

Biochar-immobilized Burkholderia pyrrocinia bacterial agent alleviates cadmium stress in pepper throughout the growth cycle: physiological and transcriptomic insights into growth promotion and decreased cadmium accumulation

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1 **Biochar-immobilized *Burkholderia pyrrocinia* bacterial agent alleviates cadmium**
2 **stress in pepper throughout the growth cycle: physiological and transcriptomic**
3 **insights into growth promotion and decreased cadmium accumulation**

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9 **Running head:** Effects of biochar-immobilized *Burkholderia pyrrocinia* agent on pepper

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Abstract: Cadmium (Cd) is a primary contaminant deteriorating agricultural soil quality in China. Pepper (*Capsicum annuum* L.) is a vegetable crop capable of accumulating high levels of Cd. Combined application of Cd-resistant plant growth-promoting rhizobacteria and biochar is a promising strategy to reduce Cd uptake. Here, we developed a biochar-immobilized *Burkholderia pyrrocinia* P10 inoculant (PC) and investigated its effects on Cd resistance mechanisms in pepper through integrated physiological and transcriptomic analyses. Under Cd stress (10–70 mg kg⁻¹), PC treatment consistently enhanced pepper growth and Cd tolerance throughout the growth period, as reflected by increased plant biomass and antioxidant enzyme activities and decreased malondialdehyde content. PC also significantly improved fruit yield and nutritional quality, while markedly reducing Cd levels in soil, plants, and fruits. These beneficial effects were superior to those of bacterial inoculation or biochar application alone. Root transcriptomic profiling at the full-fruited stage revealed that PC triggered a multifaceted molecular response, including upregulation of lignin and membrane lipid synthesis-related genes and ABCG transporter genes to reinforce Cd sequestration in the cell wall and efflux, enhanced expression of auxin and brassinosteroid biosynthesis genes to promote plant growth, induction of base excision repair, spliceosome, and heat shock protein genes to alleviate Cd-induced molecular damage and maintain protein homeostasis, and activation of glutathione and ascorbate metabolism to scavenge reactive oxygen species. These findings elucidate the molecular basis of the superior efficacy of biochar-immobilized bacterial agent in enhancing Cd tolerance and reducing Cd accumulation in pepper.

Keywords: Biochar-immobilized *Burkholderia pyrrocinia* · Cd stress · Pepper · Antioxidant defense · Transcriptomics · Cd reduction

Introduction

Cadmium (Cd), which is a highly biotoxic heavy metal that tends to accumulate, is one of the major heavy metal pollutants in agroecosystems worldwide (Yu et al., 2022). It is also the primary contaminant affecting the quality of cultivated soil in China (Hu et al., 2020; Yuan et al., 2021), especially in southwestern provinces, including Guizhou (Chen et al., 2015; Hu et al., 2021). In regions developed on carbonate rocks, Cd enrichment in soil may be due to parent materials and secondary enrichment-related processes (Yang et al., 2021; Yang et al., 2022). Moreover, Cd mainly in non-residual fractions are characterized by a high release risk and phytoavailability, leading to pronounced Cd accumulation in the soil–plant system to levels that frequently exceed safety limits for crops (Luo & Teng, 2018; Wang et al., 2019; Xia et al., 2020). Pepper (*Capsicum annuum* L.) is a major cash crop in Guizhou, with a planting area of 5.3 million mu (approximately 353,000 ha). In Guizhou, karst carbonate rocks cover 73% of the total area, making it a typical Cd geochemical anomaly zone, where the soil Cd background level is far higher than the national average for agricultural soil (Zhang et al., 2015). Previous studies identified pepper as a high-Cd accumulator, with excessive Cd levels frequently detected in pepper fruits and derived products (Xu et al., 2016; Xu et al., 2023; Zhang et al., 2025). Considering its high toxicity, mobility, persistence, and recalcitrance to degradation, Cd poses a serious threat to the safe production of pepper (Azlan et al., 2025), thereby severely constraining the sustainable development of the pepper industry.

Plant growth-promoting rhizobacteria (PGPR) are a class of beneficial microorganisms that promote plant growth (Zhang et al., 2025). They can alleviate Cd toxicity in plants through siderophore secretion, extracellular polysaccharide (EPS)-mediated biosorption and precipitation, and intracellular biotransformation, while also enhancing Cd tolerance by activating the plant antioxidant defense system (Mei et al., 2024; Manzoor et al., 2025). However, because PGPR compete with native soil microorganisms for nutrients and niches, they generally colonize soil inefficiently and are vulnerable to environmental stresses (Liu et al., 2024; Peng et al., 2025). Immobilizing free microbial cells on porous carriers via adsorption or encapsulation can generate habitable niches for colonization (Chang et al., 2025). Biochar derived from agricultural waste is an ideal carrier for microbial immobilization, serving as both a bioscaffold and a soil conditioner (Kayoumu et al., 2025). Its highly microporous structure and strong electrostatic attraction also contribute to soil Cd immobilization (Tan et al., 2020; Yin et al., 2023). Combining PGPR and biochar can decrease the soil available Cd content and enhance plant Cd

tolerance (Gururajan & Belur, 2018; Chi et al., 2024). Nevertheless, recent related research primarily focused on improving soil physicochemical properties, regulating heavy metal migration and transformation, enhancing soil microbial richness and diversity, and increasing soil enzyme activities (Qin et al., 2024; Xu et al., 2024). However, integrated physiological and transcriptomic analyses at the plant level can further elucidate the intrinsic mechanisms by which biochar-immobilized bacterial agents confer Cd tolerance in plants.

We previously isolated and identified *Burkholderia pyrrocinia* P10 as a PGPR strain that has multiple plant growth-promoting effects. Specifically, a co-fermentation broth and water-dispersible granules formulated using this strain as the key active ingredient can significantly promote pepper growth and improve fruit quality (Han et al., 2021; Han et al., 2024; Zeng et al., 2026). Moreover, strain P10 can grow normally in culture medium containing up to 800 mg L⁻¹ cadmium chloride (CdCl₂) (i.e., strong Cd resistance), which lays a solid foundation for its utility for promoting pepper growth under Cd stress conditions. In this study, we prepared a biochar-immobilized P10 inoculant and conducted a pot experiment to track its effects on pepper growth and development throughout the growth period under Cd stress conditions. By integrating physiological indices and transcriptomic data, we comprehensively dissected the mechanisms underlying the enhanced Cd tolerance and decreased Cd accumulation in pepper fruits. The findings of this study may provide a scientific basis for exploiting this Cd-tolerant P10-based biochar inoculant to enhance pepper cultivation in Cd-contaminated regions.

Materials and Methods

Experimental materials

The bacterial strain used in this study, *B. pyrrocinia* P10, is preserved in our laboratory and the China Center for Type Culture Collection (accession number CCTCC M 2019172). Biochar was purchased from Pingdingshan Tannuo Environmental Protection Materials Co., Ltd. Pepper (*C. annuum* L.) cultivar ‘Zhuojiao Xin 52’ was bred by Guizhou Zhuohao Agricultural Science and Technology Co., Ltd. The potting substrate consisted of a mixture of loess soil and specialized vegetable cultivation soil at a ratio of 3:1 (v/v). Loess soil was collected from Huaxi District, Guiyang City, Guizhou Province (26°44′ N, 106°66′ E), whereas vegetable cultivation soil was obtained from Beijing Longshuo Hongtai Trading Co., Ltd.

Preparation of biochar-immobilized bacterial agent and microstructural characterization under Cd stress conditions

A 100 μL aliquot of *B. pyrrocinia* P10 glycerol stock was used to inoculate 50 mL LB liquid medium, which was then incubated overnight at 30 °C with shaking (150 rpm). The activated bacterial suspension was adjusted to $\text{OD}_{600} = 1.0$ (approximately $1.0 \times 10^9 \text{ CFU mL}^{-1}$). Fresh LB medium was inoculated with the overnight culture at a 2% (v/v) ratio, with biochar added at the same time at a concentration of 2.0% (m/v). The mixture was incubated under the same conditions for 20 h to allow bacterial immobilization via co-culture adsorption. Biochar-immobilized P10 was harvested by centrifugation at 8,000 rpm for 15 min. The viable cell count (CFU g^{-1}) of the immobilized agent was determined using the spread plate method. For microstructural characterization, the activated P10 suspension ($\text{OD}_{600} = 1.0$) was used to inoculate LB medium containing 2.0% biochar and 0 or 600 mg L^{-1} CdCl_2 . After shaking incubation, the resulting pellets were washed with PBS buffer, fixed and examined by scanning electron microscopy (SEM; Apreo 2S, FEI, Thermo Fisher Scientific, USA). Biochar-bacterial pellets were lyophilized, ground to a fine powder, and mixed with potassium bromide at a ratio of 1:100 (w/w) to prepare pellets. Fourier transform infrared spectroscopy (FTIR) analyses were then performed using a Nicolet Summit LITE spectrometer (Thermo Fisher Scientific, USA) as described by Yan et al. (2024).

Pot experimental design and treatments

CdCl_2 solutions of varying concentrations were added to the mixed soil substrate, which was then thoroughly homogenized and equilibrated in darkness for 1 month to obtain soil contaminated with Cd^{2+} at concentrations of 0, 10, 30, 50, and 70 mg kg^{-1} . Uniformly growing pepper seedlings were transplanted into pots, with one seedling per pot. The following four treatments were initiated 3 days after transplantation: biochar-immobilized *B. pyrrocinia* P10 bacterial agent (PC), bacterial suspension (P), biochar alone (C), and untreated control (CK). For the PC, P, and C treatments, 1 g biochar-immobilized P10, pelleted P10 cells, and biochar, respectively, were suspended in 100 mL sterile water; an equal volume of sterile water alone was used for the CK treatment. Treatments for each Cd^{2+} concentration were completed using three biological replicates (three pots). Treatment solutions were applied once every 15 days under conventional cultivation practices. Plant, soil, and fruit samples were collected at the following three growth stages: seedling stage (approximately 30 days after transplantation), flowering stage (approximately 60 days after transplantation), and

full-fruiting stage (approximately 90 days after transplantation) for the subsequent analyses of various indices. Samples were designated according to the following format: Cd²⁺ concentration (0, 10, 30, 50, or 70 mg kg⁻¹) – treatment (PC, P, C, or CK) – stage (seedling, A; flowering, B; or full-fruiting, C).

Determination of growth and physiological indices of pepper plants and nutritional indices of fruits

For each treatment group, plant samples were collected at three growth stages. Soil adhering to the root system was gently removed by rinsing with water, after which the roots were blotted dry with absorbent paper before analyzing growth parameters. Total root surface area and root length were determined using a root scanner (Epson Perfection V850 Pro, Seiko Epson Corporation). The analyzed physiological indices included leaf chlorophyll, proline, malondialdehyde (MDA), and hydrogen peroxide (H₂O₂) contents. The chlorophyll content was measured using a handheld chlorophyll meter (Chlorophyll Meter SPAD-502 Plus, Konica Minolta). Proline, MDA, and H₂O₂ contents were determined using the acid ninhydrin colorimetric method, thiobarbituric acid colorimetric method, and titanium sulfate colorimetric method, respectively. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) antioxidant activities were assayed using the nitroblue tetrazolium colorimetric method, guaiacol colorimetric method, and UV absorption method, respectively. At the full-fruiting stage, the number of fruits per plant was recorded. Fruits from each plant were pooled, ground into a composite sample, and analyzed in terms of nutritional quality-related indices. Soluble sugar, soluble protein, ascorbic acid, total flavonoid, and total polyphenol contents were measured using the anthrone colorimetric method, Coomassie Brilliant Blue colorimetric method, bathophenanthroline colorimetric method, sodium nitrite–aluminum nitrate method, and Folin–Ciocalteu colorimetric method, respectively, as previously described (Cisternas-Jamet et al., 2019; Gao et al., 2022; Tuckeldoe et al., 2023).

Determination of Cd contents in plant tissues and fruits and soil available Cd contents

Roots, stems, and fruits were collected from plants that underwent different stress treatments at various growth stages. After air-drying samples, they were oven-dried at 85 °C to a constant weight, ground, and sieved. Powdered samples were then digested in 10 mL nitric acid–perchloric acid mixture (4:1, v/v). Soil samples were air-dried, after which visible debris was removed before samples were ground and sieved. Available Cd was extracted using diethylenetriaminepentaacetic acid and then the extract was centrifuged at 4,000 rpm for 10 min. The supernatant was filtered through a 0.45-μm

membrane. Cd concentrations in plant tissues and fruits as well as the available Cd content in soil were determined using an inductively coupled plasma optical emission spectrometry system (iCAP7400, Thermo Fisher Scientific, China) as described by Senila (2024).

Pepper root transcriptome sample preparation and analysis

At the full-fruiting stage, root samples were collected from pepper plants that underwent the PC, P, C, and CK treatments, each under Cd-free (0 mg kg^{-1}) and $50 \text{ mg kg}^{-1} \text{ Cd}^{2+}$ stress conditions. Each treatment–stress condition combination comprised three biological replicates (24 samples in total). Root samples were immediately frozen in liquid nitrogen and sent to Beijing Novogene Co., Ltd. for transcriptome sequencing. Total RNA was extracted using a Tiangen Polysaccharide and Polyphenol Kit (Qiagen, Germany), with RNA integrity precisely assessed using an Agilent 2100 Bioanalyzer. After removing ribosomal RNA, the remaining mRNA was fragmented into appropriate sizes. An RNA Library Prep Kit for Illumina (New England Biolabs, USA) was used to construct cDNA libraries, which were quantified and verified to be of sufficient quality. Libraries were sequenced on an Illumina NovaSeq 6000 platform (Illumina, USA). Raw reads were processed to remove adapter-containing reads, reads with ambiguous bases (N), and low-quality reads. The retained reads were assessed in terms of quality-related parameters (i.e., Q20, Q30, and GC content). They were then precisely aligned to a pepper reference genome (https://www.ncbi_capsicum_annuum_gca_000512255_2_asm51225v2) using HISAT2 software to obtain read mapping information (Hu et al., 2025). Gene expression levels were quantified and normalized using the fragments per kilobase of transcript per million mapped reads method. Differential expression was analyzed using the DESeq2 R package, with differentially expressed genes (DEGs) identified using the following criteria: $P < 0.05$ and $|\log_2(\text{fold-change})| > 1$. Significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were subsequently analyzed. Raw transcriptome data were deposited in the Genome Sequence Archive (GSA) of National Genomics Data Center (NGDC) (accession number CRA046206).

Reverse transcription quantitative real-time PCR (RT-qPCR) validation

Candidate genes were selected from the common DEGs among the PCCd vs Cd, Cd vs CK, PCd vs Cd, and CCd vs Cd comparison groups. Primers were designed using Primer-BLAST (Table S1) for RT-qPCR analyses, with *TUB* (tubulin) selected as the internal reference gene. Reaction conditions were as follows: initial denaturation at 95°C for 2 min; 40 cycles of 95°C for 15 s and annealing

temperature (Table S1) for 15 s. Target gene relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 24 software. Specifically, data underwent an analysis of variance, with significant differences among treatments compared using Duncan's new multiple range test and the least significant difference test for multiple comparisons. Data are presented herein as the mean $\pm$ standard error. Data were compiled and visualized in graphs using Microsoft Excel and Origin 2022.

Results

Preparation and microstructural characterization of biochar-immobilized *B. pyrocinia* P10

Biochar was used to adsorb and immobilize *B. pyrocinia* P10 cells. The viable cell count of PC was 2.06×10^{13} CFU g⁻¹. SEM analyses detected many P10 cells attached to the surface and surrounding the pores of biochar particles at a relatively high density, indicating that the porous structure of biochar provided favorable adhesion sites and shelter for bacterial colonization (Fig. 1A–B). In LB medium supplemented with 600 mg L⁻¹ CdCl₂ (Fig. 1D–E), P10 cells were still extensively attached to the biochar surface and pore margins, with only a slight decrease in cell density. In contrast to bacterial cells in Cd-free medium, some bacterial cells in medium containing Cd had fine granular deposits on the surface, while surface shrinkage was detected for a few cells. FTIR analyses (Fig. 1C, F) showed that under Cd stress conditions, the characteristic peak of PC (1,033.09 cm⁻¹) clearly shifted to a higher wavenumber, which was accompanied by a marked increase in peak intensity and a sharpening of the peak. Additionally, the resolution of the fine structural peaks in the 1,000–1,500 cm⁻¹ region was enhanced and the baseline absorbance decreased. Collectively, these results suggest that Cd stress induced adaptive changes in the chemical structure of surface functional groups of PC to a certain extent.

Effects of biochar-immobilized P10 on pepper growth and development under Cd stress conditions

We monitored PC treatment effects on the growth and development of pepper plants exposed to different Cd²⁺ concentrations (Fig. 2). Plants grown in soil containing 10 mg kg⁻¹ Cd²⁺ did not differ significantly from control plants in terms of growth and fruit development. However, increasing Cd

stress levels gradually inhibited growth; Cd^{2+} concentrations exceeding 50 mg kg^{-1} resulted in stunted growth as well as decreases in the number of leaves and fruits. Accordingly, we defined 10, 30, 50, and $70 \text{ mg kg}^{-1} \text{ Cd}^{2+}$ as low, moderate, high, and severe Cd stress levels, respectively. For a given Cd^{2+} concentration, plants in the PC, P, and C treatment groups grew better than CK plants to varying degrees. Notably, at Cd^{2+} concentrations exceeding 50 mg kg^{-1} , the PC treatment positively affected growth and fruit yield more than the other treatments.

Effects of biochar-immobilized P10 on the growth and physiological indices of pepper seedlings exposed to Cd stress

Pepper seedling growth indices were examined (Table 1). Regardless of the Cd stress level, the PC, P, and C treatments promoted seedling growth to varying degrees. Under Cd-free conditions, the fresh weight, root weight, root surface area, and chlorophyll content were significantly higher for plants in the PC treatment group than for plants in the P, C, and CK treatment groups. Under $10 \text{ mg kg}^{-1} \text{ Cd}^{2+}$ stress conditions, the PC treatment significantly increased the stem diameter, plant height, root weight, and chlorophyll content ($P < 0.05$), indicating that the growth-promoting effects of PC were particularly pronounced under low Cd stress conditions. When the Cd stress level increased to $30 \text{ mg kg}^{-1} \text{ Cd}^{2+}$, the chlorophyll content was significantly higher for plants in the PC treatment group than for plants in the other treatment groups ($P < 0.05$). Even under $50\text{--}70 \text{ mg kg}^{-1} \text{ Cd}^{2+}$ stress conditions, plants treated with PC, P, or C consistently had superior growth indices, with stem diameter and chlorophyll content significantly higher for PC-treated plants than for plants exposed to Cd stress alone ($P < 0.05$).

Under Cd-free conditions, PC, P, and C treatments did not significantly affect pepper seedling physiological indices or antioxidant enzyme activities. Across all treatment groups, antioxidant enzyme activities tended to increase as the Cd^{2+} concentration increased. Compared with plants exposed to Cd stress alone, plants in the PC treatment group had significantly higher POD activities at all Cd stress levels; their SOD activities increased significantly under $30\text{--}70 \text{ mg kg}^{-1} \text{ Cd}^{2+}$ stress conditions. In addition, CAT activities were significantly higher in plants treated with PC than in plants exposed to Cd stress alone under both low and severe Cd stress conditions ($P < 0.05$). Furthermore, proline and MDA contents decreased significantly in the PC, P, and C treatment groups. H_2O_2 contents also differed significantly between plants in these treatment groups and plants exposed to Cd stress alone under $10\text{--}50 \text{ mg kg}^{-1} \text{ Cd}^{2+}$ stress conditions ($P < 0.05$) (Fig. 3). Considered together, these results

indicate that these three treatments, but especially PC, alleviated Cd-induced oxidative damage in pepper seedlings by enhancing the antioxidant defense capacity to varying degrees.

Effects of biochar-immobilized P10 on the growth and physiological indices of pepper plants at the flowering stage under Cd stress conditions

At the flowering stage (Table 2), under Cd-free conditions, the growth indices of the PC, P, and C treatment groups were all superior to those of the CK group to varying degrees; the PC treatment had the greatest positive effects on growth indices, except for the chlorophyll content. Of these three treatments, PC alleviated Cd-induced growth inhibition the most, followed by P and then C. Under low Cd stress conditions, fresh weight and root weight were significantly higher for PC-treated plants than for plants exposed to Cd stress alone (49.6% and 109.0% higher, respectively). Compared with plants exposed to Cd stress alone, PC-treated plants were significantly taller, with longer roots and higher fresh weight under 30–70 mg kg⁻¹ Cd²⁺ stress conditions; root weight and stem diameter were also significantly greater under 50 mg kg⁻¹ Cd²⁺ stress conditions, while chlorophyll content was significantly higher even under 70 mg kg⁻¹ Cd²⁺ stress conditions ($P < 0.05$). Collectively, these results suggest that the PC treatment significantly promoted pepper growth and development regardless of the Cd stress level, with its growth-promoting effects particularly pronounced at the flowering stage.

The PC treatment increased physiological indices and antioxidant enzyme activities more than the other treatments. With the exception of C-treated plants under low Cd stress conditions, PC-, P-, and C-treated plants had significantly lower MDA and H₂O₂ contents than plants exposed to Cd stress alone at the same Cd²⁺ concentration ($P < 0.05$); these contents were lowest in PC-treated plants. In terms of their effects on the antioxidant enzyme system, the PC treatment resulted in significantly elevated SOD activities under all Cd stress conditions, except for low Cd stress conditions, and significantly increased POD activities under 10 and 70 mg kg⁻¹ Cd²⁺ stress conditions. CAT activities were significantly higher in PC-treated plants than in plants exposed to Cd stress alone, but only under low Cd stress conditions ($P < 0.05$). Interestingly, proline contents in PC-, P-, and C-treated plants were lower than those in plants exposed to low-to-moderate Cd stress alone to varying degrees, but were higher than those in plants exposed to high and severe Cd stress alone (50–70 mg kg⁻¹). Notably, proline contents were significantly higher in the PC treatment group than in the other treatment groups under high Cd stress conditions (Fig. 4).

Effects of biochar-immobilized P10 on the growth and physiological indices of pepper plants at the full-fruiting stage under Cd stress conditions

Upon reaching the full-fruiting stage, under Cd-free conditions, the PC treatment group had a significantly larger stem diameter, longer roots, greater root surface area, and more fruits per plant than the CK group. Under Cd stress conditions, the PC and P treatments promoted growth more than the C treatment. Under low-to-moderate Cd stress conditions, plants in the PC treatment group were significantly taller, with longer roots and greater root surface area, than plants exposed to Cd stress alone ($P < 0.05$). Under high Cd stress conditions, plants in the PC treatment group were significantly taller, with a larger stem diameter, than the other plants; relative to plants exposed to Cd stress alone, those treated with PC had a significantly larger root surface area (77.65% increase) as well as significantly more fruits per plant (400.0% increase) ($P < 0.05$), reflecting the marked yield-enhancing effect of the PC treatment. Even under severe Cd stress conditions, all growth indices, with the exception of root length and chlorophyll content, were significantly higher in the PC treatment group than in the other treatment groups; the number of fruits per plant increased by 246.42% (87.50% and 32.39% higher than the number of fruits per plant in the P and C treatment groups, respectively) (Table 3). These results demonstrate that the PC treatment effectively promoted the development of Cd-stressed plants and enhanced fruit biomass production.

During the full-fruiting stage, the three treatments had distinct effects on antioxidant enzyme activities. Specifically, POD activities were significantly higher in PC-treated plants than in plants exposed to Cd stress alone across all Cd^{2+} concentrations. SOD activities increased significantly when the Cd^{2+} concentration exceeded 30 mg kg^{-1} ($P < 0.05$). With the exception of the 50 and 70 mg kg^{-1} Cd^{2+} stress conditions, MDA and H_2O_2 contents were significantly lower in PC-treated plants than in plants exposed to Cd stress alone ($P < 0.05$). Similarly, MDA and H_2O_2 contents were lower in plants in the P and C treatment groups than in plants exposed to Cd stress alone to varying degrees. Furthermore, proline contents were higher in all three treatment groups than in the corresponding groups comprising plants exposed to Cd stress alone, with the PC and P treatments resulting in the highest proline contents ($P < 0.05$) (Fig. 5).

Effects of biochar-immobilized P10 on fruit nutritional quality-related indices of pepper under Cd stress conditions

Analyses of fruit nutritional quality-related indices revealed that under normal cultivation conditions, soluble protein, ascorbic acid, total flavonoid, and total polyphenol contents were significantly higher in the PC treatment group than in the CK group ($P < 0.05$). They were also higher than the corresponding contents in the P and C treatment groups to varying degrees; soluble sugar contents increased by 44.08% (relative to the corresponding CK content). Increases in Cd stress levels were accompanied by progressive decreases in fruit nutritional quality. However, the PC treatment had significantly positive effects on fruit nutrient contents. Under 10–70 mg kg⁻¹ Cd²⁺ stress conditions, the total flavonoid content was significantly higher in plants in the PC treatment group than in plants exposed to Cd stress alone. The PC treatment resulted in significant increases in soluble sugar and soluble protein contents under low-to-moderate Cd stress conditions as well as significant increases in the total polyphenol content under high and severe Cd stress conditions. Furthermore, compared with the P and C treatments, the PC treatment significantly increased total polyphenol contents under 10 and 50 mg kg⁻¹ Cd²⁺ stress conditions, while also significantly increasing soluble sugar contents under 30 mg kg⁻¹ Cd²⁺ stress conditions. Even under 70 mg kg⁻¹ Cd²⁺ stress conditions, the PC treatment increased soluble sugar, total polyphenol, soluble protein, ascorbic acid, and total flavonoid contents by 85.42%, 38.0%, 13.04%, 63.75%, and 52.74%, respectively (relative to the corresponding contents in plants exposed to Cd stress alone). Notably, soluble sugar and total polyphenol contents were significantly higher in PC-treated plants than in P- or C-treated plants ($P < 0.05$) (Table 4).

Effects of biochar-immobilized P10 on Cd accumulation in soil, pepper plants, and fruits

Soil available Cd contents as well as Cd contents in plant tissues (roots and stems) and fruits were determined at different growth stages (Fig. 6). Under the same Cd stress condition, Cd accumulated significantly less in fruits than in roots and stems. Soil available Cd contents remained relatively stable throughout the pepper growth cycle, which was in contrast to the gradual increase in the root Cd content as plants grew and developed, particularly from the seedling stage to the flowering stage.

At the seedling stage, soil available Cd and root Cd contents were significantly lower for PC-treated plants than for plants exposed to Cd stress alone ($P < 0.05$). They were also lower in the PC treatment group than in the P and C treatment groups to varying degrees. Under 50 mg kg⁻¹ Cd²⁺ stress conditions, the PC treatment decreased soil available Cd and root Cd contents by 38.0% and 24.70%, respectively. Under 70 mg kg⁻¹ Cd²⁺ stress conditions, the PC treatment decreased soil available Cd and root Cd contents by 14.20% and 33.14%, respectively. At the flowering stage, soil available Cd

contents decreased in all three treatment groups to varying degrees. Root Cd contents decreased significantly in all three treatment groups ($P < 0.05$), but not under severe Cd stress conditions, with the observed decreases greatest in the PC treatment group. Under high and severe Cd stress conditions, the PC treatment decreased root Cd contents by 24.09% and 13.55%, respectively. At the full-fruiting stage, soil available Cd and stem Cd contents were significantly lower in the three treatment groups than in the groups comprising plants exposed to Cd stress alone (except under low Cd stress conditions) ($P < 0.05$). Notably, under high and severe Cd stress conditions, the PC treatment decreased soil available Cd contents by 22.75% and 21.00% and stem Cd contents by 53.54% and 44.71%, respectively. Root Cd contents were also lower in plants in all three treatment groups than in plants exposed to Cd stress alone to varying degrees. The PC treatment significantly decreased root Cd contents by 54.35% under severe Cd stress conditions, whereas the C treatment decreased root Cd contents under the other Cd stress conditions.

Regarding fruit Cd contents, under the 10, 30, 50, and 70 mg kg⁻¹ Cd²⁺ stress conditions, the PC treatment significantly decreased fruit Cd concentrations by 22.9%, 25.8%, 45.3%, and 40.9%, respectively ($P < 0.05$). These decreases were greater than those observed for the C and P treatments. The PC treatment effectively blocked the stepwise translocation of Cd from the soil to the roots, stems, and edible plant parts, thereby demonstrating the positive effects of this treatment on Cd tolerance in pepper.

Effects of biochar-immobilized P10 on the root transcriptome of pepper at the full-fruiting stage

Transcriptome sequencing and KEGG enrichment analysis

For the three treatment groups (PC, P, and C) and the corresponding CK group, root samples were collected from pepper plants at the full-fruiting stage under Cd-free and 50 mg kg⁻¹ Cd²⁺ stress conditions (24 samples in total) for transcriptome sequencing analyses (Table S2). For each sample, 5.93–8.20 Gb clean data were generated, with Q30 values exceeding 96%. The proportion of mapped reads aligned to the reference genome ranged from 76.76% to 93.26%, indicating that the reference genome assembly was suitable and the data quality was sufficient for further analyses. Under Cd-free conditions, the PC vs CK, C vs CK, and P vs CK comparisons yielded 1,348 DEGs (638 upregulated and 710 downregulated), 2,312 DEGs (1,016 upregulated and 1,296 downregulated), and 916 DEGs (428 upregulated and 488 downregulated), respectively (Fig. 7A). Under Cd stress conditions, the Cd vs CK, PCCd vs Cd, CCd vs Cd, and PCd vs Cd comparisons revealed 3,532 DEGs (1,981 upregulated

and 1,551 downregulated), 3,773 DEGs (1,708 upregulated and 2,065 downregulated), 1,850 DEGs (838 upregulated and 1,012 downregulated), and 2,305 DEGs (1,132 upregulated and 1,173 downregulated), respectively. Hierarchical clustering heatmap analyses (Fig. 7B) showed that all samples formed two distinct clusters on the basis of the presence or absence of Cd stress, with P and PC treatments clustered together. Accordingly, Cd stress substantially affected plant gene expression. Moreover, transcriptional changes induced by biochar-immobilized P10 were largely attributable to the bacterial component (strain P10) rather than the biochar carrier.

KEGG enrichment analyses (Fig. 7C) showed that under Cd-free conditions, the plant hormone signal transduction pathway was significantly downregulated in the PC vs CK and P vs CK comparison groups, while the protein processing in endoplasmic reticulum pathway was significantly downregulated in the C vs CK comparison group. Notably, the phenylpropanoid biosynthesis pathway was significantly upregulated in all three comparison groups. In addition, in the PC vs CK comparison group, galactose metabolism was significantly downregulated, which was in contrast to the significant upregulation of pathways involved in fatty acid synthesis and metabolism, secondary metabolite biosynthesis, phenylalanine metabolism, and the biosynthesis of various antioxidant compounds, including flavonoids and terpenoids. These findings suggest that the effects of the PC treatment on pepper roots were not simply the sum of the effects of strain P10 and biochar alone, but were due to the synergistic effects of the two components. Under Cd stress conditions, in the Cd vs CK comparison group, the significantly upregulated pathways were ABC transporters and valine, leucine and isoleucine degradation, whereas the significantly downregulated pathways were oxidative phosphorylation and protein processing in endoplasmic reticulum. Interestingly, protein processing in endoplasmic reticulum was a significantly upregulated pathway in both the PCCd vs Cd and PCd vs Cd comparison groups. Other significantly upregulated pathways in the PCCd vs Cd comparison group were fatty acid metabolism and biosynthesis of unsaturated fatty acids. Furthermore, phenylpropanoid biosynthesis and glutathione metabolism were significantly upregulated pathways in the PCd vs Cd comparison group. By contrast, the enriched pathways in the CCd vs Cd comparison group were significantly downregulated, including terpenoid and flavonoid biosynthesis as well as pentose and glucuronate interconversions. Collectively, these results demonstrate that the PC treatment markedly activated protein processing and fatty acid metabolite synthesis in Cd-stressed roots, thereby laying a foundation for enhanced Cd stress tolerance in pepper plants.

Analysis of DEGs in pepper roots under different treatment and Cd stress conditions

Under the same Cd stress condition, all three treatments (PC, P, and C) reversed the Cd-induced transcriptomic changes to a certain extent; however, the PC treatment significantly induced the expression of genes involved in multiple signal transduction, hormone response, and biosynthetic pathways (Table S3). Specifically, the exposure to Cd stress alone (Cd vs CK) significantly upregulated the expression of genes encoding transcription factor VIP1, calcium-binding protein CML19, RIN4, ethylene-responsive factor ERF1B, and auxin repressor AUX22, while downregulating the expression of an indole-3-acetic acid amide synthetase gene (*GH3.1*). By contrast, PCCd, PCd, and CCd treatments downregulated the expression of transcription factor genes and *AUX22*, but significantly upregulated the expression of genes encoding an abscisic acid receptor (*PYL1*), calcium-binding proteins (*PC15* and *CML19*), and *GH3.1*. All three treatments also induced the expression of a gene encoding UDP-glucosyltransferase 73C (*UGT73C*), which contributes to zeatin biosynthesis, but the PCCd treatment increased expression more than the other treatments. *UGT73C* expression was significantly downregulated in plants exposed to Cd stress alone. Additionally, the upregulated expression of a cis-zeatin O-glucosyltransferase gene (*cisZOGT*) was greater in plants exposed to Cd stress alone than in plants that underwent the PCCd treatment. The PCCd and PCd treatments upregulated the expression of genes encoding a heat shock protein (*HSP83A*) and brassinosteroid resistant 1/2 (*BZR1/2*), but downregulated the expression of genes encoding jasmonate–amino acid synthetase (*JAR1*) and jasmonate ZIM domain-containing protein (*JAZ*); however, *JAR1* expression was upregulated in plants exposed to Cd stress alone. These two treatments significantly upregulated the expression of amidase-encoding genes in the tryptophan biosynthesis pathway (4.57-fold increase in PCCd-treated samples and up to 5.88-fold increase in PCd-treated samples) as well as genes encoding OPC-8:0 CoA ligase 1 (*OPCL1*) and acetyl-CoA acyltransferase 1 (*ACAAL1*) involved in α -linolenic acid metabolism. The expression levels of these genes were significantly downregulated in plants exposed to Cd stress alone. The expression of a chloroplastic oxoene reductase gene (*ceQORH*) was upregulated only in plants exposed to Cd stress alone, whereas enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase gene (*MFP2*) expression was significantly upregulated exclusively in C-treated plants (6.88-fold increase). In the diterpenoid biosynthesis pathway, the expression of a gibberellin 3-beta-dioxygenase gene (*GA3ox*) was significantly downregulated under Cd stress conditions, whereas it was upregulated following PCCd and CCd treatments. The expression

of a gibberellin 2-beta-dioxygenase gene (*GA2ox*) was significantly upregulated following an exposure to Cd stress alone, but downregulated after the PCCd treatment. Furthermore, the PCCd treatment significantly upregulated the expression of a 3-epi-6-deoxocathasterone 23-monooxygenase gene (*CYP90C1*) (up to 6.68-fold increase) in the brassinosteroid biosynthesis pathway. Considered together, these results indicate that the PC treatment strengthened signal transduction and hormone responses to suppress excessive downstream stress-related signaling, while also effectively shifting hormone signals from stress responses toward growth promotion.

Biopolymer synthesis, protein processing in the endoplasmic reticulum, and oxidative phosphorylation were pathways that effectively reflected the differences among treatment groups in terms of gene and protein homeostasis as well as energy metabolism. Among base excision repair-related genes, those encoding endonuclease III (*NTH*), DNA-3-methyladenine glycosylase II (*AlkA*), and exodeoxyribonuclease III (*Xth*) had expression levels that were significantly upregulated by all three treatments (PC, P, and C). This was in contrast to their significantly downregulated expression levels in plants exposed to Cd stress alone, in which only the expression of a DNA-3-methyladenine glycosylase I gene (*Tag*) was slightly upregulated. Additionally, the expression of a replication factor C subunit 3/5 gene (*RFC*) was upregulated by the P and C treatments. In the spliceosome pathway, genes encoding pre-mRNA-splicing factor SYF2 (*Syf*), pre-mRNA-splicing factor ATP-dependent RNA helicase DHX38/PRP16 (*Prp16*), ATP-dependent RNA helicase DHX8/PRP22 (*Prp22*), THO complex subunit 1 (*THOC*), and splicing factor U2AF 65 kDa subunit (*U2AF*) had markedly upregulated expression levels in the three treatment groups, whereas their expression levels were predominantly downregulated in plants exposed to Cd stress alone. In terms of the protein processing in endoplasmic reticulum pathway, the PCCd and PCd treatments upregulated the expression of multiple heat shock protein genes, including *DNAJ3*, *HSP22*, *HSP26.5*, *CDC48*, and *DPPA1*, as well as genes encoding soluble inorganic pyrophosphatases (compared with the corresponding expression levels in plants exposed to Cd stress alone). By contrast, the corresponding genes in the Cd vs CK comparison group had significantly downregulated expression levels. These findings imply that PC and P treatments maintained gene and protein homeostasis in Cd-stressed plants, while effectively restoring root energy metabolism to sustain nutrient transport in roots.

In terms of heavy metal transport and detoxification, Cd stress significantly upregulated the expression of ABC transporter genes, including *PDR2*, *PDR4*, *PDR5*, *PDR12*, *ABCG27*, *ABCG10*, and

450 *ABCG25*. The PCd and CCd treatments downregulated the expression of most *PDR*-type genes,
451 whereas the PCCd treatment had a bidirectional regulatory effect, simultaneously upregulating the
452 expression of *ABCG19*, *ABCG6*, *PDR5*, and *PDR1*, while downregulating the expression of *PDR4*,
453 *PDR12*, *ABCG27*, and *ABCG11*. These expression level changes reflect an active stress response
454 involving Cd efflux or sequestration in roots, suggesting that the PC treatment may have altered Cd
455 translocation. In the glycerolipid metabolism pathway, the PCd and PCCd treatments significantly
456 upregulated the expression of an endoplasmic reticulum omega-7 fatty acid desaturase gene (*FAD2*)
457 and related genes (relative to the corresponding expression in samples exposed to Cd stress alone), with
458 the PCCd treatment upregulating expression (up to 5.36-fold increase) more than the other treatment.
459 The CCd treatment also significantly upregulated the expression of certain *FAD2*-related genes.
460 Furthermore, the PCCd treatment upregulated the expression of genes encoding caffeoyl shikimate
461 esterase (*CSE*) and lipid phosphate phosphatase (*LPP3*), while downregulating the expression of an
462 alcohol dehydrogenase gene (*ADH1*). Accordingly, all three treatments enhanced membrane stability in
463 Cd-stressed plants by increasing membrane lipid desaturation, with the PC treatment having the most
464 pronounced positive effect on membrane lipid formation. In terms of cell wall modifications, in the
465 phenylpropanoid biosynthesis pathway, Cd stress suppressed the expression of genes encoding
466 peroxidases (*PER4* and *PER21*) and cytochrome P450 98A3 (*CYP98A3*). By contrast, all three
467 treatments significantly upregulated the expression of these genes, with the greatest increases in
468 expression detected for the PCCd treatment (increasing *CYP98A3* and *PER4* expression 4.28- and
469 1.56-fold, respectively), followed by the PCd and then CCd treatments. Hence, the PC treatment
470 effectively promoted lignin synthesis and cell wall reinforcement, thereby enhancing the effectiveness
471 of the cell wall as a physical barrier. In the pentose and glucuronate interconversion pathway, Cd stress
472 significantly upregulated the expression of genes encoding a pectin methylesterase (*PME11*),
473 polygalacturonase (*PGLR*), and peroxidase (*PER21*). Compared with the effects of an exposure to Cd
474 stress alone, all treatments significantly downregulated the expression of these genes to varying
475 degrees (up to 3.10-fold decrease in expression following the PCCd treatment). Regarding antioxidant
476 defense, the PCd and PCCd treatments significantly upregulated *APX1*, *MGST3*, and *GST* expression
477 levels (genes contributing to glutathione metabolism), while the CCd treatment upregulated the
478 expression of these genes to a lesser extent. The expression levels of most of these genes were
479 downregulated in plants exposed to Cd stress alone. Among the genes in the ascorbate and aldarate

metabolism pathway, *APX* expression was upregulated by the PC treatment, but downregulated by the other two treatments and the exposure to Cd stress alone; *GST* expression was upregulated by the PC and C treatments, but especially the PC treatment, while being downregulated by the exposure to Cd stress alone. These findings suggest that the PC and P treatments increased the antioxidant capacity and scavenging of reactive oxygen species (ROS).

Common DEGs among treatment groups were selected for RT-qPCR analyses to validate expression data. The expression patterns for the selected genes determined by RT-qPCR were highly consistent with the trends observed in the transcriptome sequencing data (Fig. 8), thereby confirming the reliability of the experimental results.

Discussion

Analyses of the Cd resistance-related characteristics of biochar-immobilized *B. pyrocinia* P10

B. pyrocinia strain P10 is highly resistant to Cd stress (grows well even in medium containing 800 mg L⁻¹ CdCl₂), while also having multiple plant growth-promoting effects. Selecting an appropriate carrier material is critical for enhancing microbe-based heavy metal bioremediation efficiency, while also directly influencing bacterial survival and colonization in soil. Owing to its well-developed porous structure, large specific surface area, and favorable biocompatibility, biochar has recently been widely used as an immobilization carrier for microorganisms (Li et al., 2025). In the present study, SEM examinations revealed that most P10 cells were embedded within biochar pores or aligned along pore walls; the strong affinity of this strain for the biochar carrier enabled it to form a compact bacterium–biochar composite structure (Lai et al., 2022). Under Cd stress conditions, the number of bacterial cells within biochar pores decreased to some extent; however, morphologically intact cells were still present inside pores, suggesting that the physical sheltering effect of biochar partially alleviated the direct toxic effect of Cd on bacterial cells (Ju et al., 2025; Wang et al., 2024). In addition, the surface of some bacterial cells was shrunken and had fine granular deposits. Previous research showed that under high Cd stress conditions, *Burkholderia* sp. SRB-1 can decrease Cd toxicity by forming granular precipitates (e.g., CdS and CdCO₃) on their cell surface (Liu et al., 2023a). FTIR analyses detected a distinct shift and marked increase in the intensity of the characteristic peak at 1,033.09 cm⁻¹, which is attributable to C–O stretching vibrations of polysaccharides and P=O stretching vibrations, likely reflecting enhanced secretion of EPS by bacterial cells under Cd stress

conditions (Yin et al., 2024). Moreover, no distinct characteristic peaks of free Cd^{2+} or CdO were detected below 700 cm^{-1} , suggesting that Cd^{2+} immobilization by the P10 strain and biochar was mainly due to complexation or mineralization (Chang et al., 2025). This is supported by our study data that revealed the overexpression of *cysD*, *algA*, and *epsP* in strain P10, which confirmed that sulfate precipitation-induced CdS formation and EPS secretion are important mechanisms underlying the Cd resistance of this strain (unpublished data). Collectively, these microstructural observations provide a mechanistic basis for the subsequent alleviation of the detrimental effects of Cd stress on pepper by biochar-immobilized P10 in a pot experiment.

Biochar-immobilized P10 promotes the growth and development of Cd -stressed plants by activating antioxidant defense and inhibiting Cd accumulation

Cd stress induces the excessive generation of ROS in plants (Anwar et al., 2024), disrupts redox homeostasis, and subsequently triggers oxidative stress and cytotoxicity (AbdElgawad et al., 2020), which is manifested as lipid peroxidation, H_2O_2 production, and increased ion leakage (Riaz et al., 2021). Proline is a critical osmoregulator in plant responses to environmental stress; its accumulation contributes to maintaining cellular osmotic balance, stabilizing proteins and membrane structures, and scavenging ROS (Hosseinifard et al., 2022). In the present study, throughout the pepper growth period, increasing Cd stress levels resulted in increased MDA and H_2O_2 contents to varying degrees; however, the proline content and antioxidant enzyme activities (POD, SOD, and CAT) also increased, with particularly notable increases in enzyme activities at the seedling and flowering stages. These changes reflect a plant adaptive strategy for mitigating Cd -induced damage in which the osmotic balance is maintained and the antioxidant defense system is activated (Song et al., 2025). Accordingly, at the seedling stage, low-to-moderate Cd^{2+} concentrations did not markedly inhibit plant growth, although the chlorophyll content decreased. By contrast, under high Cd stress conditions, the root surface area decreased significantly. Inhibited plant growth became increasingly obvious as the exposure to Cd stress was prolonged. At the full-fruiting stage, growth indices were generally lower under Cd stress conditions than under Cd -free conditions. For example, under $50\text{ mg kg}^{-1}\text{ Cd}^{2+}$ stress conditions, plant height, fresh weight, and number of fruits per plant decreased by 54.97%, 67.33%, and 85.37%, respectively (relative to the corresponding data for samples under CK conditions). Accordingly, Cd stress at certain levels suppresses photosynthesis, leading to decreased carbon fixation (Huang et al., 2021), ultimately causing root damage and impaired plant growth and development (Haider et al.,

2021). Interestingly, our results showed that low Cd stress conditions promoted pepper growth. In an earlier study by Shao et al. (2021), treating plants with 10 mg kg⁻¹ Cd²⁺ promoted root growth in three of four tested pepper varieties, possibly because Cd²⁺ can activate cytokinin metabolism-related enzymes, thereby accelerating plant growth (Feng et al., 2020).

Throughout the pepper growth period, MDA and H₂O₂ contents were lower in treated (PC, P, and C) plants than in the corresponding plants exposed to Cd stress alone (at the same Cd²⁺ concentration), with the lowest contents detected after the PC treatment. Compared with pepper plants exposed to Cd stress alone, plants that underwent the PC treatment had significantly higher POD and SOD activities in all three analyzed growth stages, thereby strengthening antioxidant defenses, decreasing ROS levels, and alleviating Cd-induced oxidative damage (Awan et al., 2020; Junaid et al., 2023; Zhang et al., 2024). These results are consistent with the findings of a recent study by Alshaal et al. (2024). Moreover, as the duration of the exposure to Cd stress increased, distinct proline contents suggestive of an adaptive response were detected in the three treatment groups. At the seedling stage, proline contents were significantly lower in treated plants (i.e., PC, P, or C) than in plants exposed to Cd stress alone. At the flowering stage, proline contents in treated plants were generally lower than those in the corresponding plants exposed to low-to-moderate Cd stress levels, but higher than those in the corresponding plants under high and severe Cd stress conditions (50 and 70 mg kg⁻¹ Cd²⁺, respectively). At the full-fruiting stage, proline contents in treated plants were higher than in the corresponding plants exposed to Cd stress alone, with significant increases in proline contents induced by the PC and P treatments ($P < 0.05$). These differential responses may be attributed to the relatively short duration of Cd exposure and the relatively mild damage caused by low-to-moderate Cd stress levels at the seedling stage, with the PC and P treatments alleviating Cd-induced oxidative stress primarily by enhancing antioxidant enzyme activities. This is in accordance with the observation that inoculating with *Burkholderia contaminans* and *Arthrobacter humicola* can mitigate the increase in the proline content of *Ageratum conyzoides* seedlings under Cd stress conditions (Wang et al., 2024). By contrast, high Cd²⁺ concentrations caused relatively severe damage to plants at the flowering stage, with a prolonged exposure to stress leading to persistent Cd-induced injury at the full-fruiting stage. Therefore, the PC and P treatments also increased proline synthesis at the later stages. Proline maintains cellular osmotic balance, while also serving as a heavy metal chelator to decrease Cd toxicity (Pramanik et al., 2021). Similar increases in proline contents have been observed in *Trichoderma* sp. strain-inoculated wheat

under Ni stress conditions (Bibi et al., 2026). Nevertheless, the precise regulatory mechanisms underlying the differential modulation of proline accumulation by bacterial or biochar-P10 agents in response to varying stress intensities and durations warrant further investigation.

After being taken up by plant roots, Cd is loaded into the xylem and translocated upward, eventually accumulating in various tissues, organs, and fruits (Zhang et al., 2020; Chtouki et al., 2021). During this process, a substantial proportion of Cd²⁺ is retained and immobilized in the roots, primarily through cell wall binding, vacuolar compartmentalization, and decreased root-to-shoot translocation (Chen et al., 2023). In the present study, root Cd contents increased sharply from the seedling stage to the flowering stage, implying that Cd accumulates rapidly during this period in pepper. This observation differs slightly from that reported by Zhao et al. (2021), who revealed that Cd accumulates fastest during the pre-vigorous seedling stage; this discrepancy may be attributed to the diversity in Cd adsorption and accumulation among pepper cultivars. At the full-fruiting stage, the rank order of Cd contents was as follows: roots > stems > fruits. This is consistent with the findings of previous studies (Wang et al., 2019; Luo et al., 2022). Moreover, it further underscores how pepper roots contribute to Cd immobilization. All three treatments decreased soil and plant Cd accumulation throughout the growth period to varying degrees, with the PC treatment having the most prominent effects. For example, the PC treatment significantly decreased soil available Cd and stem Cd contents at the full-fruiting stage (except under low Cd stress conditions), while also decreasing the fruit Cd content by 22.9%–45.3% ($P < 0.05$) (compared with the Cd contents of the respective samples exposed to Cd stress alone). We previously determined that *B. pyrrocinia* P10 produces a range of beneficial metabolites, including siderophores, EPS, and organic acids (Han et al., 2021), which can promote plant growth and alter Cd mobility in the rhizosphere microenvironment (Khanna et al., 2019; Zhou et al., 2024). Under Cd stress conditions, strain P10 complexed and adsorbed Cd²⁺ through functional groups, including hydroxyl and carboxyl moieties on polysaccharide and protein components of EPS. Overexpressing EPS synthesis-related genes (*algA* and *epsP*) can enhance the Cd resistance of strain P10 (unpublished data), which is consistent with the fact that Cd-resistant bacterial strains complex Cd²⁺ through protein components of EPS to prevent it from entering cells (Hu et al., 2025). Furthermore, siderophores secreted by PGPR alleviate the inhibition of iron uptake by Cd, while also chelating Cd²⁺ and decreasing its phytoavailability (Dong et al., 2025). Collectively, these factors may jointly contribute to decreases in the soil available Cd content and upward transport of Cd from soil to

plants and fruits following an inoculation with biochar-immobilized P10. However, the molecular mechanisms by which this agent decreases Cd accumulation in plant tissues and fruits remain to be thoroughly investigated.

In the present study, the PC treatment decreased the fruit Cd content under Cd stress conditions exceeding 50 mg kg⁻¹ Cd²⁺ (45.3% reduction) significantly more than the P treatment (20.5%) and C treatment (27.3%); however, the C treatment was more effective under low-to-moderate Cd stress conditions. This suggests that the physicochemical passivation by biochar and the metabolic regulation by Cd-resistant PGPR may involve temporally distinct mechanisms during the Cd immobilization and translocation mediated by the biochar-P10 agent. The physical adsorption capacity of biochar is most effective in the early stages, but its active functional groups may become saturated over time (Xie et al., 2022). By contrast, the bacterial agent introduced into soil must go through a colonization period in which it competes with rhizosphere microorganisms for resources before it can sustain metabolic activities (e.g., secretion of EPS, IAA, and other metabolites) that modulate the rhizosphere microenvironment, with critical effects during the fruit development stage (Wu et al., 2019). Consequently, the PC treatment effectively decreased Cd accumulation in fruits under continuous stress conditions, while simultaneously increasing fruit yield and enhancing nutritional quality.

By activating the antioxidant defense system in plants and restricting Cd accumulation, all three treatments alleviated Cd-induced damage and promoted the growth and development of Cd-stressed pepper to varying degrees. The PC treatment consistently had better effects than the other treatments, with the most pronounced growth-promoting and stress-alleviating effects. Even under severe Cd stress conditions (70 mg kg⁻¹ Cd²⁺), the PC treatment increased the number of fruits per plant by 87.50% and 32.39% compared with the P and C treatments, respectively. Similar findings have been reported for studies that combined biochar with *Bacillus* sp. in barley and pepper as well as biochar with *Trichoderma* in tobacco (Andrey et al., 2019; Tan et al., 2024; Ren et al., 2025).

Synergistic regulation of intracellular and extracellular multi-molecular pathways by biochar-immobilized P10 enhances Cd tolerance and inhibits Cd accumulation in pepper roots at the full-fruiting stage

Cell wall sequestration, membrane lipid remodeling, and heavy metal Cd efflux

Cell wall chelation of heavy metals, which is a mechanism protecting plant cells from heavy metal stress, can enhance Cd²⁺ immobilization through pectin demethylesterification and lignin deposition.

During these processes, pectin, which serves as a substrate for reactions catalyzed by pectin methylesterase (PME) and pectin acetyesterase, becomes the most critical component that can bind to Cd^{2+} (Muller et al., 2026). Transcriptomic analyses in the present study revealed that an exposure to Cd stress alone (Cd vs CK) significantly upregulated *PME11* and *PGLR* expression, reflecting adaptive changes in the cell wall to prevent Cd from entering the cytoplasm. However, these extensive changes may adversely affect cell wall structural integrity and its ability to function as a protective barrier. Compared with the effects of Cd stress alone, all three treatments downregulated *PME11* and *PGLR* expression to varying degrees, with the greatest decrease observed for the PCCd treatment (up to 3.10-fold decrease). By contrast, the PC treatment upregulated the expression of key lignin biosynthesis genes in the phenylpropanoid pathway, including *PER4* (1.56-fold increase) and *CYP98A3* (4.28-fold increase), more than the PCd treatment or the CCd treatment, which had relatively weak effects on the expression of these genes. Peroxidases catalyze the polymerization of lignin monomers to generate lignin macromolecules, whereas CYP98A3 is a key enzyme involved in the hydroxylation of lignin precursors (p-coumaroyl shikimate/quinate) (Bhatt et al., 2022). The PC treatment effectively promoted lignin synthesis and cell wall reinforcement, thereby strengthening the physical barrier preventing Cd^{2+} from entering root cells. This finding is in accordance with the significantly lower Cd accumulation in the roots of PC-treated plants than in the roots of plants exposed to Cd stress alone at the full-fruiting stage, while also partially explaining why the PC treatment was the most effective treatment for restricting root Cd accumulation under moderate-to-high Cd stress conditions.

At the membrane lipid level, the PC treatment significantly upregulated the expression of *FAD2* (up to 5.36-fold increase); this gene encodes a key enzyme (fatty acid desaturase) contributing to unsaturated fatty acid synthesis. Increased membrane lipid desaturation can directly enhance the physical stability of cell membranes, enabling root cells to maintain membrane fluidity and integrity under high Cd stress conditions, while also ensuring membrane-associated proteins are formed and function properly, including ion channels and ABC transporters (Zheng et al., 2025b). The PC treatment promoted Cd^{2+} transport and detoxification by upregulating *ABCG19*, *ABCG6*, *PDR1*, and *PDR5* expression levels and downregulating the expression of *PDR4*, *PDR12*, and *ABCG27*. *ABCG* family members are frequently implicated in active heavy metal efflux and vacuolar sequestration. For example, *ABCG2* expression is reportedly upregulated in Cd-stressed *Ageratina adenophora* inoculated with *Burkholderia* sp. SRB-1 (Liu et al., 2023). PDR-type transporters play critical roles in extruding

Cd²⁺ to the apoplast or sequestering it in vacuoles (Naaz et al., 2023). Under Cd stress conditions alone, the significant upregulated expression of these *PDR* genes reflected an active stress-induced efflux response of plants exposed to Cd²⁺. By contrast, the PC treatment downregulated the expression of most *PDR*-type genes, but upregulated the expression of a distinct subset of *ABCG* family members. These differential regulatory changes may be associated with the significant decrease in soil available Cd contents and the decreased Cd accumulation in roots and fruits following the PC treatment.

Signal transduction and hormonal regulation, maintenance of gene/protein homeostasis, and ROS scavenging

After entering a cell, Cd rapidly triggers an increase in the cytosolic Ca²⁺ concentration, which activates downstream signaling cascades and subsequently induces the expression of stress-responsive genes (Zhang et al., 2024). As a core second messenger in plant cells, Ca²⁺ protects plants from Cd toxicity in multiple ways, including alleviating the inhibitory effects of Cd on growth, regulating heavy metal uptake and translocation, improving photosynthesis, mitigating oxidative damage, and modulating signal transduction (Huang et al., 2017). In the current study, the PC treatment significantly upregulated the expression of the calcium-binding protein-encoding gene *CML19* and the abscisic acid receptor-encoding gene *PYL1*, while downregulating the expression of genes encoding ethylene/auxin stress-responsive factors (e.g., *ERF1B* and *AUX22*). This is consistent with the findings of an earlier study in which soybean was treated with the Cd-resistant PGPR *Leclercia adecarboxylata* strain HW04 combined with biochar; the expression of calcium signaling and MAPK signaling pathway-related genes enabled plants to adapt to stress conditions, thereby decreasing the inhibitory effects of stress on growth (Woo et al., 2025). Phytohormones, including IAA, gibberellins, brassinosteroids, and jasmonic acid, directly regulate plant growth and stress tolerance (Manoj et al., 2020). The PCCd treatment downregulated *GA2ox* expression, but upregulated the expression of *GA3ox* and UGT73C-encoding genes, thereby decreasing gibberellin and zeatin degradation and maintaining gibberellin-mediated stem elongation. The PC treatment induced the expression of jasmonic acid synthesis-related genes, such as *OPCL1* and *ACAA1*, while downregulating the expression of *ERF1B*, *JAR1*, and *JAZ*. The coordinated regulation of ethylene and jasmonic acid signaling is typically associated with enhanced defense responses, modulated ROS signaling, and induced stress-related gene expression. More importantly, the PC treatment upregulated the expression of genes involved in IAA and brassinosteroid biosynthesis and signaling, including *amiE* and *GH3.1* (related to IAA), as well as *CYP90C1* and

BZR1/2 (related to brassinosteroids). Brassinosteroids promote cell elongation and regulate organ growth and development (e.g., roots, stems, leaves, and fruits) (Percio et al., 2025), whereas IAA stimulates root cell division and elongation, while promoting lateral root and root hair development (Al-khayri et al., 2024). IAA-producing PGPR can indirectly decrease Cd²⁺ accumulation in plant roots and shoots by modulating the Cd–ROS–MAPK signaling pathway (Pramanik et al., 2021). These findings at the molecular level confirm that biochar-immobilized P10 promoted the growth of Cd-stressed plants by enhancing IAA and brassinosteroid biosynthesis. Furthermore, strain P10 produces ACC deaminase, which degrades the ethylene precursor ACC, thereby effectively alleviating Cd-induced ethylene overaccumulation and mitigating its inhibitory effect on root elongation (Kumar et al., 2025). Thus, at the full-fruited stage, PCCd-treated plants were significantly taller, had a larger stem diameter and root surface area, and more fruits per plant than the corresponding plants exposed to Cd stress alone (50 mg kg⁻¹ Cd²⁺), which was in accordance with the transcriptomic findings at the physiological and root architectural levels.

Various processes, such as DNA transcription, pre-mRNA splicing, and mRNA translation, directly influence the synthesis of biological macromolecules (Wahl et al., 2009). Cd stress severely disrupts the synthesis of biological macromolecules as well as protein folding and assembly, leading to the accumulation of misfolded proteins, thereby triggering endoplasmic reticulum stress and impairing multiple plant growth- and development-related activities, including root energy metabolism and nutrient transport (Li et al., 2024). In the present study, Cd stress alone significantly downregulated the expression of genes involved in the base excision repair pathway (*NTH*, *AlkA*, and *Xth*) and the spliceosome pathway (*Syf*, *Prp16*, *Prp22*, *THOC*, and *U2AF*). By contrast, the three treatments upregulated the expression of most of these genes, with the most pronounced upregulation observed for the PC treatment. These results indicate that Cd²⁺ impairs plant physiological functions by suppressing base excision repair and spliceosomal activities, whereas the biochar-P10 agent can mitigate Cd-induced molecular damage by enhancing these activities, which is consistent with the results of an earlier study on vetiver grass inoculated with *Burkholderia* sp. SRB-1 (Liu et al., 2023). Furthermore, in plants exposed to Cd stress alone (Cd vs CK), the expression levels of genes associated with the protein processing in endoplasmic reticulum pathway were significantly downregulated. However, PCd and PCCd treatments upregulated the expression of multiple heat shock protein-encoding genes, including *DNAJ3*, *HSP22*, *HSP26.5*, *CDC48*, and *DPPA1*, as well as genes encoding soluble inorganic

pyrophosphatases. Heat shock proteins, which function as molecular chaperones, help refold or degrade misfolded proteins under endoplasmic reticulum stress conditions and are critical for maintaining protein homeostasis (Vos et al., 2011). *HSP* expression was upregulated the most by the PC treatment, implying that the biochar-P10 agent alleviated endoplasmic reticulum stress and maintained efficient protein synthesis and processing. At the energy metabolism level, the PC treatment significantly enhanced oxidative phosphorylation and nitrogen metabolism. Oxidative phosphorylation is the core process of mitochondrial ATP synthesis, with Cd stress typically decreasing the mitochondrial membrane potential and adversely affecting the electron transport chain, with detrimental effects on the root energy supply (Finger-Teixeira et al., 2021). The upregulated expression of oxidative phosphorylation-related genes and *HSP* family members suggests that the biochar-P10 agent can restore root energy metabolism and ensure that mitochondrial proteins assemble properly, thereby maintaining normal pepper root physiological functions under high Cd stress conditions.

Excessive ROS accumulation directly leads to Cd-induced cellular damage. Under 50 mg kg⁻¹ Cd²⁺ stress conditions, the PC treatment significantly enhanced the activities of antioxidant enzymes (SOD, POD, and CAT) and increased the proline content, while markedly decreasing the MDA content in pepper plants at the full-fruiting stage. Transcriptomic analyses further revealed that the PC treatment significantly activated the glutathione and ascorbate metabolism pathways, upregulating the expression of *APX1*, *MGST3*, and *GST* considerably more than the P or C treatments. These two metabolic pathways are critical for scavenging H₂O₂ in plants: ascorbate peroxidase catalyzes the reaction between H₂O₂ and ascorbate, while GST catalyzes the conjugation of glutathione (GSH) with Cd²⁺ to form low-toxicity complexes that are subsequently transported into vacuoles (Rai et al., 2023). In accordance with our findings, Anbuganesan et al. (2024) reported that applying peanut shell biochar along with *Aspergillus pseudoterreus* and *Bacillus* sp. can significantly increase SOD, CAT, and ascorbate peroxidase activities in maize, while decreasing the MDA content and heavy metal accumulation. These observations strongly support the efficacy of the PC treatment for enhancing antioxidant activities and ROS scavenging.

Conclusion

The results of this study demonstrate that the biochar-immobilized bacterial agent prepared using *B. pyrrocinia* P10 effectively decreased Cd-induced damage and significantly promoted plant growth,

750 biomass accumulation, and fruit yield and quality throughout the pepper growth period mainly by
751 inducing antioxidant enzyme activities and regulating the proline content. Furthermore, the PC
752 treatment significantly decreased the soil available Cd content and inhibited the translocation of Cd
753 from the soil to roots, stems, and fruits, thereby maintaining the fruit Cd content at consistently low
754 levels. The overall performance of the PC treatment was superior to that of the P or C treatments. At
755 the transcriptional level, biochar-immobilized P10 decreased Cd accumulation in plants by inducing the
756 expression of genes involved in cell wall sequestration, membrane lipid remodeling, and heavy metal
757 Cd efflux, while concurrently protecting against Cd stress by upregulating the expression of genes
758 associated with calcium signal transduction, hormonal regulation and biosynthesis, and ROS
759 scavenging. These findings reflect the potential utility of biochar-immobilized P10 for mitigating Cd
760 stress and ensuring safe production of crops, including pepper cultivated on Cd-contaminated soil.
761 Future research should focus on the mechanisms regulating proline synthesis at various Cd stress levels
762 as well as the colonization of soil by the P10-based biochar agent and its effects on soil microecology.
763 The generated results may facilitate a more comprehensive characterization of the mechanistic basis of
764 the enhanced Cd tolerance of pepper conferred by biochar-immobilized P10.

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768 **Statements and Declarations**

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773 **Conflict of interest**

774 The authors declare no conflict of interest.

775 **Author contributions**

776 Lizhen Han designed the experiments and reviewed the manuscript, Tingting Yang and Lingxiang Zeng
777 performed all experiments, Tingting Yang, Lingxiang Zeng and Yudie Zhao analyzed the data, Tingting
778 Yang wrote the manuscript, all authors read and approved the final manuscript.

779 **Data availability**

780 The data underlying this article are available in the article and in its online supplementary material.

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Figure legends

Fig. 1 Microscopic characterization of biochar-immobilized *Burkholderia pyrrocinia* P10 agent. **(A)**: SEM observation of biochar immobilized P10 inoculant ($\times 1000$ magnification); **(B)**: SEM observation of biochar inoculant ($\times 5000$ magnification); **(C)**: FTIR analysis of biochar inoculant without cadmium stress; **(D)**: SEM observation of biochar inoculant under 600 mg/L cadmium stress ($\times 1000$); **(E)**: SEM observation of biochar inoculant under 600 mg/L cadmium stress ($\times 5000$); **(F)**: FTIR analysis under 600 mg/L cadmium stress

Fig. 2 Plant growth performance and fruit phenotypes of pepper plants in different treated groups under a gradient of cadmium stress concentrations (0, 10, 30, 50, and 70 mg/kg). CK: control without cadmium stress; P: single bacterial inoculant; C: single biochar; PC: biochar-immobilized P10 agent under a gradient of cadmium concentrations (0, 10, 30, 50, and 70 mg/kg)

Fig. 3 Effect of biochar-immobilized P10 agent on physiological indicators and antioxidant enzyme activities of pepper plants at the seedling stage. A, B, C, D, E, and F represent the contents of malondialdehyde(MDA), proline(Pro), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and hydrogen peroxide(H_2O_2) in pepper plants under different cadmium stress concentrations, respectively

Fig. 4 Effect of biochar-immobilized P10 agent on physiological indicators and antioxidant enzyme activities of pepper plants at the flowering stage. A, B, C, D, E, and F represent the contents of MDA, Pro, POD, SOD, CAT and H_2O_2 in pepper plants under different cadmium stress concentrations, respectively

Fig. 5 Effect of biochar-immobilized P10 agent on physiological indicators and antioxidant enzyme activities of pepper plants at the full fruiting stage. A, B, C, D, E, and F represent the contents of MDA,

Pro, POD, SOD, CAT and H₂O₂ in pepper plants under different cadmium stress concentrations, respectively

Fig. 6 Effect of biochar-immobilized P10 agent on available cadmium content in soil, cadmium contents in roots, stems, and fruits of pepper plants with different treatments at these three stages under Cd²⁺ stress. (A) and (B) represent the seedling stage; (C) and (D) represent the flowering stage; and (E) to (H) represent the fruiting stage

Fig. 7 Transcriptomic analysis of pepper roots at the full fruiting stage under cadmium stress and different treatments (CK, P, C, PC). (A): number of differentially expressed genes(DEGs) among comparison groups; (B): cluster heatmap showing sample relationships based on DEGs; (C): KEGG pathway enrichment analysis of DEGs in selected comparisons.

Fig. 8 Expression of 10 differential genes in the chilli roots by RT-qPCR.

Fig. 1 Microscopic characterization of biochar-immobilized Burkholderia pyrrocinia P10 agent.

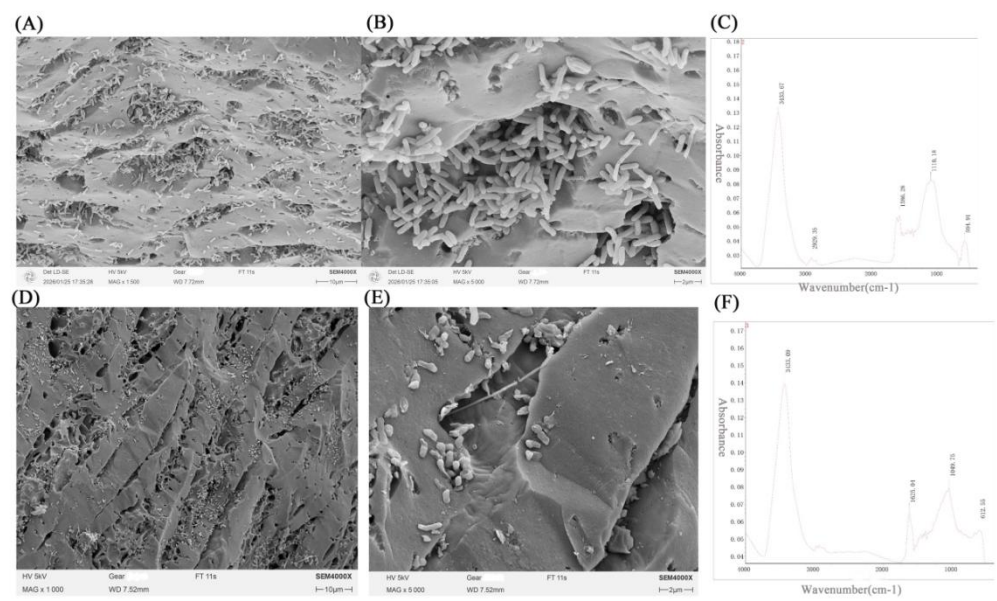


Fig. 2 Plant growth performance and fruit phenotypes of pepper plants in different treated groups under a gradient of cadmium stress concentrations (0, 10, 30, 50, and 70 mg/kg).

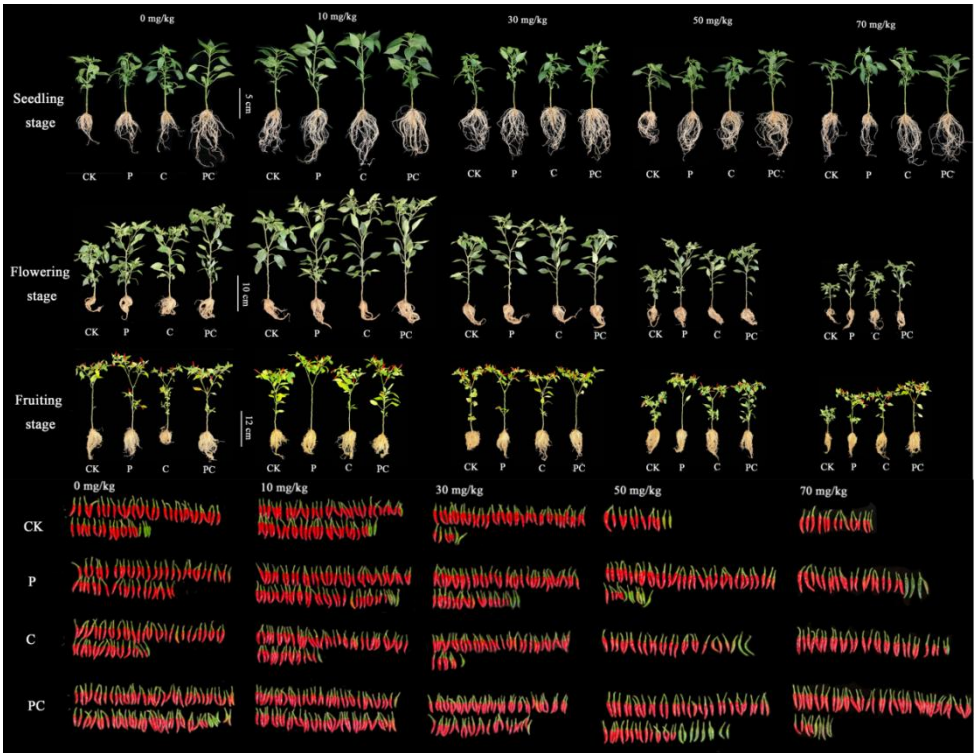


Fig. 3 Effect of biochar-immobilized P10 agent on physiological indicators and antioxidant enzyme activities of pepper plants at the seedling stage.

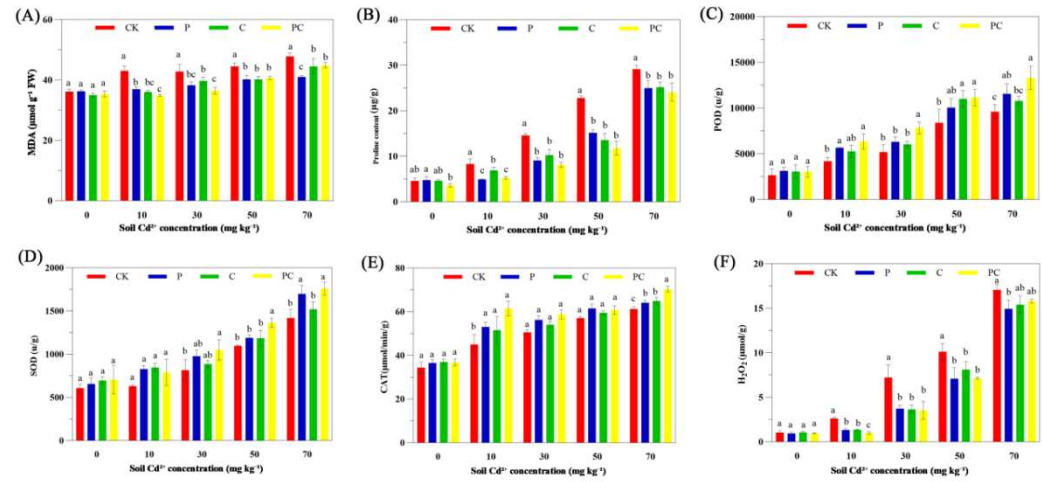


Fig. 4 Effect of biochar-immobilized P10 agent on physiological indicators and antioxidant enzyme activities of pepper plants at the flowering stage.

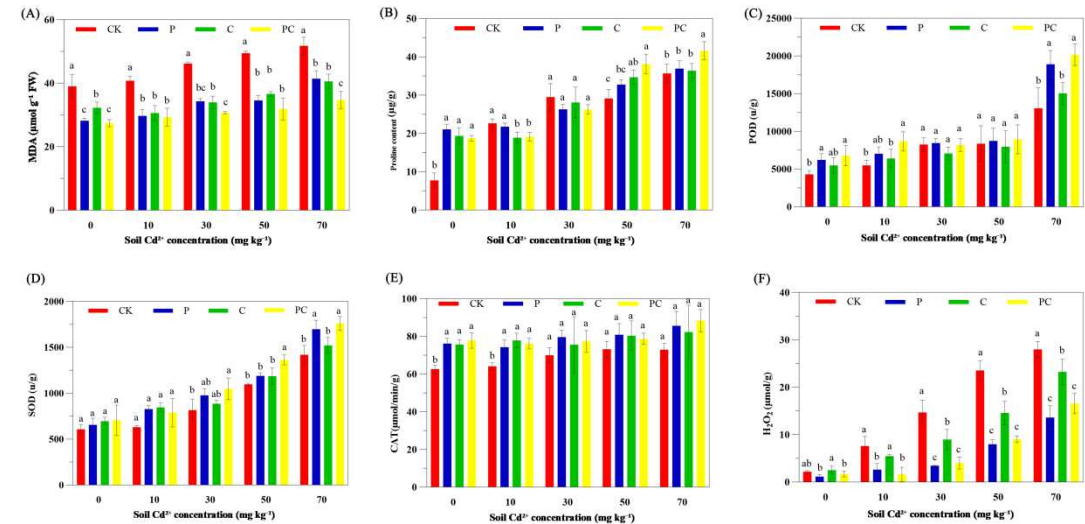


Fig. 5 Effect of biochar-immobilized P10 agent on physiological indicators and antioxidant enzyme activities of pepper plants at the full fruiting stage.

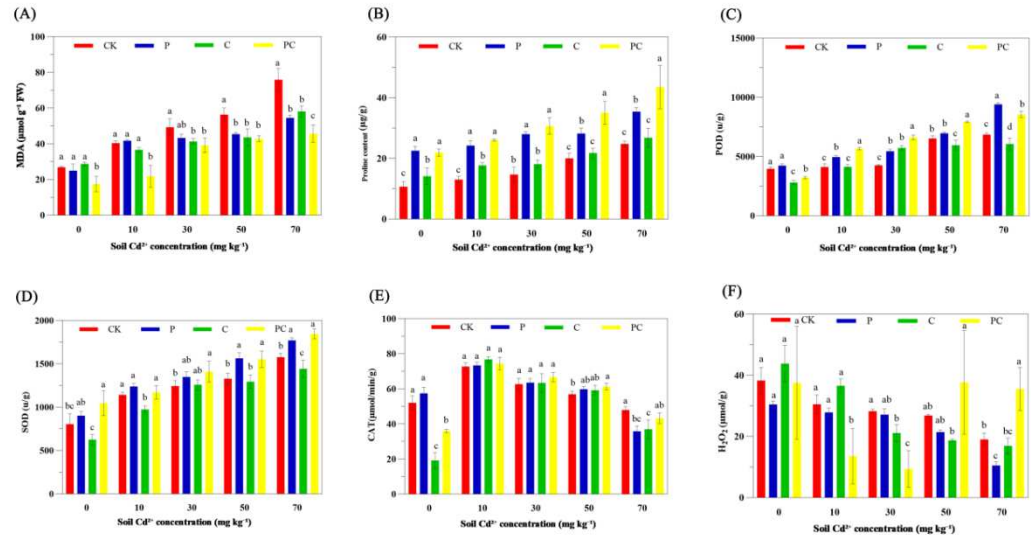


Fig. 6 Effect of biochar-immobilized P10 agent on available cadmium content in soil, cadmium contents in roots, stems, and fruits of pepper plants with different treatments at these three stages under Cd²⁺ stress.

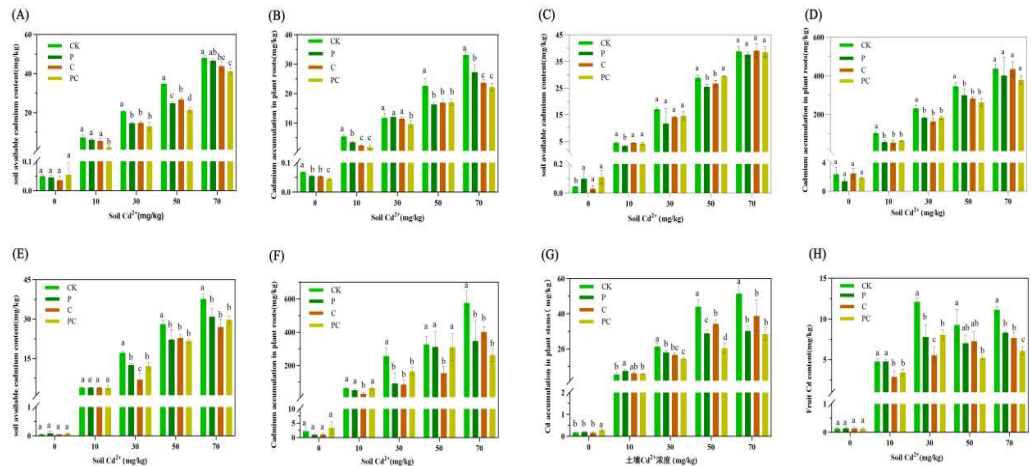


Fig. 7 Transcriptomic analysis of pepper roots at the full fruiting stage under cadmium stress and different treatments (CK, P, C, PC).

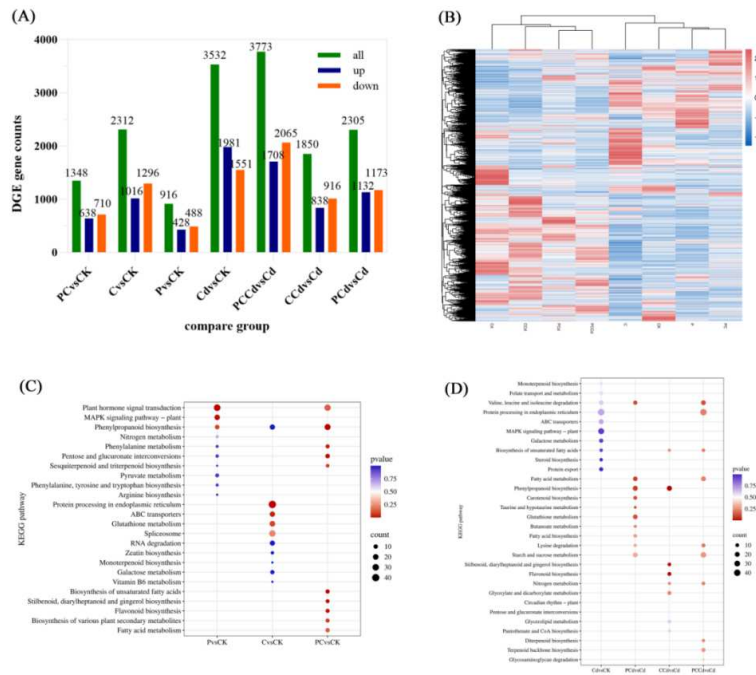


Fig. 8 Expression of 10 differential genes in the chilli roots by RT-qPCR.

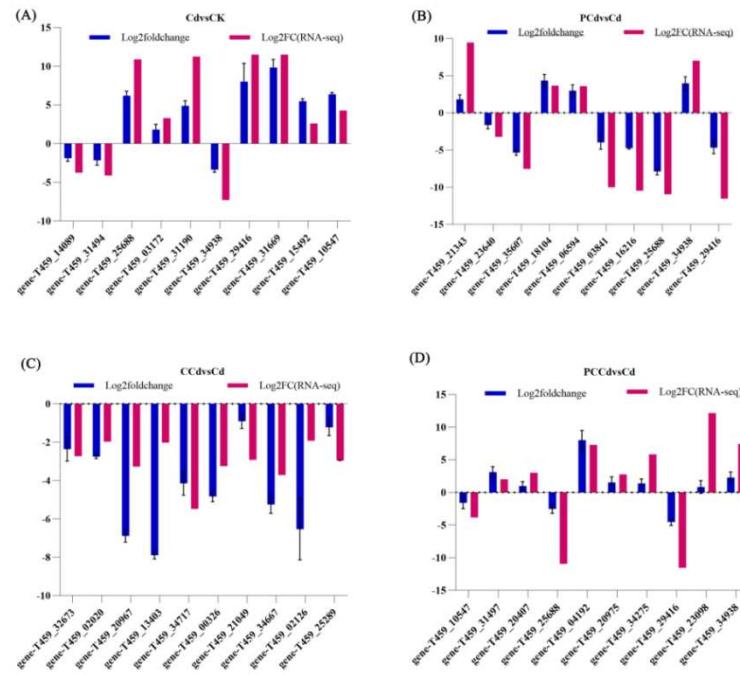


Table 1 Effect of biochar-immobilized P10 agents on the growth indices of pepper plants at the seedling stage

Treatment	Stem thickness (cm)	Plant height (cm)	Fresh weight (g)	Root weight (g)	Root length (cm)	Root surface area (cm ²)	Leaf chlorophyll (SPAD)
CK	3.53±0.07c	19.07±0.74b	8.34±0.37c	1.98±0.24b	18.87±0.34a	240.00±2.71b	58.60±0.78c
Pck	4.48±0.30a	25.27±1.89a	11.51±0.44b	3.68±0.58a	17.98±0.61a	232.74±10.45b	62.97±0.92b
Cck	3.87±0.04bc	19.16±0.70b	10.87±0.70b	2.05±0.45b	20.81±1.67a	242.41±21.16b	60.37±0.39c
PCck	4.22±0.09ab	26.89±2.05a	13.69±0.54a	4.94±0.32a	21.43±1.27a	349.75±20.13a	66.80±0.85a
10	3.69±0.15c	20.61±0.60b	10.34±0.17ab	2.65±0.05c	22.89±0.74ab	289.87±9.87b	54.53±0.49c
10P	3.99±0.02b	24.05±0.73ab	12.92±0.50a	4.73±0.44bc	21.14±1.00b	353.58±16.94a	58.67±0.59b
10C	3.34±0.08d	20.37±1.18b	8.78±1.55b	3.81±0.37bc	21.41±0.48ab	369.55±5.07a	59.30±0.55b
10PC	4.41±0.07a	26.13±1.67a	12.34±0.22a	6.26±0.75a	23.69±0.36a	351.63±5.60a	63.27±0.23a
30	3.48±0.03a	19.01±0.40b	9.27±0.52ab	3.15±0.13a	20.76±0.64a	259.37±4.44b	54.60±0.46c
30P	3.55±0.02a	24.4±1.11a	10.79±0.46a	2.93±0.44a	20.83±0.61a	294.81±14.42ab	57.43±0.27b
30C	3.46±0.15a	19.33±0.32b	10.08±0.89a	3.25±0.67a	20.33±0.71a	314.91±22.52a	56.90±1.02b
30PC	3.59±0.07a	18.89±2.34b	7.84±0.25b	2.53±0.07a	20.60±0.54a	282.83±5.76ab	59.77±0.57a
50	3.48±0.06a	18.29±1.07a	8.94±0.39a	2.28±0.25a	19.88±0.70a	239.77±16.61b	53.50±0.67c
50P	3.27±0.19a	17.92±1.08a	10.17±1.15a	2.65±0.30a	20.43±1.34a	305.19±12.55a	55.17±0.44bc
50C	3.39±0.18a	17.31±2.00a	9.06±1.22a	2.05±0.23a	19.96±0.50a	237.14±13.28b	58.23±0.83a
50PC	3.61±0.07a	18.73±0.96a	9.73±1.15a	2.70±0.23a	20.31±0.33a	244.51±10.19b	57.47±1.42a
70	2.52±0.24b	16.70±0.30ab	5.32±0.60b	1.87±0.42a	18.17±0.38a	143.37±6.35b	48.80±0.72b
70P	3.38±0.11a	15.11±0.62b	8.61±0.33a	2.32±0.56a	19.61±1.46a	223.26±28.21a	53.13±0.50a
70C	3.14±0.10a	17.73±0.55a	6.72±0.69b	1.82±0.33a	17.75±1.05a	206.87±20.47a	54.93±0.57a
70PC	3.22±0.12a	15.49±0.57b	6.11±0.25b	1.92±0.09a	18.79±0.37a	199.30±5.81ab	54.90±1.16a

1133 concentration ($P<0.05$); Identical letters (e.g., a, a) indicate no significant difference, while different letters (e.g., a, b) indicate a significant difference. Treatments that share
1134 the same letter (e.g., ab, ab) do not differ significantly, The same below. The numbers preceding the uppercase letters represent the cadmium stress concentrations in the soil,
1135 which are 10 mg/kg, 30 mg/kg, 50 mg/kg, and 70 mg/kg, respectively. CK: no cadmium stress; P: P10 inoculation treatment; C: biochar treatment; PC: biochar-bacterial
1136 agent treatment. The same below.

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Table 2 Effect of biochar-immobilized P10 agents on the growth indices of pepper plants at the flowering stage

Treatment	Stem thickness (cm)	Plant height (cm)	Fresh weight (g)	Root weight (g)	Root length (cm)	Root surface area (cm ²)	Leaf chlorophyll (SPAD)
CK	4.59±0.20b	34.36±1.98d	21.66±0.60b	4.24±0.09b	19.82±0.39b	248.82±12.81c	68.20±1.59a
Pck	5.41±0.18a	42.68±1.09c	38.05±3.26a	5.90±0.48a	22.91±0.30a	327.46±5.57b	69.63±1.77a
Cck	5.95±0.10a	48.27±0.93b	37.34±2.06a	5.50±0.54ab	20.97±0.75b	300.20±11.32b	57.63±1.98b
PCck	6.02±0.23a	54.86±1.99a	38.98±2.26a	6.61±0.58a	23.43±0.29a	385.83±14.38a	68.57±1.44a
10	5.43±0.07a	54.55±0.33a	30.57±2.90b	3.65±0.12c	24.43±0.65ab	340.46±10.14a	65.57±1.24b
10P	5.96±0.42a	59.48±1.75a	43.44±1.37a	6.47±0.15a	24.61±0.49ab	370.83±6.68a	69.47±0.98a
10C	5.56±0.05a	59.34±2.69a	33.39±0.67b	5.13±0.38b	22.57±0.59b	353.09±11.15a	65.00±1.56b
10PC	5.30±0.13a	60.55±3.27a	45.72±0.62a	7.63±0.68a	25.22±0.69a	371.96±8.70a	67.83±0.52ab
30	5.53±0.17ab	44.05±0.16b	21.62±0.48d	3.24±0.15ab	22.17±0.38b	329.33±3.09b	63.23±2.19a
30P	5.73±0.14a	52.80±2.05a	38.74±0.89a	3.61±0.14a	24.21±0.48a	360.75±8.93a	64.33±1.96a
30C	5.07±0.06bc	48.67±2.31ab	26.83±2.23c	2.85±0.25b	21.00±0.50b	338.15±5.30b	60.77±3.06a
30PC	5.01±0.18c	49.74±0.23a	32.23±1.32b	3.32±0.21ab	22.66±0.87ab	364.32±3.63a	65.23±1.28a
50	3.55±0.27b	31.78±0.55b	17.32±0.80c	1.99±0.15b	20.64±0.37a	255.53±7.83b	66.33±0.68ab
50P	5.00±0.07a	47.37±3.37a	32.24±1.29a	2.37±0.31ab	21.24±0.55a	250.67±17.41b	62.20±0.21c
50C	4.94±0.51a	43.07±3.34a	24.18±3.60b	1.86±0.03b	20.52±0.39a	245.79±4.69b	64.43±0.37bc
50PC	4.86±0.36a	46.71±2.97a	28.77±1.61ab	2.74±0.11a	20.98±0.41a	307.17±10.35a	68.37±1.66a
70	2.98±0.30b	24.17±2.43b	16.31±0.75b	1.44±0.06b	18.99±0.73a	206.87±20.47a	57.07±0.82c
70P	4.25±0.08a	37.04±0.93a	17.48±1.09b	1.53±0.09b	19.99±1.15a	222.87±25.02a	63.30±1.56b
70C	3.91±0.24a	21.76±1.57b	18.55±1.99b	1.67±0.24b	18.93±0.62a	210.75±6.47a	70.57±0.39a
70PC	4.53±0.19a	35.30±2.65a	24.27±1.62a	2.46±0.37a	20.13±0.16a	237.22±16.15a	68.53±0.63a

Table 3 Effect of biochar-immobilized P10 agents on the growth indices of pepper plants at the full fruiting stage

Treatment	Stem thickness (cm)	Plant height (cm)	Fresh weight (g)	Root weight (g)	Root length (cm)	Root surface area (cm ²)	Leaf chlorophyll (SPAD)	Number of fruits per plant
CK	5.54±0.24b	62.83±1.39a	50.66±0.66a	3.46±0.53a	20.79±0.34b	346.71±4.13b	58.07±0.17ab	13.67±1.20b
Pck	5.99±0.13ab	67.59±1.27a	54.11±1.88a	3.71±0.44a	20.50±0.61b	453.12±6.93a	61.03±4.49ab	14.33±0.88b
Cck	6.40±0.22a	63.35±1.15a	41.12±0.55a	3.71±0.46a	23.83±1.67ab	361.56±3.21b	54.97±1.48b	14.00±1.15b
PCck	6.31±0.08a	67.24±1.71a	58.74±1.83a	4.03±0.36a	25.62±1.27a	436.86±5.37a	64.53±0.24a	22.33±2.03a
10	5.84±0.10a	61.98±2.45b	49.75±0.94a	2.86±0.35a	24.37±0.74b	385.92±3.74c	56.67±2.06a	18.33±1.20a
10P	6.17±0.20a	69.25±0.73a	54.31±1.56a	3.28±0.31a	23.66±1.00b	484.37±3.81a	55.43±0.50a	19.67±1.45a
10C	6.17±0.14a	63.75±1.65b	50.63±0.97a	4.11±0.94a	24.51±0.48b	442.26±8.63b	55.17±1.02a	16.67±1.20a
10PC	6.27±0.02a	72.52±0.44a	56.76±1.72a	3.65±0.04a	27.82±0.36a	479.83±6.86a	59.53±1.89a	20.00±1.15a
30	5.05±0.02b	50.37±3.33b	34.30±1.29c	2.59±0.23b	21.78±0.64b	327.63±2.97c	56.87±1.28ab	12.00±1.53c
30P	5.70±0.08a	62.77±0.99a	46.79±0.89ab	3.21±0.26ab	22.84±0.61b	428.79±9.44a	51.93±1.94bc	17.33±0.88a
30C	5.62±0.06a	53.15±3.09b	42.24±1.09b	3.50±0.24a	22.54±0.71b	358.29±4.63b	47.03±2.13c	14.00±0.58b
30PC	5.75±0.09a	63.58±1.96a	48.48±0.52a	2.59±0.20b	25.40±0.54a	421.2±10.65a	60.53±2.22a	16.33±1.76ab
50	4.31±0.07b	28.29±1.55c	16.55±0.93c	1.36±0.58c	20.89±0.70a	229.98±5.24c	66.50±1.00a	2.00±1.00c
50P	5.29±0.03a	55.10±0.93a	42.99±1.08b	3.56±0.30b	22.44±1.34a	328.98±11.02b	60.10±2.48b	11.00±1.53a
50C	5.06±0.24a	45.66±0.79b	44.12±1.09a	4.49±1.38a	22.07±0.50a	356.44±10.83b	66.13±0.09a	8.67±1.86b
50PC	5.35±0.16a	54.96±0.59a	45.31±1.26a	2.62±0.33bc	23.49±0.33a	408.56±5.22a	67.23±2.22a	10.00±5.03a
70	3.77±0.04c	29.67±1.82b	15.75±0.32c	1.34±0.50b	19.19±0.38a	204.88±12.90c	74.30±3.75a	4.33±0.67b
70P	4.45±0.14b	33.16±2.24b	32.02±0.63b	2.39±0.14ab	18.85±2.11a	334.36±4.88b	72.57±0.70ab	8.00±3.79ab
70C	5.27±0.12a	45.79±2.13a	40.72±1.60a	3.13±0.35a	19.09±0.85a	345.81±7.03b	63.97±3.29b	11.33±2.73ab
70PC	5.14±0.18a	52.40±1.97a	42.50±1.36a	3.48±0.65a	20.96±0.37a	392.23±11.10a	68.60±0.83ab	15.00±1.15a

Table 4 Effect of biochar-immobilized P10 agents on the nutritional indices of pepper fruits

Treatment	Soluble sugar (mg/g)	Soluble protein (mg/g)	Ascorbic acid (mg/g)	Total flavonoids (mg/g)	Total polyphenols (mg/g)
CK	35.39±1.04a	1.33±0.02c	9.66±0.49b	48.57±1.2c	0.99±0.05c
Pck	46.35±0.23a	1.68±0.08b	9.65±0.24b	73.65±2.05a	1.48±0.06ab
Cck	48.4±0.67a	1.49±0.09bc	13.73±0.82a	66.48±1.47b	1.6±0.01a
PCck	50.99±0.66a	2.63±0.15a	13.98±1.38a	78.65±1.89a	1.37±0.07b
10	65.97±0.47a	1.29±0.04d	11.49±0.95b	63.32±1.5d	1.23±0.02b
10P	77.75±2.96a	2.05±0.05b	11.78±0.32b	93.98±2.73b	1.14±0.05bc
10C	66.71±1.22a	1.49±0.01c	16.84±0.58a	86.23±1.59c	1.03±0.08c
10PC	76.29±1.58b	3.57±0.09a	10.82±1.2b	124.57±1.82a	1.48±0.05a
30	56.21±1.45a	1.08±0.05b	12.7±2.32a	53.57±1.46c	1.3±0.07a
30P	43.24±1.49ab	1.71±0.21a	13.27±1.69b	83.73±1.23b	1.04±0.02b
30C	53.5±1.15b	1.66±0.03a	9.52±0.54a	77.4±2.31b	1.41±0.03a
30PC	62.8±1.78c	1.59±0a	11.75±0.52a	108.98±2.52a	1.45±0.05a
50	32.78±2.07ab	1.02±0.01b	10.74±0.21a	40.98±0.71c	1.1±0.04b
50P	33.78±0.13ab	1.43±0.02a	10.34±0.09a	55.65±1.51b	0.94±0.01c
50C	47.41±0.52b	1.08±0.04b	10.14±0.91a	52.65±0.72b	0.91±0.04c
50PC	53.92±0.51a	1.49±0.11a	9.78±0.46a	66.73±0.6a	1.53±0.01a
70	23.25±0.24b	0.92±0.05bc	4.8±1.08a	31.25±2.47c	1±0.03b
70P	26.95±0.4c	0.86±0.04c	7.92±0.29a	60.48±4.8a	0.95±0.07b
70C	41.86±1.12bc	1.1±0.03a	5.78±0.62a	46.48±0.71b	0.77±0.05c
70PC	43.11±1.24a	1.04±0.03ab	7.86±1.35a	47.73±0.74b	1.38±0.07a

1145 **Table S1 RT-qPCR primers of chili differential expression genes**

1146 **Table S2 Quality assessment of transcriptome sequencing data for chilli samples**

1147 **Table S3 Significantly enriched pathways and related differentially expressed gene expression levels in PCCdvsCd, CCdvsCd, PCdvsCd, and CdvsCK**

Supplementary Files

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- [SupplementaryTable.xlsx](#)