscoparin-2''- β -D-glucopyranoside, and isoscoparin concentrations declined compared with T6 and T12, showing no difference from CK, whereas szechenyin A and total active compounds increased.

3.8 Transcriptomic analysis of leaf tissues

Transcriptome sequencing of leaves under different biochar treatments revealed substantial transcriptional changes. Compared to CK, T6 showed 7179 differentially expressed genes (DEGs: 3561 upregulated, 3618 downregulated; Fig. 8a), T12 had 3488 DEGs (2424 upregulated, 1064 downregulated; Fig. 8b), and T18 had 4564 DEGs (2463 upregulated, 2101 downregulated; Fig. 8c). KEGG enrichment analysis indicated that DEGs were mainly enriched in the starch and sucrose metabolism

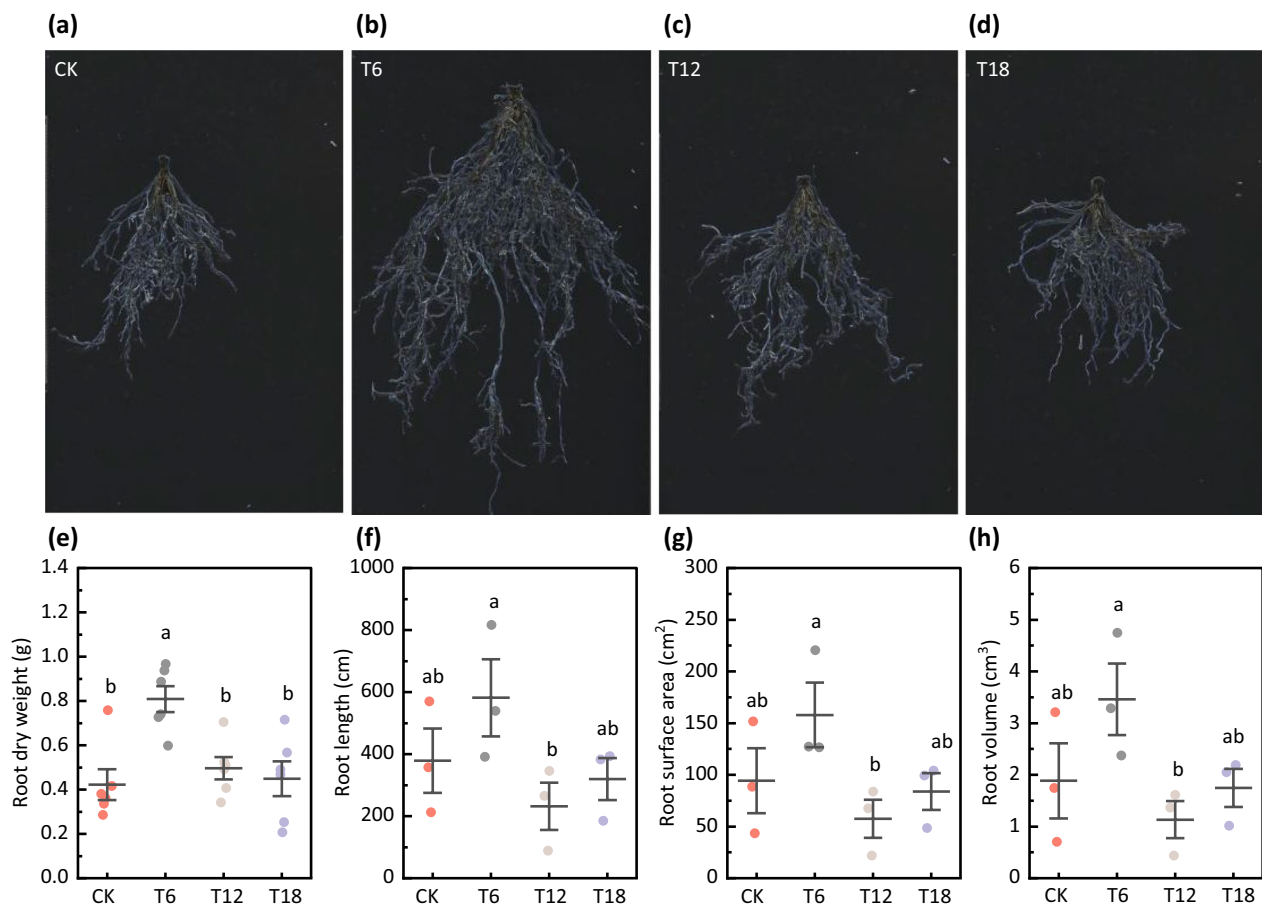


Fig. 5 Root morphology. **a–d** Photos of roots 142 days after biochar application; **e** Root dry weight, **f** Root length, **g** Root surface area, and **h** Root volume. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK). Different lowercase letters mean significant differences ($p < 0.05$) and error bars show the standard deviation of the mean. Short cross lines represent the mean

pathway (Fig. 8d–f). GO analysis highlighted sugar transmembrane transporter activity and carbohydrate transmembrane transporter activity as the top enriched categories (Fig. 8g–i).

3.9 Expression levels of key genes

Based on transcriptome analysis and functional annotation, genes related to flowering, plant hormone, and floral meristem development were identified. Among flowering-related genes, TRINITY_DN1143_c0_g1, TRINITY_DN1582_c0_g1, TRINITY_DN1296_c1_g1, TRINITY_DN3178_c0_g2, TRINITY_DN11435_c2_g1, and TRINITY_DN377_c0_g2 were upregulated in T18 and T12 compared with CK (Fig. 9a). In hormone-related genes, TRINITY_DN17480_c0_g1 showed higher expression in T18 and T12 than in CK, while TRINITY_DN26091_c0_g1 was upregulated in T12 (Fig. 9b). For floral meristem-related genes, TRINITY_DN5852_c0_g1

and TRINITY_DN29230_c1_g1 exhibited higher expression levels in T18 and T12 than in CK (Fig. 9c).

ROS-related genes (TRINITY_DN35564_c0_g1 and TRINITY_DN16872_c0_g1) were upregulated (Fig. 9d). Similarly, several SOD-related genes (TRINITY_DN23459_c0_g2, TRINITY_DN23459_c0_g1, TRINITY_DN59738_c0_g1, TRINITY_DN1893_c0_g1, and TRINITY_DN62891_c1_g1) also showed increased expression levels (Fig. 9e).

3.10 Expression and validation of genes related to sucrose and starch metabolism and transport

Triose-P, the precursor for sucrose and starch biosynthesis, is exported from the chloroplast by the Triose-P/Pi translocator (TPT). Expression of TPT-related genes (TRINITY_DN18301_c0_g1_i6) did not differ significantly among treatments. Genes involved in sucrose synthesis (TRINITY_DN77407_c0_g1_i1/i3, TRINITY_DN37125_c0_g1_i1) were upregulated in T12 and T18.

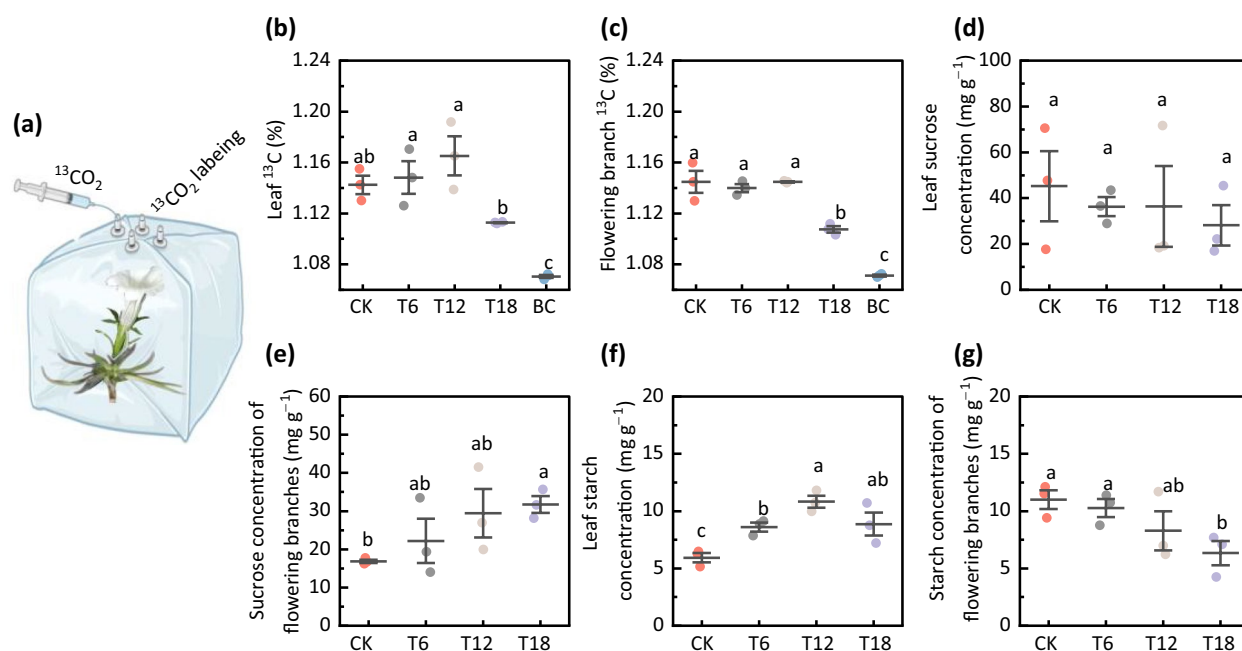


Fig. 6 ^{13}C content and sugar concentration in leaves and flower branches. **a** Schematic diagram of $^{13}\text{CO}_2$ -labeled plants; **b** ^{13}C content in leaves; **c** ^{13}C content in flower branches; **d** Sucrose concentration in leaves; **e** Sucrose concentration in flower branches; **f** Starch concentration in leaves; **g** Starch concentration in flower branches. BC: blank control, natural abundance isotope in organs. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK). Different lowercase letters mean significant differences ($p < 0.05$) and error bars show the standard deviation of the mean. Short cross lines represent the mean

Starch synthesis genes (TRINITY_DN7881_c0_g1_i16, TRINITY_DN10786_c0_g1_i13/i12) showed increased expression in T18. Carbohydrate transporter genes (TRINITY_DN6532_c0_g1_i12, TRINITY_DN40997_c0_g1_i1) were upregulated in all biochar treatments. Sugar transporter genes (TRINITY_DN872_c0_g1_i2/i3/i10) were more highly expressed in T18 (Fig. 10).

qRT-PCR validation of seven sugar metabolism and transport-related genes, using TRINITY_DN16781_c0_g1 (Stable expression) as an internal control, showed strong correlation with RNA-seq data ($R^2 = 0.874$), confirming the accuracy of the transcriptomic results (Fig. 11a–h).

4 Discussion

4.1 Migration pathway and uptake of BCNPs in soil

Biochar exists in soil in two forms: particles smaller than $0.45 \mu\text{m}$ are considered soluble and mobile, while those larger than $0.45 \mu\text{m}$ are insoluble (Yang et al. 2021). In this study, the powdered biochar particles had an average diameter of 122.42 nm , with 19.85% of particles below 100 nm , classifying them as nanoparticles capable of migrating through soil (Fig. 1e). The uptake of nanoparticles by plants is influenced by factors such as particle size, plant species, nanoparticle type, and growth stage. Among these, particle size is the most critical

determinant of root absorption. However, reported size thresholds for nanoparticle uptake vary across studies. For instance, tobacco roots can absorb 3.5-nm AuNPs, while 18-nm AuNPs tend to aggregate on the root surface (Sabo-Attwood et al. 2012). In contrast, *Arabidopsis* roots fail to absorb 7–108 nm AuNPs (Taylor et al. 2014), but can internalize 200-nm SiO_2 nanoparticles, with fewer particles observed as size increases (Slomberg and Schoenfisch 2012). Most studies report the mean size of pristine nanoparticles, yet plant uptake likely depends on the smallest fraction of particles rather than the average size. Once released into the environment, nanoparticles undergo various biotic and abiotic transformations that alter their physicochemical properties (Lowry et al. 2012), meaning that environmental nanoparticles may differ substantially from their original form.

Additionally, contact between nanoparticles and plant roots typically occurs through surface adsorption. Root hairs secrete mucilage and organic acids, resulting in a negatively charged root surface. Consequently, positively charged nanoparticles are more readily adsorbed onto root surfaces and subsequently absorbed (Lv et al. 2019). Nanoparticle uptake is positively correlated with root surface area. However, under conditions of sufficient water and nutrients, biochar application did not significantly affect root surface area in *G. szechenyii*

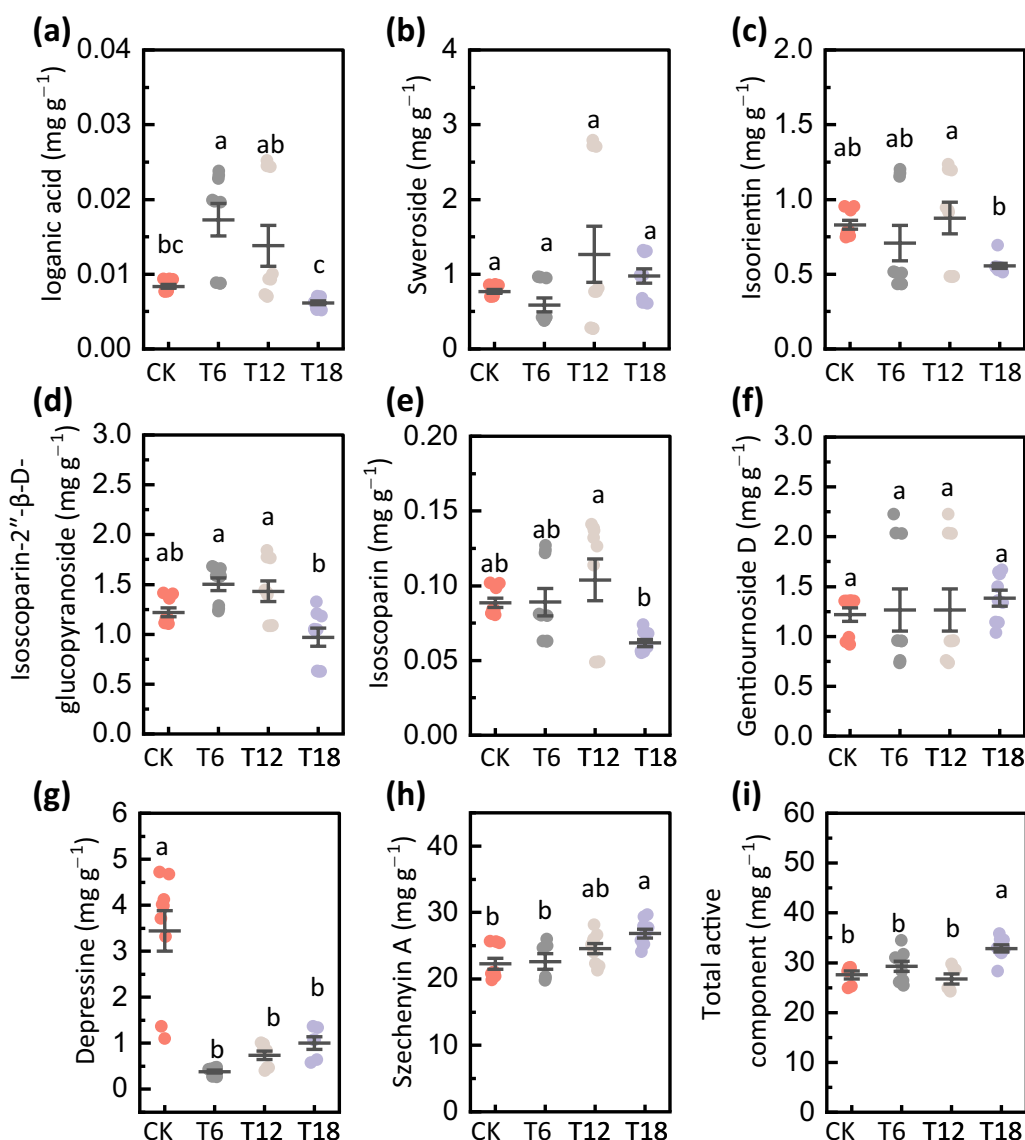


Fig. 7 Active compound concentration in flowers. **a–h** Loganic acid, sweroside, isoorientin, isoscoparin-2''- β -D-glucopyranoside, isoscoparin, gentiouranoside D, depressine, and szechenylin A; **i** Total active compounds. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK). Different lowercase letters mean significant differences ($p < 0.05$) and error bars show the standard deviation of the mean. Short cross lines represent the mean

(Fig. 5g), suggesting that the plant's capacity to absorb nanoparticles remained unchanged. Thus, the level of nanoparticle accumulation in the plant likely depended on their concentration in the soil rather than on morphological changes in the roots.

The epidermal layers of root tips and root hairs are often not fully developed, allowing nanoparticles to enter the root stele or xylem (Péret et al. 2009; Rajput et al. 2020). Once inside plant tissues, nanoparticles may be taken up by cells via ion channels or endocytosis (Lv et al. 2019). Protoplasts are capable

of internalizing particles smaller than $1 \mu\text{m}$ through endocytosis (Liu et al. 2009; Ma and Yan 2018). In this study, ultrastructural observations revealed no nanoparticle accumulation in root cells, while BCNPs were detected in chloroplasts of leaf cells (Fig. 3a–e). The absence of observed nanoparticles in roots may be due to transverse sectioning, which captures only a single cross-section and may have missed regions where nanoparticles were actually enriched. While previous studies have primarily focused on the effects of biochar on soil physicochemical properties (Lopes

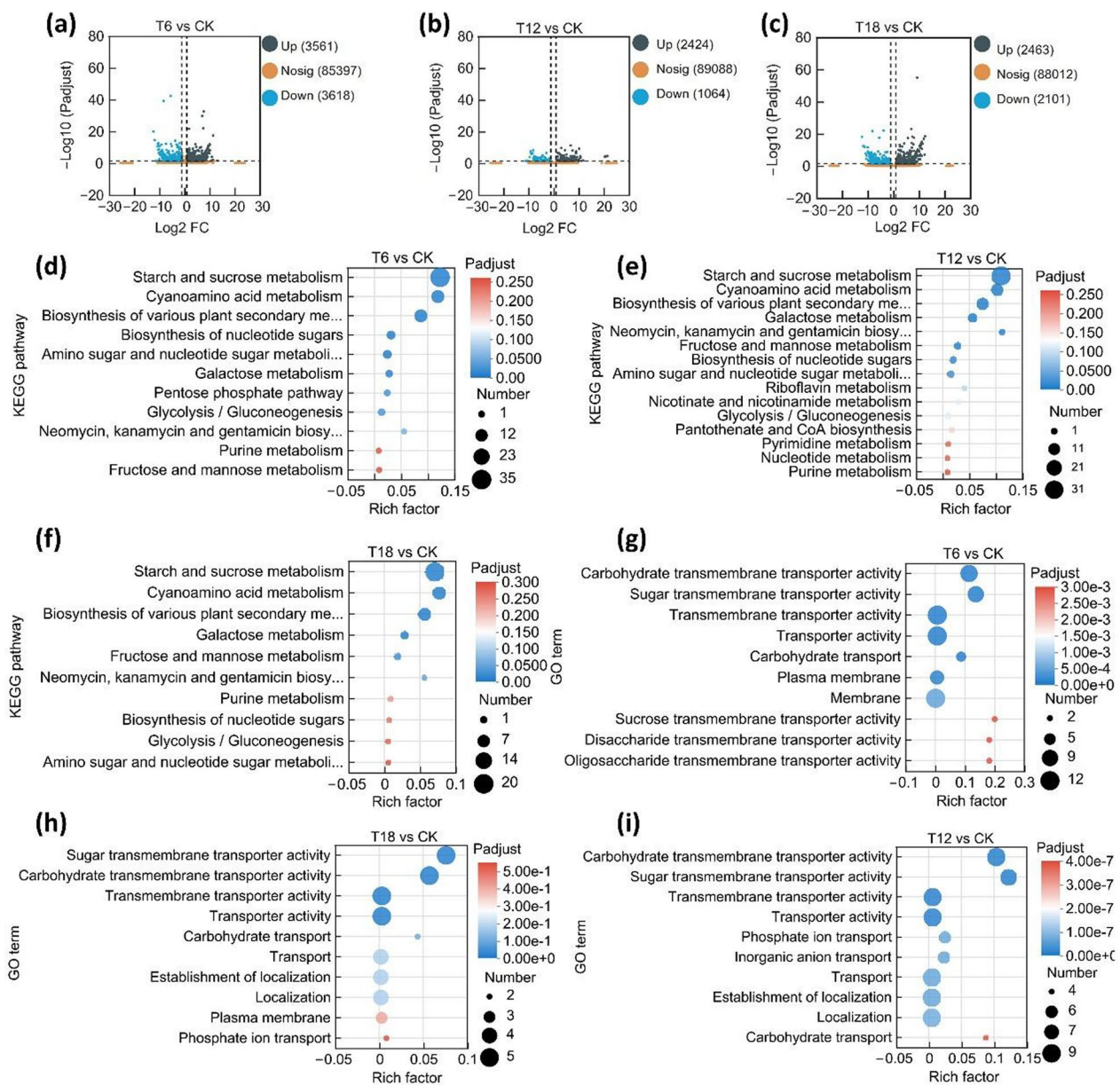


Fig. 8 Analysis of differential gene expression (DEGs) and pathway enrichment. **a** A volcano plot showing DEGs between T6 and CK (T6 vs CK); **b** A volcano plot showing DEGs between T12 and CK (T12 vs CK); **c** A volcano plot showing DEGs between T18 and CK (T18 vs CK); **d-f** KEGG enrichment plots of DEGs between the treatment and the control (T6 vs CK, T12 vs CK, T18 vs CK). The top 15 KEGG pathway enrichments with DEGs are listed here; **g-i** The top 10 enrichment GO terms of DEGs between the treatment and the control (T6 vs CK, T12 vs CK, T18 vs CK). The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK)

et al. 2021; Mendes Gde et al. 2014), our findings provide new evidence that nano-scale biochar particles can detach from bulk biochar in soil, be absorbed by roots, and enter plant cells, where they may participate in metabolic and developmental processes.

4.2 Regulation of sucrose and starch metabolism by BCNPs

Sucrose synthesis in plants is a critical biochemical process primarily catalyzed by the enzymes sucrose-phosphate synthase (SPS) and sucrose-phosphatase (SPP) (Hu et al. 2022). This pathway is not only essential for plant growth and development but also for responding

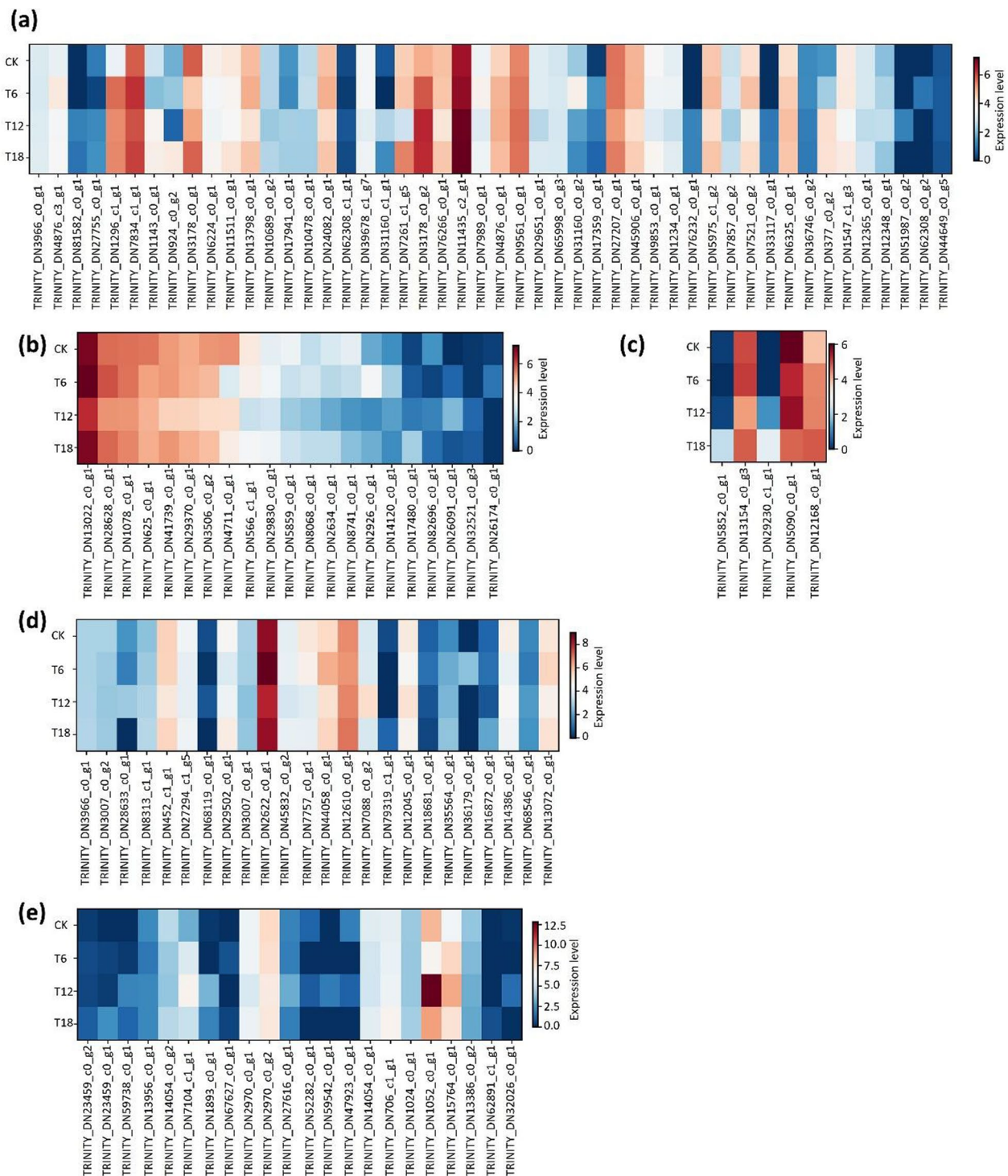


Fig. 9 Expression levels of key genes. **a** Expression of flowering-related genes; **b** Differential expression of genes involved in plant hormones; **c** Expression of genes associated with floral meristem; **d** Expression of genes associated with ROS; **e** Expression of genes associated with SOD. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK)

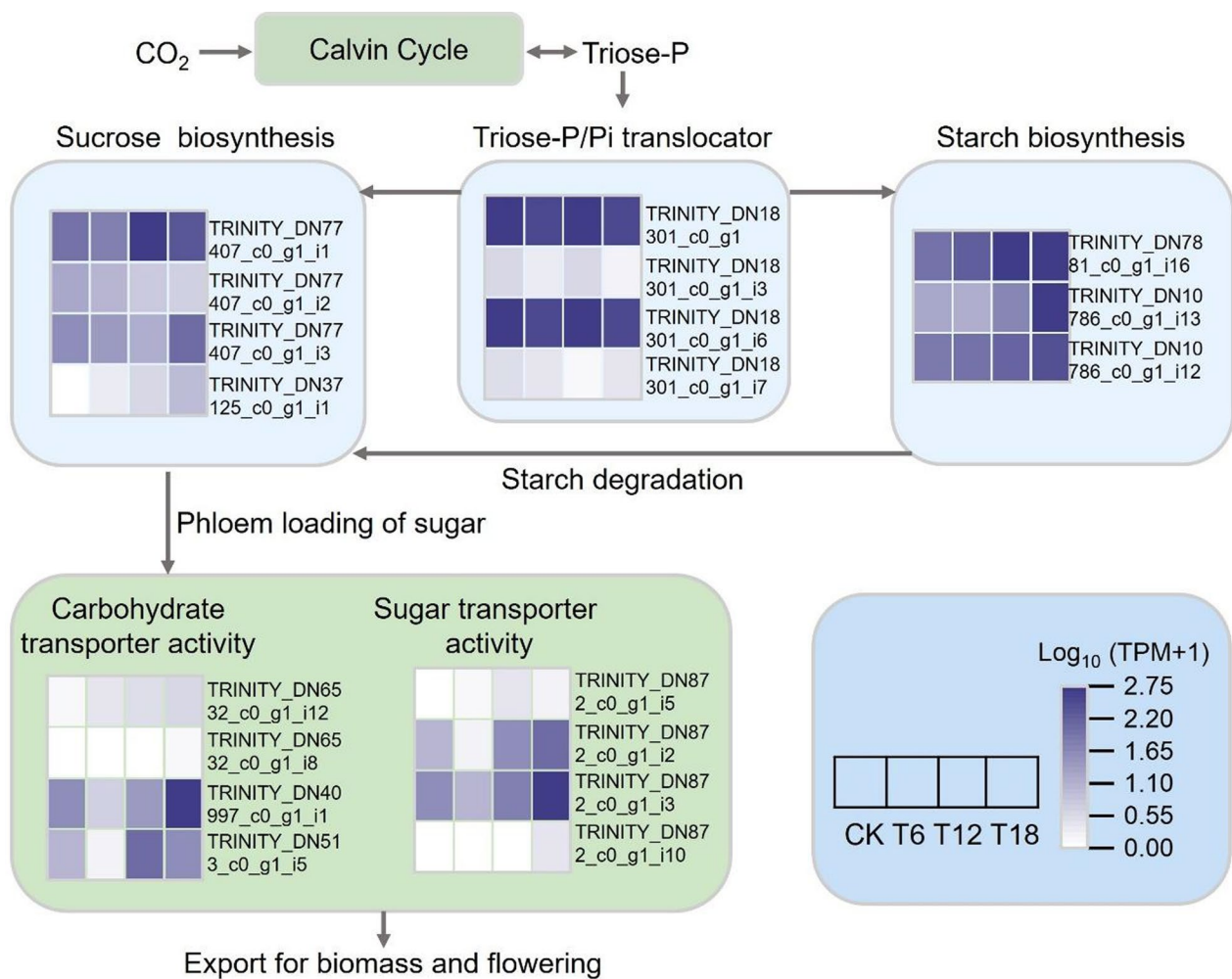


Fig. 10 Changes in the expression of differentially expressed genes involved in sucrose and starch biosynthesis and sugar transport processes. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK)

to environmental stresses (Maloney et al. 2015). Nanoparticles can significantly enhance carbon metabolism and promote sugar biosynthesis in plants. SiO₂ nanoparticles have been reported to upregulate key genes involved in soluble sugar metabolism, including *SPS*, *SUS*, and *α-glucosidase* (Li et al. 2023). In maize, Fe₃O₄ nanoparticles promote sucrose translocation from leaves to grains, reducing leaf sucrose and starch levels (Yan et al. 2020). In this study, biochar application modulated carbohydrate metabolism through nanoparticle-mediated mechanisms. KEGG pathway enrichment analysis revealed significant enrichment of the sucrose and starch metabolism pathway (Fig. 8d–i). In T18 leaves, transcript levels of key sucrose synthesis genes were markedly upregulated (Fig. 10). Despite this, leaf sucrose levels remained comparable to controls, likely

due to a dynamic equilibrium between sucrose production and export.

Transcriptomic analysis revealed upregulation of key sucrose transporter genes, indicating enhanced sucrose export from leaves (Fig. 10). Consistently, sucrose concentration increased in flower branches, while leaf levels remained unchanged (Fig. 6d and e). ¹³C isotope tracing supported this, showing reduced ¹³C accumulation in T18 leaves, consistent with increased export of photosynthates (Fig. 6b). Nanoparticles are known to improve photosynthetic activity; in pepper, foliar application of graphene nanosheets enlarged chloroplasts and elevated leaf sucrose and starch concentration (Younes et al. 2019). Together, these findings suggest that BCNPs enhance both the synthesis and translocation of sucrose in source leaves.

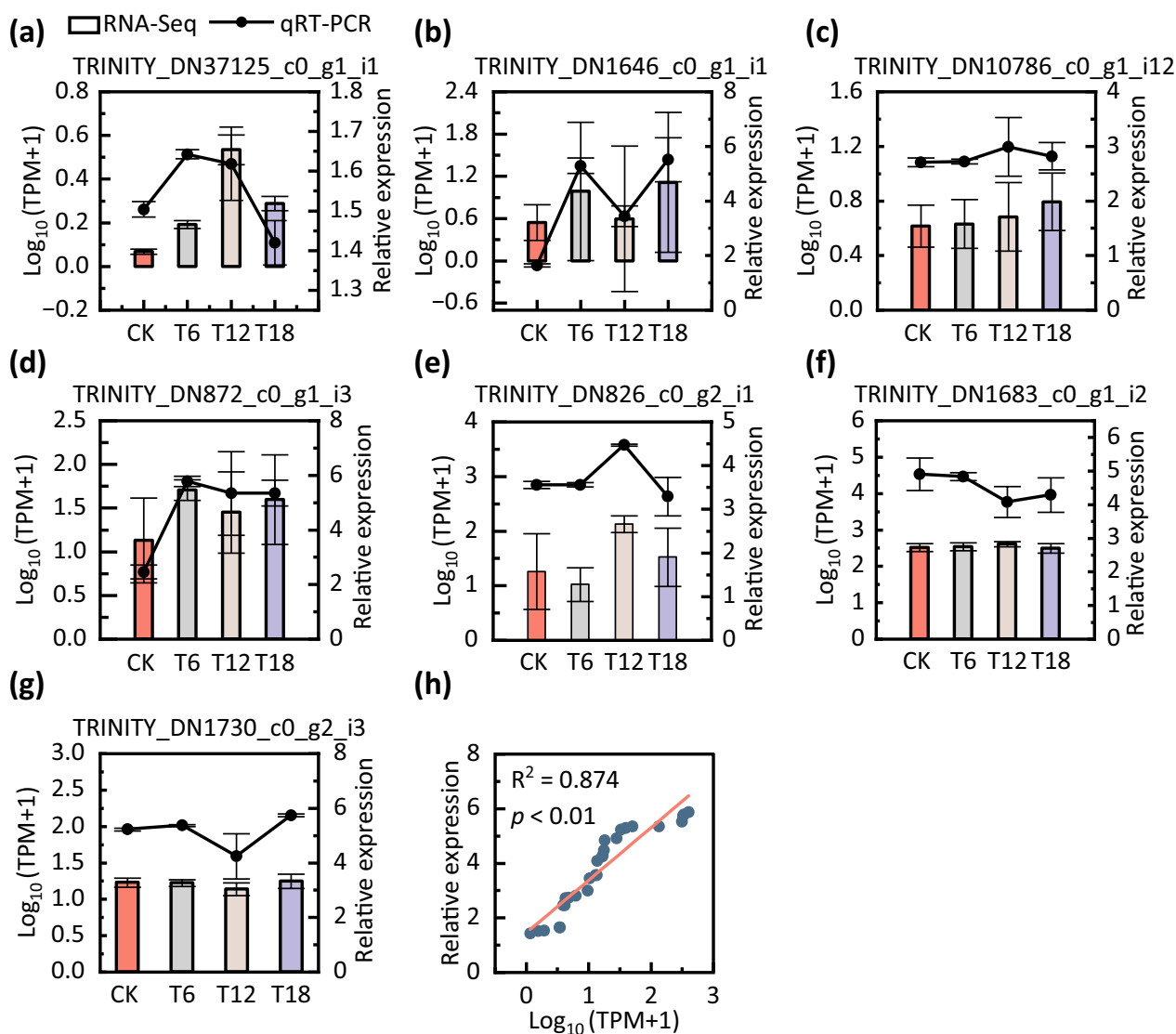


Fig. 11 Validation of DEGs by qRT-PCR. **a–g** Seven randomly selected DEGs related to sucrose and starch metabolism were validated by qRT-PCR. Data represent the mean $\pm$ SD; **h** Correlation between qRT-PCR and RNA-Seq. The application rates of biochar per plant were 6 g (T6), 12 g (T12), and 18 g (T18), and the treatment without biochar application served as the control (CK)

Starch is another major carbohydrate in plants. BCNPs increased both the number of starch granules and the overall starch concentration in *G. szechenyii* chloroplasts (Fig. 3g and Fig. 6f). In contrast, toxic copper oxide nanoparticles reduced chloroplast starch granule abundance in barley (Rajput et al. 2019). Key enzymes in starch biosynthesis include ADP-glucose pyrophosphorylase (AGPase) and granule-bound starch synthase (GBSS). Zn-chitosan nanoparticles have been shown to enhance sink activity in wheat via various pathways (Kumar et al. 2021). In this study, biochar application upregulated the expression of starch

biosynthesis-related genes in T18 (Fig. 10). Starch functions as an energy reserve, temporarily storing excess photosynthates and being degraded to meet energy demands when necessary (Liu et al. 2023). The increase in leaf starch concentration following biochar application suggests sufficient photosynthate production and enhanced source activity. The reduced starch concentration in flower branches is likely due to an increased flower number and corresponding higher sugar demand, resulting in starch degradation and redistribution to support flower growth.

Notably, biochar application increased flower number but reduced the dry weight of individual flowers,

suggesting that BCNPs promote flowering upon entering plant tissues (Fig. 4g and h). Although photosynthate increased, the greater number of flowers likely led to reduced carbohydrate allocation per flower, resulting in decreased flower dry weight. The source-sink balance influences carbon partitioning, biomass accumulation, and yield (van den Herik et al. 2021). These findings further demonstrate that BCNPs enhance both source activity and sink capacity, with a preferential strengthening of sink strength.

4.3 BCNPs promote flowering in *G. szechenyii*

This study aimed to distinguish whether flowering in *G. szechenyii* is regulated by biochar itself or by biochar-derived nanoparticles. Although previous studies have demonstrated that biochar enhances soil nutrient availability, particularly nitrogen and phosphorus, thereby promoting flowering (Conversa et al. 2015; Malińska et al. 2014; Mandal et al. 2016), the role of BCNPs in directly modulating plant development remains poorly understood. To exclude the confounding effect of improved nutrient availability, we applied repeated fertilization with N, P, K, and micronutrients to ensure nutrient sufficiency. Post-fertilization analysis showed no significant differences in available soil N, P, K, Mg, Si, Zn, or Mn between biochar-treated and control groups (Fig. 2a–p). Notably, BCNPs were detected within leaf chloroplasts of treated plants, with a significantly higher nanoparticle density compared to the control. Concurrently, T18 plants exhibited a marked increase in flower number (Fig. 4g). These findings suggest that BCNPs, rather than nutrient effects alone, may act as active agents in promoting flowering.

It is undeniable that biochar not only alters soil nutrient availability but also affects the composition of soil microbial communities (Enebe et al. 2025). Soil microorganisms decompose organic matter, fix nitrogen, and solubilize phosphorus and potassium (Kallenbach et al. 2016). In the rhizosphere of *P. tabuliformis*, the dominant genera *Acidobacteriales_uncultured*, *Holophaga*, and *Bradyrhizobium* are involved in organic matter degradation (Banerjee et al. 2016). Biochar application has been shown to increase the abundance and activity of nitrogen-fixing bacteria such as *Azospirillum* and *Bradyrhizobium* (Abujabhah et al. 2018). *Bacillus* species contribute to phosphorus and potassium solubilization (Shahid et al. 2022). As key decomposers and transformers, microorganisms convert unavailable nutrients into plant-accessible forms. However, since this study was conducted under conditions of adequate nutrient and water supply, the microbial contribution to *G. szechenyii* flowering was minimal, indicating that biochar nanoparticles played the dominant regulatory role.

Carbohydrates serve not only as energy sources but also as signaling molecules that regulate flowering. In rice, sucrose promotes flowering by triggering the K48-linked polyubiquitination and subsequent degradation of the flower repressor Ghd7, a process mediated by the E3 ligase IPA INTERACTING PROTEIN1 (IPI1) (Cho et al. 2024). In this study, BCNPs accumulated in leaves and enhanced the export of photosynthates. While the number of flower branches remained unchanged compared to the control, flower number increased, indicating that the nanoparticles do not influence flower branch differentiation but instead promote flowering by facilitating sucrose allocation to flower tissues. Consistent with this mechanism, ZnO nanoparticles have been shown to upregulate sucrose transporter genes in rice (Ghouri et al. 2023), and salicylic acid-chitosan nanoparticles enhance sucrose metabolism and improve source-to-sink transport, supporting reproductive growth in cotton (Savani et al. 2023). Nanoparticles regulated sucrose translocation to floral branches, providing the material basis for flower development.

Flowering-related gene expression and hormone levels are also key factors influencing floral induction. In this study, several flowering-related, floral meristem-related, and hormone-associated genes were upregulated in T18 and T12 (Fig. 9a–c). Once transported to the shoot apical meristem, sucrose functions as a long-distance signal that regulates the expression of *single flower truss* (*SFT*) and modulates hormone levels (Christiaens et al. 2016). *SFT*, expressed in leaves, encodes a mobile florigen that promotes flowering (Lu et al. 2020). Sucrose can thus influence *SFT* expression and its subsequent translocation. In addition to carbohydrate availability, gibberellins are required to trigger the transition of the shoot apical meristem to an inflorescence meristem (Vanhaelewyn et al. 2019). Application of MnFe_2O_4 nanomaterials in tomato was reported to enhance photosynthetic rate, upregulate gibberellin biosynthesis and flowering induction genes, and promote earlier flowering (Yue et al. 2022). Because no reference genome is currently available for *Gentiana macrophylla*, genes related to flowering, hormone biosynthesis, and sucrose metabolism have not yet been cloned. Here, functional annotation of transcriptome data enabled the identification of flowering-related, floral meristem-related, and hormone-associated genes and their expression patterns. Together, these findings suggest that BCNPs promote flowering in *G. szechenyii* through coordinated regulation of sucrose, flowering genes, and hormone pathways, ultimately increasing flower number.

4.4 Effects of BCNPs on secondary metabolite production

Different types of nanoparticles induce distinct secondary metabolites through diverse regulatory mechanisms. Silver nanoparticles, for instance, have been shown to enhance the accumulation of anthocyanins, paclitaxel, and gibberellin III in plants (DeRosa et al. 2010; Jamshidi and Ghanati 2017; Syu et al. 2014). They also activate chlorophyll synthesis-related proteins, increasing chlorophyll content and photosynthetic activity. Elevated starch synthesis and subsequent degradation to glucose enhance the tricarboxylic acid (TCA) cycle in mitochondria, generating more energy to support cellular metabolism. This energy facilitates the synthesis of tocopherols from chlorophyll degradation products and mitigates excessive reactive oxygen species (ROS), maintaining cellular homeostasis (Galazzi et al. 2019). Moreover, silver nanoparticles can activate membrane proteins such as thioredoxin (TRX) and gene-modifying enzymes, leading to ROS generation. Acting as key signaling molecules, ROS trigger the expression of enzymes involved in secondary metabolism, ultimately enhancing the biosynthesis of secondary metabolites (Zhang et al. 2022).

Graphene nanoparticles (GNPs) activate antioxidant defenses in plants. In pepper, GNP treatment enhanced the activities of antioxidant enzymes such as superoxide dismutase (SOD) and induced antioxidant molecules, thereby reducing hydroxyl ($\cdot\text{OH}$) and superoxide ($\text{O}_2^{\cdot-}$) radicals. This alleviated lipid peroxidation and electrolyte leakage. In addition, GNPs increased phenylalanine ammonia-lyase (PAL) activity, promoting the biosynthesis of secondary metabolites (Younes et al. 2019). Thus, metallic and graphene nanoparticles induce oxidative signaling that upregulates synthase genes and increases polyphenol and flavonoid accumulation. In this study, BCNPs at the T18 level elevated szechenyin A and total active compound contents. BCNPs accumulated in chloroplasts, enhanced photosynthesis and sucrose levels, and promoted starch degradation to supply energy for tocopherol synthesis. Upregulation of ROS-related genes indicates ROS-mediated stimulation of szechenyin A biosynthesis.

Additionally, five SOD-related genes were upregulated, indicating a strong SOD-mediated response to BCNPs. Previous studies have shown that SOD positively contributes to secondary metabolite biosynthesis. For example, AgNPs enhanced SOD activity in *Pennisetum glaucum*, mitigating salt stress and promoting secondary metabolite accumulation (Khan et al. 2023). Therefore, both ROS and SOD play important roles in regulating bioactive compound synthesis in this study.

5 Conclusion

This study demonstrates that biochar application promotes flowering in *G. szechenyii* through the action of biochar-derived nanoparticles. These nanoparticles translocate from roots to leaf chloroplasts, where they modulate sucrose and starch metabolism. Upregulation of genes related to carbohydrate synthesis and transport enhanced both sugar production and export. Increased allocation of sugars to flower branches supported flower development. Moreover, BCNPs regulated the expression of flowering-related and hormone-associated genes. The coordinated action of sugars, flowering genes, and hormones collectively promoted flowering and increased flower number. While previous studies have linked the flowering-promoting effects of biochar to improved soil physicochemical properties and nutrient availability, our findings reveal an additional mechanism: biochar-derived nanoparticles directly influence flowering regulation. This previously overlooked pathway expands the understanding of how biochar shapes plant reproductive development and underscores the broader potential of biochar nanotechnology in agriculture. Nonetheless, the study is limited by the lack of genetic evidence (e.g., knockout or overexpression analyses) to confirm the molecular pathways involved. Furthermore, the mechanisms by which BCNPs act within chloroplasts—particularly their roles in photosynthesis and carbohydrate metabolism—remain unresolved and warrant further investigation.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-026-00570-7>.

Supplementary Material 1.

Author contributions

Writing-original draft, Guopeng Chen; Data curation, Lame Zeren, Chenghui Wang, Xuemei Wu and Guopeng Chen; Methodology, Yue Xu and Jie Zhang; Data analysis, Rong Ding, Hongmei Jia and Guopeng Chen; Supervision, Rui Gu; Resources, Shihong Zhong.

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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