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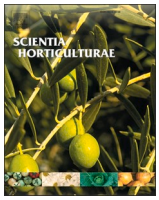

Research Paper

Effects of cadmium stress on the growth, physiology, mineral uptake, cadmium accumulation and fruit quality of 'Sharpblue' blueberry

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ABSTRACT

Cadmium (Cd) contamination is becoming a widespread environmental problem, and Cd toxicity risk is increasing in blueberries, typical acidophilous plants. However, studies on the physiological mechanisms of Cd toxicity affecting the growth of blueberry plants and fruits remain limited. Herein, a pot experiment involving four treatments, CK (0 $mg{\cdot}kg^{-1}$), Cd1 (2.5 $mg{\cdot}kg^{-1}$), Cd2 (15 $mg{\cdot}kg^{-1}$) and Cd3 (50 $mg{\cdot}kg^{-1}$), was conducted to investigate the effects of Cd stress on the growth, fruit quality, physiological characteristics, mineral composition and Cd uptake of 'Sharpblue' blueberry cuttings, and relationships between physicochemical indices and phenotypic data under Cd stress were analyzed via principal component analysis and Mantel's test. Compared with CK, Cd treatment led to chlorosis (decrease in SPAD and N content) in 'Sharpblue' leaves and inhibited increases in plant height, basal stem, crown width and biomass (dry weight), with the most significant changes in the 50 $mg{\cdot}kg^{-1}$ Cd treatment. The size and thickness of the upper epidermal cells, as well as the thickness of the palisade mesophyll and spongy tissues of 'Sharpblue' were significantly reduced at Cd > 15 $mg{\cdot}kg^{-1}$, while the density of stomata was significantly increased. Moreover, Cd stress reduced the yield (decrease in size and single-fruit weight) and quality (decrease in anthocyanin, flavonoid and total phenol content and increase in titratable acid content) of 'Sharpblue' fruits, and Cd content in fruit exceeded the Chinese food safety threshold of 0.05 $mg{\cdot}kg^{-1}$ when Cd > 15 $mg{\cdot}kg^{-1}$. The Cd enrichment capacity of different organs of 'Sharpblue' plants was in the following order: root > shoot > leaf > fruit, and the 50 $mg{\cdot}kg^{-1}$ Cd treatment significantly inhibited the uptake of Fe, Mn, Cu, and Zn. Additionally, 'Sharpblue' plants responded to H_2O_2 and malondialdehyde accumulation in different organs under Cd stress by regulating levels of osmoregulatory substances (soluble protein (SP), soluble sugar, and proline) and altering the activities of catalase, superoxide dismutase, ascorbic acid, and glutathione in the antioxidant system. Finally, correlation analysis revealed that Mg, Fe, Mn, Cu, Cd, H_2O_2, SP, SPAD, N, anthocyanin, titratable acid, flavonoids, and total phenols were strongly correlated with 'Sharpblue' growth, leaf and fruit phenotype under Cd stress. These findings provide theoretical information for soil safety management and the breeding of Cd-tolerant cultivars during blueberry cultivation, as well as for the study of Cd stress response mechanisms in acidophilous plants.

1. Introduction

Due to modern agricultural practices, industrial development and changes in human activities, heavy metal pollution in soils has become a severe problem, which has had a strong impact on the environment worldwide. Currently, 25 % of China's agricultural soils are contaminated with heavy metals, with cadmium (Cd) levels exceeding the threshold of 7 % (Shifaw, 2018; Wang et al., 2019). In contrast, in Europe, approximately 21 % of agricultural soils contain Cd above the safety threshold (0.1 $mg{\cdot}kg^{-1}$) (Kuang et al., 2024). Cd is highly toxic,

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highly mobile, and extremely easy for plants to absorb. Excessive Cd accumulation damages cell structure, accelerates chlorophyll degradation, affects metabolic processes such as protein synthesis and enzyme activity, and leads to the abnormal expression of related genes, inhibiting the growth and development of plants and even causing death (Sterckeman and Thomine, 2020). More importantly, in agricultural production, Cd stress not only affects crop yield and quality but also leads to Cd accumulation in animals and humans through the food chain, ultimately posing a serious threat to human health and food safety (Meharg et al., 2013). Therefore, it is vital to study the Cd enrichment characteristics of different plants, especially crops, as well as mechanisms of Cd stress response to guide production practices and ensure food safety.

Blueberry plants (*Vaccinium* spp.) are perennial berry bushes in the family Ericaceae and are known as the "king of berries" due to their unique fruit flavor and abundance of anthocyanins, phenols, and other active antioxidant substances (Wu et al., 2023). Due to the economic benefits of commercial blueberry cultivation, blueberries are grown in many countries, and since 2020, China has ranked first in terms of blueberry planting area (Yu et al., 2020). However, blueberry plants are typical acid-loving plants (suited to soils with pH 4.4–5.5), and an acidic soil environment increases the solubility and mobility of Cd; as a result, there is an increase in the activity of Cd^{2+} that enters soils with the application of sulfur powder, phosphorus fertilizers, and composite fertilizers during the cultivation process, which further increases Cd toxicity risks (Chen et al., 2019; Jiang et al., 2017). Chen et al. (2020) and Manquian-Cerda et al. (2016) showed via in *vitro* studies that Cd stress significantly inhibits the growth of blueberry plants and antioxidant enzyme activities and phenolic compounds play important roles in blueberry plant response to Cd stress. Cultivation experiments have shown that Cd at a certain concentration (5–15 $mg{\cdot}kg^{-1}$) contributes to the accumulation of rabbiteye blueberry biomass as well as an increase in catalase (CAT) and peroxidase (POD) activities (Song et al., 2023). Li et al. (2021) investigated and analyzed the distribution of heavy metals in blueberries and soil in the blueberry production area of Majiang, Guizhou, and found that although the concentration of Cd and other heavy metals in the soil exceeded the value set by the national standard, this did not substantially impact the growth of blueberries or increase Cd toxicity risk in the fruits. In addition, Chen et al. (2021) reported that Cd stress interfered with the uptake and transport of elements in different tissues of 'Bluegold' blueberry, and Cd stress significantly increased the abundance of several extremely tolerant bacterial populations in the interroot region of 'Northland' blueberry (*Acetobacteraceae*-unclassified, *Acidibacter, Acidiphilium, Granulicella*, etc.) (Chen et al., 2023). Yu et al. (2024) used *Arabidopsis thaliana* to verify that the 'Brigitta' blueberry metal transporter protein-encoding gene family (NRAMP) genes *VcNramp4, VcNramp6*, and *VcNramp8.2* were linked to plant response to Cd stress, and the *VcCXIP4* and *VcYSL6* genes also performed certain functions in response to Cd stress (Chen et al., 2019). However, to date, few systematic studies have been reported on the effects of Cd stress on the growth phenotype, physicochemical characteristics, and fruit quality of blueberries (Chen et al., 2021; Manquian-Cerda et al., 2016).

Therefore, to address this gap, we comprehensively investigated the effects of Cd stress on growth phenotypic indices, elemental absorption, fruit quality and physiological stress tolerance responses in 'Sharpblue' blueberry plants (two-year-old cuttings) and evaluated Cd enrichment characteristics in different organs and the Cd content of the fruits. Overall, our results initially revealed the potential physiological mechanisms of Cd stress inhibiting the growth and fruit quality of 'Sharpblue'. In summary, this study improved our understanding of Cd toxicity in blueberries, provided systematic guidance for their cultivation and safe production, and established a theoretical basis for enhancing blueberry resistance to Cd stress and studying Cd tolerance mechanisms in acidophilous plants.

2. Materials and methods

2.1. Experimental materials

Two-year-old 'Sharpblue' blueberry cuttings (*Vaccinium corymbosum* L.) with good, consistent growth and no pests or diseases were selected as potting materials, and each plastic pot (top diameter 26 cm, bottom diameter 20 cm, height 30 cm, with tray) was evenly filled with 2.0 kg of well-mixed soilless substrate ($V_{coarse\ charcoal}$: $V_{fine\ charcoal}$: $V_{perlite}$: $V_{pine\ bark}$ = 3:3:1:1). The blueberry materials used were obtained from the Baima Blueberry Experimental Base, Lishui District, Nanjing, Jiangsu Province (31°36′5.66″N, 119°11′49.39″E).

2.2. Experimental design and sampling

According to the environmental risk control standard for soil contamination of agricultural land (GB 15618-2018) and risk control standard for soil contamination of development land (GB36600-2018), four treatments were established with uniform applications of a $CdCl_2{\cdot}5/2H_2O$ solution and distilled water, with exogenous Cd concentration of 0 (CK), 2.5 (Cd1), 15 (Cd2) and 50 (Cd3) $mg{\cdot}kg^{-1}$ in the cultivation substrate. Four pots were set up as replicates for each treatment, and one blueberry cutting was planted in each pot. This experiment was conducted in an artificial glass greenhouse, and all the blueberry pot treatments were set up with a randomized block design. The blueberries were consistently watered, fertilized (After 15 d of Cd treatment, 500 mL of 1 ‰ compound fertilizer aqueous solution) and managed during the experiment. Physicochemical indicators of the substrate are shown in Table S1. After 150 d of growth, fresh tissues (root, shoot, and leaf) from plants in each treatment group were collected for physiological analysis, and fresh leaves ($2\times2\ mm^2$) from plants in each treatment group were cut and fixed in 2.5 % glutaraldehyde solution for scanning electron microscopy (SEM) analysis. Moreover, during the experiment, mature blueberry fruits from each treatment were collected for growth and physicochemical analyses. After completion of sampling, three blueberry plants exhibiting an intermediate level of growth were randomly selected from each treatment group for biomass determination.

2.3. Determination of plant growth and fruit indices

The plant height and the crown width of the blueberry plants in each treatment group were measured with a tape measure, and digital Vernier calipers were used to determine basal stem width (n = 4). The relative chlorophyll content (SPAD value) and nitrogen content (n = 6) of the upper, middle and lower mature leaves of the blueberry plants in each treatment group were determined with a TYS-4 N portable plant nutrient tester (Zhejiang, China) (n = 15); the single-fruit weight, transverse diameter, and vertical diameter were measured by an electronic balance and a digital Vernier calipers for each treatment (n = 6); and the firmness of the blueberry fruits was determined with a KM-5 hardness tester (Kyoto, Japan) (n = 6). The blueberry plants collected from each treatment group were washed, dried, divided into 3 parts—roots, shoots and leaves—using branching scissors, put into marked envelopes, placed in an oven at 105 °C for 30 min, dried at 70 °C to a constant weight, and weighed on an analytical balance to determine the dry weights of the tissues (n = 3).

2.4. Scanning electron microscopy analysis

Leaves from each treatment were fixed in 2.5 % glutaraldehyde solution, dehydrated with different concentrations of ethanol (10 %, 20 %, 30 %, 50 %, 70 %, 90 % and 100 %), dried with supercritical CO_2, coated with a gold spray and subsequently analyzed by scanning electron microscopy (SEM; Quanta 200, FEI, USA). The upper epidermis, lower epidermis and cut surfaces of the blueberry leaves were imaged. The

epidermal cell size, stomatal size and density were quantitatively analyzed using ImageJ 2 (2.8.0) software according to Zhang et al. (2019). Three samples per treatment were prepared as replicates ($n = 3$) for observation.

2.5. Determination of Cd and mineral content

Roots, shoots, leaves and fruits of blueberry plants from each treatment were dried at 105 °C for 30 min at 70 °C to a constant weight and were subsequently ground and passed through a sieve (100 mesh). An inductively coupled plasma emission spectrometer (ICP–OES, Avio220, PE, USA) was used to determine the K, Ca, Mg, Fe, Mn and Zn contents of each tissue, and inductively coupled plasma–mass spectrometry (ICP–MS, Nexion300X, PE, USA) was used to determine the Cu and Cd contents of each tissue. Three replicates were analyzed for each sample ($n = 3$). The specific methods used were described previously in Guo et al. (2021) and the determination of multiple elements in food (GB 5009.268-2016).

2.6. Physiological indices

Fresh tissues (roots, shoots, leaves and fruits) were taken from each treatment and were washed, dried, and ground in an ice bath for physiological index determination. Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) method (Heath and Packer, 1968); soluble protein (SP) content was determined by the Coomassie brilliant blue method (Bradford, 1976); soluble sugar (SS) content was determined by the anthrone colorimetry method (Seifter and Dayton, 1950); anthocyanins were determined by a pH difference method (Giusti and Wrolstad, 2001); and titratable acids were determined by an acid–base potential titration method (Nour, 2022). In addition, 0.1 g of fresh plant tissue was mechanically homogenized with PBS (0.9 mL, pH 7.0, 0.1 M) and centrifuged at 12,000 rpm for 10 min (4 °C) under ice-bath conditions. Kits provided by Nanjing Jiancheng Bioengineering Institute were used for the determination of H_2O_2, proline (Pro), SOD, CAT, reduced glutathione (GSH), ascorbic acid (AsA), flavonoids and total phenol content. Three replicates ($n = 3$) were analyzed for each sample.

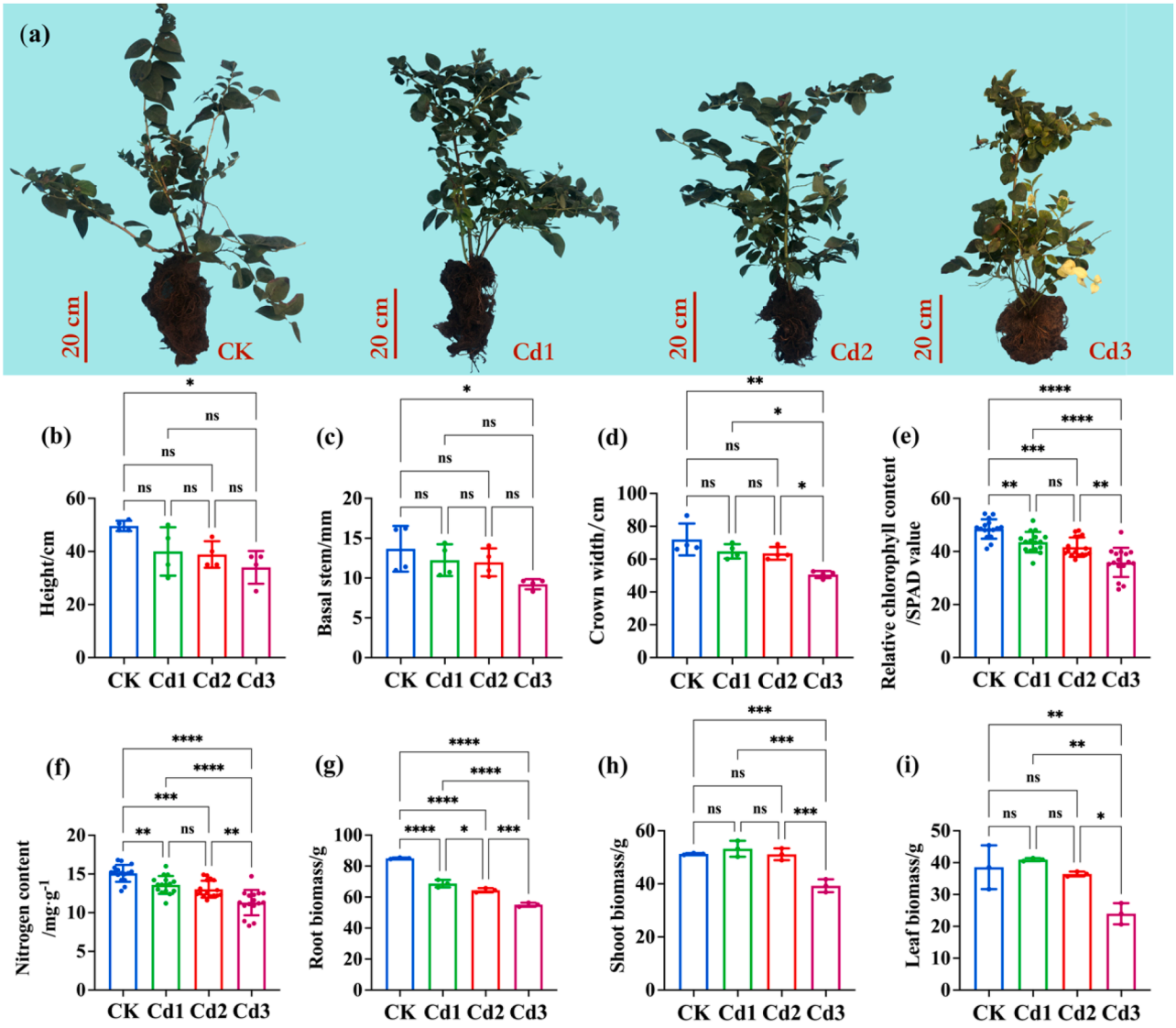

Fig. 1. Phenotypes of 'Sharpblue' plants under Cd stress (a), height (b), basal stem (c), crown width (d), relative chlorophyll content (SPAD) (e), nitrogen content (leaf) (f), root biomass (dry weight) (g), shoot biomass (dry weight) (h) and leaf biomass (dry weight) (i). The data shown in the bar graphs are the means ± SDs. Differences between treatments were analyzed by one-way ANOVA (Tukey's multiple comparisons test). ns, nonsignificant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

2.7. Data processing and analysis

The data were processed and analyzed by ANOVA using SPSS statistics 29. 0 (IBM Corp., Armonk, NY, USA) and Prism 9.0 (GraphPad Software, San Diego, CA, USA). Duncan's multiple comparisons test was used to determine the significance of the differences in the tissue mineral data with SPSS 29.0 ($p < 0.05$). Tukey's multiple comparison test was used to evaluate the significant effects of Cd stress on the growth, physiology and fruit phenotype of the blueberry plants with Prism 9.0 ($p < 0.05$). Graphs were constructed and edited using Prism 9.0 and Origin 2024 (Origin Lab, Inc., USA).

3. Results

3.1. Effects of Cd stress on the growth of 'Sharpblue' plants

The growth of 'Sharpblue' was poisoned and inhibited with increasing substrate Cd concentration (Fig. 1a), and the plant height, basal stem, and crown width of the plants decreased in all the treatments, with the corresponding indices in the Cd3 treatment significantly reduced by 31.49, 32.51, and 29.69 %, respectively, compared to those in the CK treatment (Fig. 1b–d). Moreover, Cd stress significantly reduced the chlorophyll and N contents of 'Sharpblue' leaves, with the lowest values of 35.88 (SPAD) and 11.30 mg·g^{-1}, respectively, under the Cd3 treatment (Fig. 1e–f). In addition, Cd stress significantly inhibited dry matter accumulation in 'Sharpblue' roots, whereas a low concentration of Cd (2.5 mg·kg^{-1}) promoted dry matter accumulation in shoots and leaves; however, the values were not significantly different from those of CK. At substrate Cd concentrations greater than 15 mg·kg^{-1}, the dry weights of the shoots and leaves significantly decreased, and the seedling root, shoot, and leaf biomass significantly decreased by 35.23 %, 23.32 % and 37.82 %, respectively, compared with those of CK (Fig. 1g–i).

3.2. Effect of Cd stress on 'Sharpblue' leaves

Cd stress significantly affected the leaf phenotype of 'Sharpblue'. Compared with those in CK, the size and thickness of the upper epidermal cells in Cd-treated plants increased and then decreased with increasing Cd stress; the greatest changes in the size and thickness of the upper epidermal cells were found in the Cd2 treatment (2055.78 μm^2 and 16.82 μm, respectively), the smallest change in the upper epidermal cell size was found in the Cd3 treatment (817.60 μm^2), and an increase in Cd stress significantly contributed to the increase in upper epidermal thickness (Fig. 2a–c). Moreover, compared with those in the CK treatment, the thickness of palisade mesophyll and spongy tissues in the leaves of the plants in the Cd2 treatment significantly increased by 47.78 % and 6.48 %, respectively; however, the Cd3 treatment resulted in significant reductions of 23.16 % and 18.23 %, respectively, in the corresponding indices, and the chloroplastic tissues became more loosely packed when the substrate Cd concentration was >15 mg·kg^{-1} (Fig. 2d–f). Overall, Cd stress increased leaf stomatal size and decreased stomatal density; interestingly, leaf stomatal size (39.77 μm) was greater ($p>0.05$) in the Cd3 treatment than in the CK (35.97 μm) treatment, whereas stomatal density was significantly greater (33.33 %) than in CK (Fig. 2g–i).

3.3. Effect of Cd stress on 'Sharpblue' fruits

Cd stress inhibited the growth of 'Sharpblue' fruits (Fig. 3a). Compared to the CK treatment, the Cd1, Cd2 and Cd3 treatments significantly reduced the single-fruit weight by 20.66, 28.17 and 31.73 %, respectively, and the fruit transverse diameter by 10.55, 13.72 and 14.09 %, respectively; however, the vertical diameter of the fruit did not significantly differ (Fig. 3b, d and e). Moreover, the Cd2 treatment increased fruit firmness, but the results were not significantly different from those of CK, while the Cd3 treatment significantly decreased fruit firmness, yielding the lowest value of 0.28 kg·cm^{-2} (Fig. 3c). In addition, Cd stress affected the quality of 'Sharpblue' fruits. Compared with

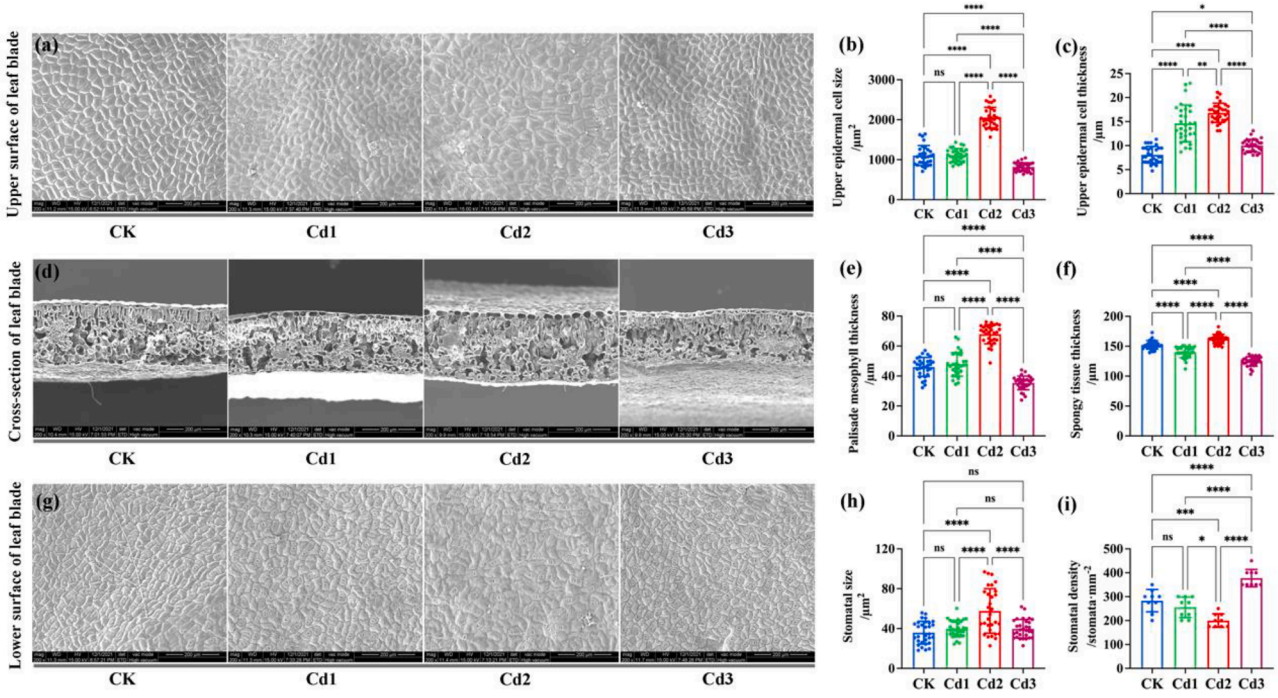

Fig. 2. Upper surface phenotype (a), upper epidermal cell size (b), upper epidermal cell thickness (c), leaf cross-sectional phenotype (d), palisade mesophyll thickness (d), spongy tissue thickness (f), lower surface phenotype (g), stomatal size (h) and stomatal density (i) of 'Sharpblue' leaves under Cd stress. Scale bar for electron microscopy images: 200 μm. The data shown in the bar graphs are the means ± SDs. Differences between treatments were analyzed by one-way ANOVA (Tukey's multiple comparison test). ns, nonsignificant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

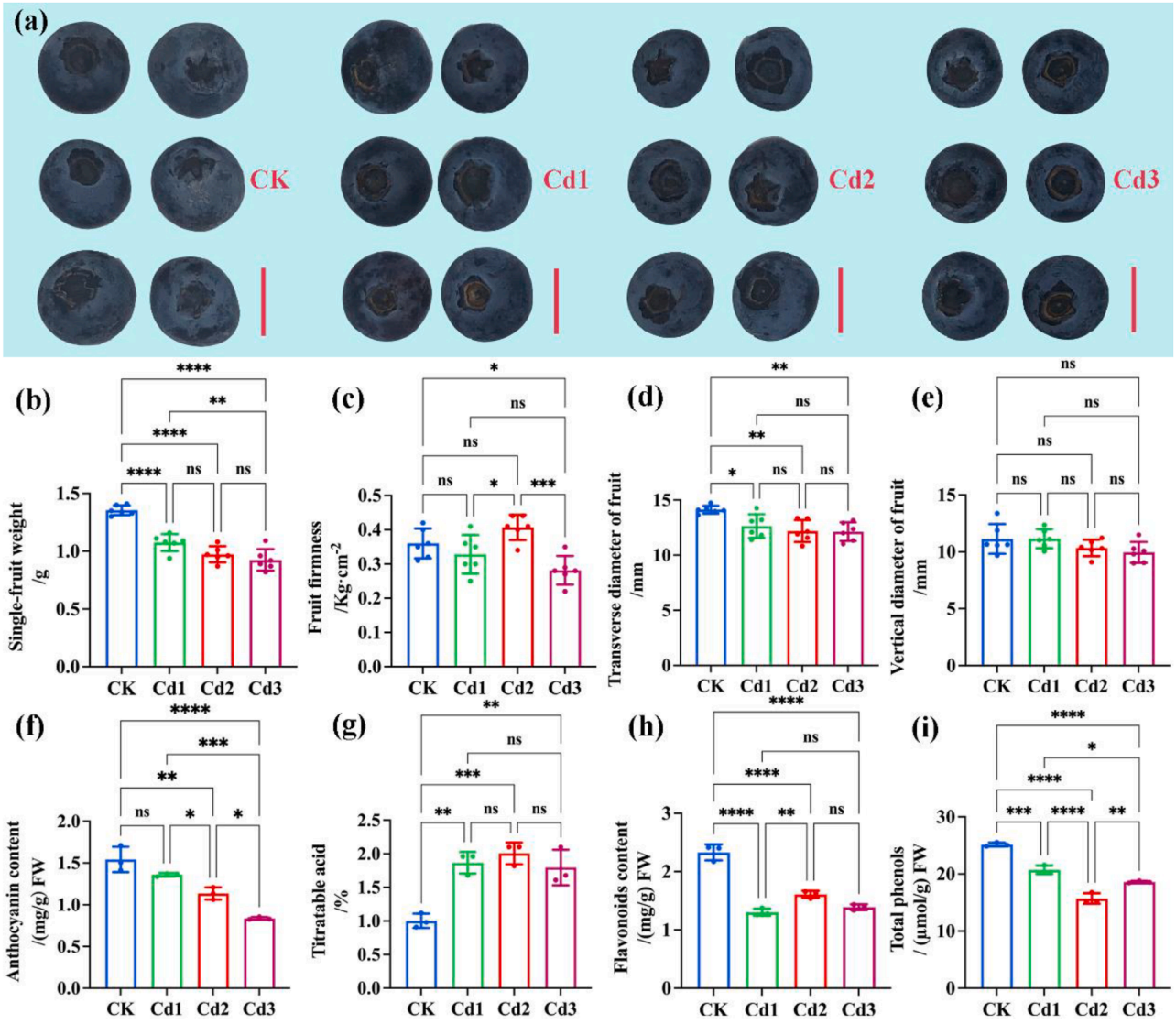

Fig. 3. 'Sharpblue' fruit phenology (a), single-fruit weight (b), fruit firmness (c), transverse diameter of fruit (d), vertical diameter of fruit (e), anthocyanin content (f), titratable acid (g), flavonoid content (h) and total phenols (i) under Cd stress. Scale bar for the fruit picture: 1 cm. The data shown in the bar graphs are the means ± SDs. Differences between treatments were analyzed by one-way ANOVA (Tukey's multiple comparison test). ns, nonsignificant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

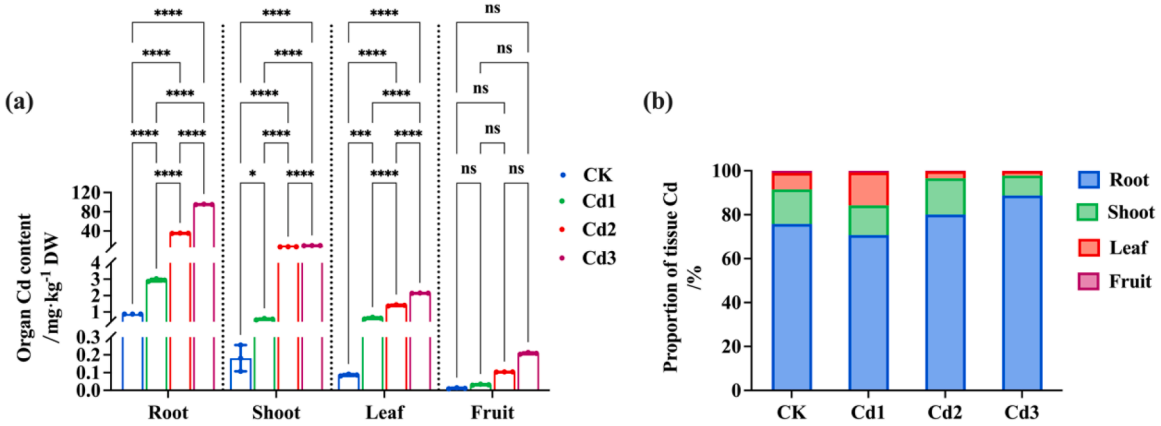

Fig. 4. Cd content (a) and proportion (b) in different organs (roots, shoots, leaves, and fruits) of 'Sharpblue' plants under Cd stress. The data shown in the bar graphs are the means ± SDs (a). Differences among treatments were analyzed by two-way ANOVA (Tukey's multiple comparisons test). ns, no significant difference; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

CK, the Cd treatments significantly reduced the fruit anthocyanin content, with the lowest anthocyanin content (0.84 mg·kg^{-1} FW) in Cd3-treated fruits (Fig. 3f); however, compared with those in CK, the fruit titratable acid content in Cd3-treated fruits significantly increased by 86.05 %, 100 %, and 79.07 %, respectively (Fig. 3g). Cd stress also significantly inhibited the accumulation of total phenolics and flavonoids in fruits, with the lowest flavonoid content (1.30 mg·g^{-1} FW) occurring in fruits under the Cd1 treatment and a low total phenol content (15.71 μmol/g FW) occurring in fruits under the Cd2 treatment (Fig. 3h and i).

3.4. Effect of Cd stress on the Cd and mineral content of different tissues of 'Sharpblue' plants

As the substrate Cd concentration increased, the Cd content in each organ of 'Sharpblue' plants increased, with the highest Cd content occurring in the roots (70.71 %-88.73 % of the whole-plant Cd content), followed by the shoots, leaves, and fruits (Fig. 4a and b). Notably, at a substrate Cd concentration of 15 mg·kg^{-1}, the fruit content was 0.10 mg·kg^{-1}. Moreover, Cd stress affected the absorption and transport of elements in 'Sharpblue'. Overall, substrate Cd concentration within a certain range was beneficial for promoting the uptake of K, Ca and Mg by 'Sharpblue' plants, but the opposite effect was observed when the Cd concentration exceeded the tolerance capacity of the plants. In addition, the uptake of trace elements (Fe, Mn, Cu and Zn) in different organs of 'Sharpblue' plants under Cd stress showed a pattern in which low concentrations of Cd led to promotion and high concentrations of Cd led to inhibition. In particular, compared with those in the CK treatment, the absorption and transport of Cu and Zn in the Cd3 treatment were significantly inhibited; in addition, the contents of Cu in the roots, shoots, leaves and fruits were reduced by 48.46 %, 46.15 %, 27.60 % and 21.46 %, respectively; and the Zn content in the fruits was reduced by as much as 78.75 % (Table 1).

3.5. Effects of Cd stress on oxidative damage in different tissues of 'Sharpblue' plants

The levels of reactive oxygen species (ROS) and lipid peroxidation in 'Sharpblue' roots, shoots, leaves and fruits increased continuously with increasing Cd stress. Overall, 'Sharpblue' leaves and fruits displayed greater damage from Cd stress. Compared with CK, the H_2O_2 contents of the roots, shoots, leaves and fruits were significantly increased by 135.39 %, 102.28 %, 54.20 % and 100.98 % under Cd3 treatment, while the MDA contents were 2.07, 2.44, 3.65 and 2.21 times higher than those of CK, respectively (Fig. 5 a and b).

3.6. Effect of Cd stress on osmoregulatory substances in different tissues of 'Sharpblue' plants

With increasing substrate Cd concentration, the SP content in 'Sharpblue' plants increased in the roots, while in the shoots, leaves and fruits, it first increased and then decreased; the SP content in the roots, shoots and leaves under Cd3 treatment was significantly greater than that under CK by 168.74 %, 24.95 %, and 43.17 %, respectively; however, in fruits, there was no significant difference in the SP content (Fig. 6 a). Furthermore, compared to CK, the SS content in the roots and leaves under the Cd3 treatment significantly increased by 38.81 % and 9.59 %, while the SS content in the shoots and fruits significantly decreased by 12.86 % and 7.88 % (Fig. 6 b). In addition, Cd stress significantly promoted the accumulation of Pro in the roots, shoots, leaves and fruits, with the Pro content increasing by 77.74 %, 45.05 %, 39.95 % and 2.67 %, respectively, in all tissues under the Cd3 treatment compared with that in the CK treatment (Fig. 6 c).

Table 1
Changes in the mineral content of 'Sharpblue' plants under Cd stress.

Treatment		CK	Cd1	Cd2	Cd3
K/ mg·kg^{-1} DW	Root	2710 ± 102.9^{b}	2123 ± 23.64^{c}	1753 ± 97.44^{d}	3621 ± 12.56^{a}
	Shoot	4387 ± 29.31^{ns}	4386 ± 88.14^{ns}	4519 ± 166.3^{ns}	4559 ± 30.34^{ns}
	Leaf	13505 ± 72.30^{c}	15475 ± 655.2^{a}	14613 ± 2.03^{b}	13932 ± 1.59^{c}
	Fruit	8271 ± 73.46^{a}	7625 ± 230.5^{b}	8110 ± 41.48^{a}	6164 ± 110.7^{c}
Ca/ mg·kg^{-1} DW	Root	15678 ± 370.4^{b}	22279 ± 495.2^{a}	10765 ± 291.5^{c}	6692 ± 91.74^{d}
	Shoot	6227 ± 44.96^{a}	4782 ± 75.56^{b}	6196 ± 160.8^{a}	4564 ± 16.78^{c}
	Leaf	14148 ± 209.4^{b}	14027 ± 302.0^{b}	13267 ± 143.9^{c}	15555 ± 104.6^{a}
	Fruit	648.9 ± 3.54^{b}	555.7 ± 44.06^{b}	1454 ± 241.2^{a}	656.5 ± 12.91^{b}
Mg/ mg·kg^{-1} DW	Root	2181 ± 44.92^{c}	2465 ± 2.25^{b}	2746 ± 22.19^{a}	1519 ± 9.38^{d}
	Shoot	1211 ± 4.30^{b}	1242 ± 14.28^{a}	1263 ± 20.03^{a}	902.9 ± 11.80^{c}
	Leaf	3644 ± 45.28^{c}	4568 ± 65.90^{a}	3860 ± 37.15^{b}	3906 ± 14.72^{b}
	Fruit	379.3 ± 5.50^{c}	546.3 ± 29.98^{b}	785.1 ± 12.52^{a}	534.2 ± 12.17^{b}
Fe/ mg·kg^{-1} DW	Root	1900 ± 70.88^{c}	2661 ± 1.50^{a}	2159 ± 63.38^{b}	1452 ± 223.9^{d}
	Shoot	126.0 ± 2.91^{ab}	109.6 ± 10.95^{b}	145.2 ± 27.48^{a}	105.3 ± 3.35^{b}
	Leaf	235.5 ± 10.29^{c}	286.6 ± 3.13^{a}	272.8 ± 0.36^{b}	226.5 ± 5.26^{c}
	Fruit	48.60 ± 0.94^{a}	45.26 ± 2.85^{b}	42.32 ± 1.74^{b}	36.65 ± 0.93^{c}
Mn/ mg·kg^{-1} DW	Root	32.65 ± 0.50^{c}	37.6 ± 0.62^{b}	45.59 ± 1.13^{a}	21.65 ± 0.31^{d}
	Shoot	226.3 ± 2.08^{b}	212.9 ± 1.01^{c}	247.8 ± 0.02^{a}	139.9 ± 2.67^{d}
	Leaf	124.7 ± 0.36^{b}	164.4 ± 4.19^{a}	128.6 ± 2.71^{b}	125.0 ± 0.79^{b}
	Fruit	30.86 ± 0.55^{b}	26.46 ± 1.06^{d}	89.88 ± 0.79^{a}	28.46 ± 1.22^{c}
Cu/ mg·kg^{-1} DW	Root	2.27 ± 0.02^{a}	2.08 ± 0.07^{a}	1.34 ± 0.24^{b}	1.17 ± 0.06^{b}
	Shoot	1.95 ± 0.10^{a}	1.49 ± 0.18^{b}	1.29 ± 0.11^{b}	1.05 ± 0.02^{c}
	Leaf	1.92 ± 0.02^{a}	1.66 ± 0.03^{ab}	1.37 ± 0.45^{b}	1.39 ± 0.08^{b}
	Fruit	2.05 ± 0.13^{a}	1.63 ± 0.07^{c}	1.83 ± 0.00^{b}	1.61 ± 0.05^{c}
Zn/ mg·kg^{-1} DW	Root	36.57 ± 3.77^{a}	33.6 ± 1.44^{a}	28.62 ± 3.04^{b}	23.41 ± 1.04^{c}
	Shoot	80.99 ± 1.78^{a}	66.42 ± 0.75^{b}	68.11 ± 4.74^{b}	50.97 ± 0.05^{c}
	Leaf	40.11 ± 2.81^{b}	32.51 ± 1.64^{c}	53.58 ± 3.81^{a}	32.89 ± 0.38^{c}
	Fruit	21.36 ± 1.21^{a}	14.22 ± 1.09^{b}	17.43 ± 3.82^{b}	4.54 ± 0.37^{c}

Note: The data shown are the means ± SDs. Different lowercase letters in the same row of values (between treatments) indicate significant differences at the $p < 0.05$ level according to Duncan's multiple range test; ns, nonsignificant.

3.7. Effect of Cd stress on antioxidant substances in different tissues of 'Sharpblue' plants

In contrast to CK, Cd treatment increased CAT activity and inhibited SOD activity in the roots, shoots, leaves and fruits of 'Sharpblue' Notably, the SOD activities of 'Sharpblue' leaves were significantly greater under Cd stress than those of CK plants; the highest values of CAT in different tissues were 13.26 U/mg pro (roots, Cd2), 10.91 U/mg pro (shoots, Cd1), 11.23 U/mg pro (leaves, Cd3), and 32.56 U/mg pro (fruit, Cd3), whereas, under Cd stress, the SOD activities of different tissues had the lowest values under the Cd3 treatment, at 294.78 U/g, 326.97 U/g, 357.79 U/g, and 311.02 U/g, respectively (Fig. 7a and b).

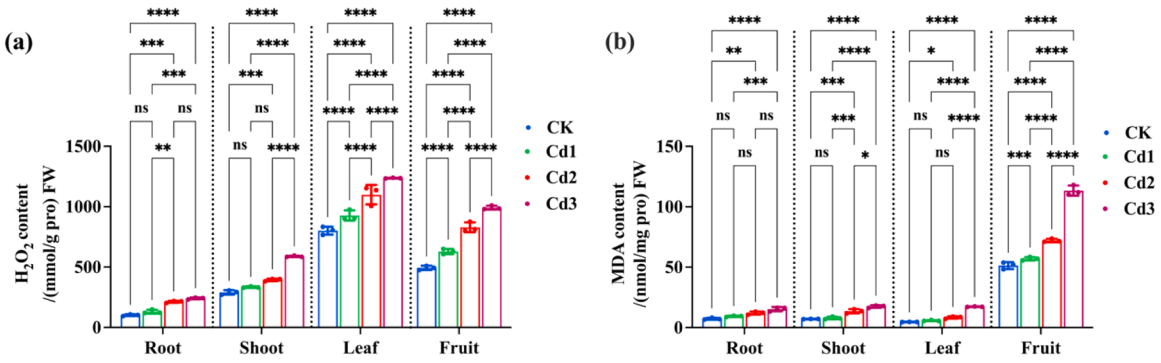

Fig. 5. Changes in hydrogen peroxide (H_2O_2) (a) and malondialdehyde (MDA) (b) levels in different tissues of 'Sharpblue' plants under Cd stress. The data shown in the bar graphs are the means ± SDs. Differences between treatments were analyzed by two-way ANOVA (Tukey's multiple comparison test). ns, no significant difference; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

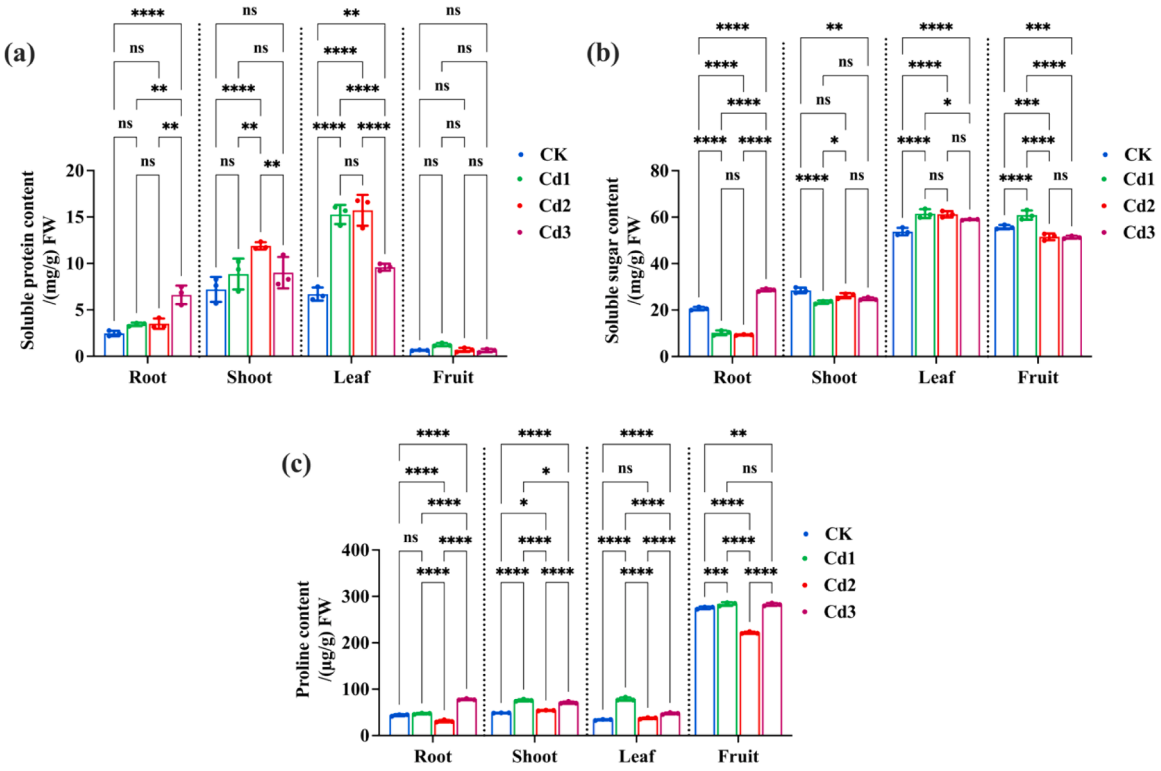

Fig. 6. Changes in soluble protein (SP) (a), soluble sugar (SS) (b) and proline (Pro) (c) contents in different tissues of 'Sharpblue' plants under Cd stress. The data shown in the bar graphs are the means ± SDs. Differences between treatments were analyzed by two-way ANOVA (Tukey's multiple comparison test). ns, no significant difference; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Moreover, the contents of AsA and GSH exhibited similar trends in 'Sharpblue' roots, shoots, leaves and fruits, and the contents of both AsA and GSH in fruits were significantly greater than those in other tissues. Overall, Cd stress had a greater effect on the changes in GSH content in the different tissues of 'Sharpblue' than AsA (Fig. 7c and d).

3.8. PCA and correlation analysis of Cd stress on various growth and physiological indices of 'Sharpblue' plants

To further investigate the relationships between 'Sharpblue' plants in the different Cd treatment and their growth indices, 40 indices were analyzed via PCA, and two principal components, PC1 (31.9 %) and PC2 (15.8 %), were extracted. Among them, the indices in the different Cd treatments were significantly different from those in the CK treatment; the most significant difference occurred between Cd3 and the other treatments, and a smaller difference was found between the Cd1 and Cd2 treatments. Overall, most of the growth, elemental and fruiting indices were positively correlated with CK treatments and negatively correlated with Cd treatments (Fig. 8a).

Moreover, we further analyzed the associations between the phenotypic data of 'Sharpblue' and physicochemical indices under Cd stress, and we found that the Mg, Fe, Mn and Cu contents were significantly positively correlated with fruit anthocyanins (ANC), flavonoids (FC) and total phenols (TP), while they were significantly negatively correlated with titratable acid (TA) according to Mantel's test analysis. In addition, Cu was significantly positively correlated with SPAD and N, which indicated that the fruit phenotype under Cd stress was strongly correlated with these physicochemical indices. Similarly, the plant phenotypic indices [plant height (PH), crown width (CW), basal stem (BS), root biomass (RS), stem biomass (SB), and leaf biomass (LS)] of

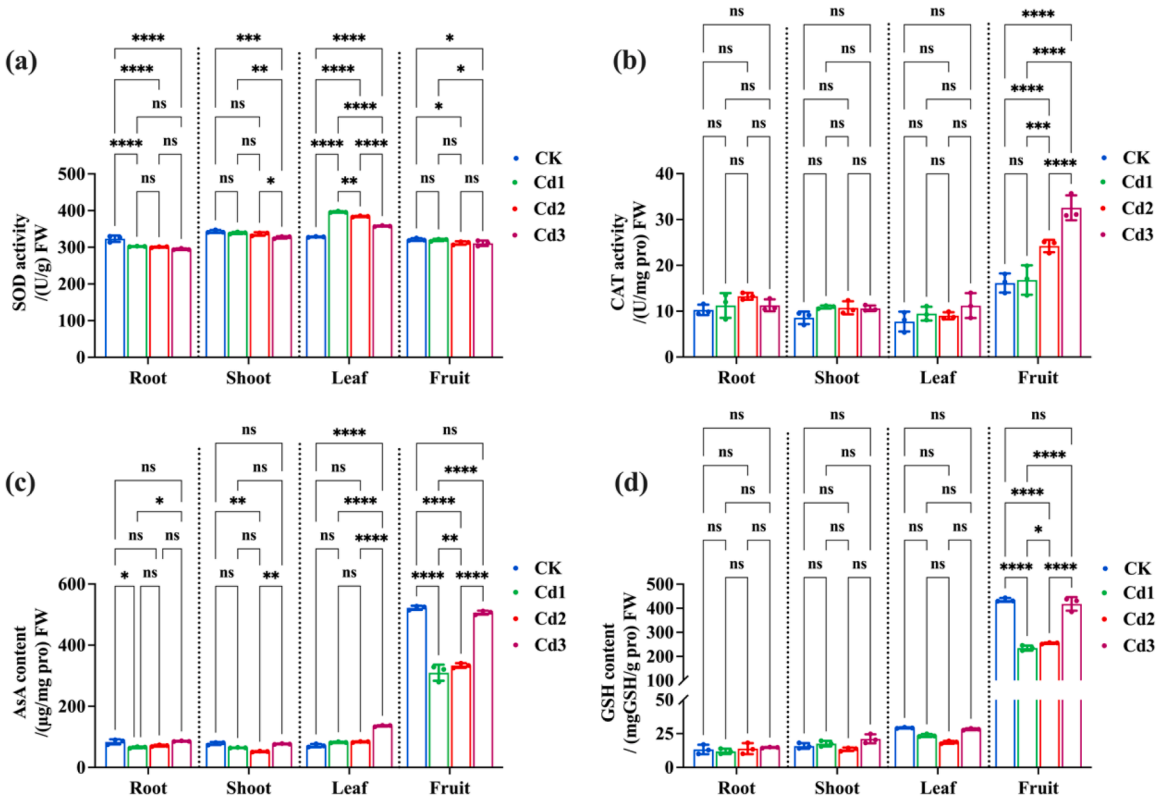

Fig. 7. Changes in superoxide dismutase (SOD) (a) and catalase (CAT) (b) activities and ascorbic acid (AsA) (c) and glutathione (GSH) (d) contents in different tissues of 'Sharpblue' plants under Cd stress. The data shown in the bar graphs are the means ± SDs. Differences between treatments were analyzed by two-way ANOVA (Tukey's multiple comparison test). ns, no significant difference; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

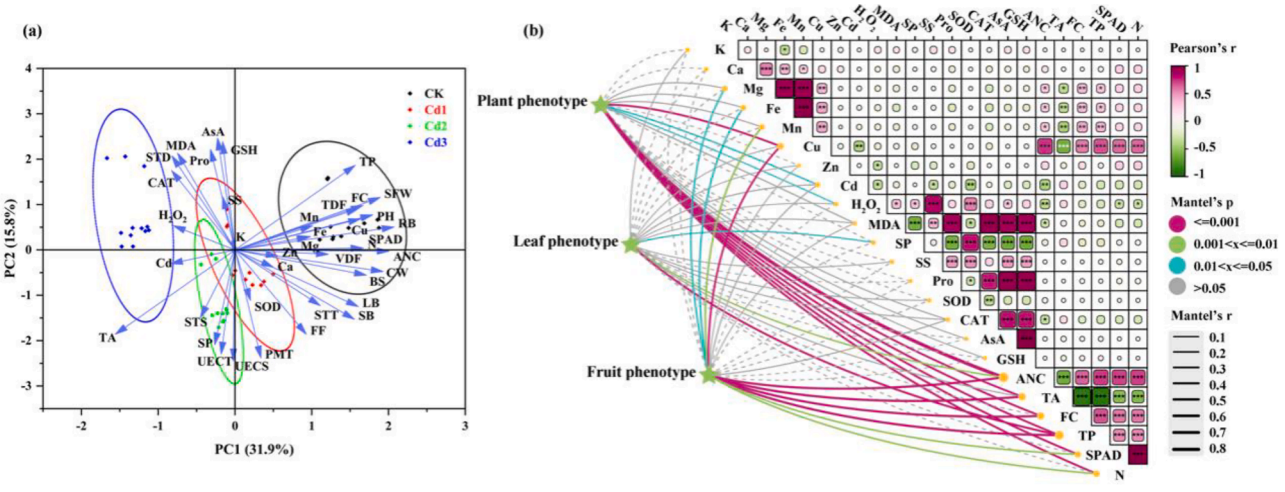

Fig. 8. PCA of each 'Sharpblue' indices under control conditions (CK) and under different Cd treatments (a); correlation analysis of phenotypic and physicochemical indices (b), showing pairwise comparisons of physicochemical indices under different treatments; the color gradient indicates Person's correlation coefficient, * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; Plant phenotypic indices (plant height, basal stem, crown width, root biomass, stem biomass, and leaf biomass); leaf phenotypic indices (upper epithelial cell size, upper epithelial cell thickness, palisade mesophyll thickness, spongy tissue thickness, stomatal density and size); and fruit phenotypic indices (single-fruit weight, firmness, transverse and longitudinal diameters) were tested for correlation with each physicochemical index by the Mantel test; the edge widths correspond to Mantel's r statistic for distance correlation; and the edge colors indicate statistical significance based on 999 permutations. PH, plant height; BS, basal stem; CW, crown width; RB, root biomass; SB, shoot biomass; LB, leaf biomass; UECS, upper epidermal cell size; UECT, upper epidermal cell thickness; PMT, palisade mesophyll thickness; STT, spongy tissue thickness; STS, stomatal size; STD, stomatal density; SFW, single-fruit weight; FF, fruit firmness; TDF, transverse diameter of fruit; VDF, vertical diameter of fruit; K, potassium; Ca, calcium; Mg, magnesium; Fe, iron; Mn, manganese; Cu, copper; Zn, zinc; Cd, cadmium; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; SP, soluble protein; SS, soluble sugar; Pro, proline; SOD, superoxide dismutase; CAT, catalase; AsA, ascorbic acid; GSH, glutathione; ANC, anthocyanin; TA, titratable acid; FC, flavonoids; TP, total phenols; SPAD, relative chlorophyll content; N, nitrogen.

'Sharpblue' under Cd stress were also strongly correlated with Cu, Cd, H_2O_2, SPAD, N, and fruit phenotypic indices [single-fruit weight (SFW), fruit firmness (FF), transverse diameter of fruit (TDF), and vertical diameter of fruit (VDF)]. Cd treatment was significantly negatively correlated with SS, SOD, and ANC, and H_2O_2 was significantly negatively correlated with ANC, SPAD, and N, while being significantly positively correlated with MDA, SP, SS, SOD, and AsA. In addition, leaf phenotypic indices [upper epidermal cell size (UECS), upper epidermal cell thickness (UECT), palisade mesophyll thickness (PMT), spongy tissue thickness (STT), and stomatal size (STS) and stomatal density (STD)] were strongly correlated with SP and TP, suggesting that the changes in 'Sharpblue' phenotype due to Cd stress were related to elemental uptake and fruit quality; in addition, SP was significantly negatively correlated with Pro, CAT, AsA, and GSH and significantly positively correlated with SOD, whereas SPAD was significantly negatively correlated with TA (Fig. 8b).

4. Discussion

Cd, a heavy metal that is nonessential and highly toxic in plants, is highly mobile in soil and other substrates and is readily taken up by plants and translocated to different tissues (Kubier et al., 2019). Excessive accumulation of Cd can damage the structure and function of plant cells, interfere with the absorption and transport of nutrients, impede chlorophyll synthesis and photosynthesis processes, cause cellular oxidative damage and disruption of physiological processes related to antioxidant systems, which can reduce plant biomass accumulation and disrupt the process of plant growth and development (Ghuge et al., 2023; Hu et al., 2022). In this study, Cd stress inhibited the growth of 'Sharpblue', and its plant height, basal stem, and crown width were significantly lower in response to 50 $mg{\cdot}kg^{-1}$ Cd stress, which was similar to the findings of Song et al. (2023) and Chen et al. (2020). Compared with that in CK, dry mass accumulation in 'Sharpblue' roots was significantly suppressed under Cd stress; however, the root-to-shoot ratio decreased significantly, indicating that 'Sharpblue' roots are more sensitive to Cd stress and that they can respond to Cd stress to a certain extent by regulating the distribution of aboveground and belowground biomass (Wu et al., 2020). PCA showed that 'Sharpblue' growth indicators (plant height, basal stem, crown width, root biomass, shoot biomass, and leaf biomass) were linearly and negatively correlated with Cd (Fig. 8a), which also indicated that high concentrations of Cd stress had a toxic effect on 'Sharpblue' plants. Moreover, as an important center for photosynthesis, respiration and transpiration, leaves are sensitive to environmental changes. Cd stress can damage mesophyll cells and affect stomatal closure (Sandalio et al., 2001). Tran et al. (2013) reported that 25 μM Cd significantly inhibited the thickness of epidermal cells, palisade tissue and spongy tissue of pea plants and increased stomatal density and guard cell width. In this study, compared with those in the CK treatment, the upper epidermal cell size and thickness in 'Sharpblue' leaves significantly increased under 15 $mg{\cdot}kg^{-1}$ Cd treatment and significantly decreased to their lowest values under 50 $mg{\cdot}kg^{-1}$ Cd treatment; the palisade mesophyll thickness and spongy tissue thickness showed the same trend, which may be attributed to the fact that the high concentration of Cd stress inhibited cell division and expansion during leaf primordia development (Wang et al., 2014). Notably, the stomatal size of 'Sharpblue' leaves under the Cd3 treatment was not significantly different from that under CK, but the stomatal density was significantly greater than that under the Cd3 treatment, which was closely related to the improvement in water use efficiency and the maintenance of the CO_2 concentration required for photosynthesis (Melo et al., 2007; Poulos et al., 2007). In addition, Cd stress reduced the SPAD and N contents of 'Sharpblue' leaves, and these leaves presented more obvious chlorosis under the Cd3 treatment, which was similar to the results of Liu et al. (2023a) and Shah et al. (2023). Inhibition of chlorophyll-related enzyme activities by Cd stress, disruption of photosynthetic pigment structures and competition for Mg^{2+}-active sites led to an increase in chlorophyll content and was also a potential cause of low chlorophyll content in the leaves of these plants (Baweja et al., 2020; Parmar et al., 2013).

Fruit quality directly determines the economic value of fruit crops, and abiotic stresses have been found to affect fruit yield and quality. Wang et al. (2022b) showed that Cd stress significantly inhibited the anthocyanin content of *Cucumis melo* L. fruit and that cherry tomato fruit size and weight were significantly reduced and TA content was significantly increased with 50 μM Cd treatment (Xie et al., 2021). Additionally, average strawberry fruit weight and soluble solids content severely decreased with Cd stress (Zhang et al., 2020). In this study, Cd stress significantly reduced the single-fruit weight and transverse diameter of fruit in 'Sharpblue' plants, but there was no significant effect on the vertical diameter of fruit; notably, compared to those in the CK treatment, the fruit firmness in the Cd3 treatment significantly decreased (Fig. 3a–e and Table 1), possibly because Cd stress affected the Mg, Zn, and Cu content of the fruits, contributing to cell membrane peroxidation and ethylene synthesis (Pennazio and Roggero, 1991). Moreover, Cd stress significantly increased the titratable acid content and decreased the soluble sugar content of 'Sharpblue' fruits, suggesting that Cd stress reduces the flavor and texture of these fruits (Figs. 3g and 6b). In addition, Cd stress significantly reduced the anthocyanin, flavonoids and total phenols contents of 'Sharpblue' fruits (Fig. 3f, h and i), which are important secondary metabolites of plant phenylpropanoid biosynthesis and flavonoid biosynthesis; are important bioactive compounds in blueberries; are closely related to fruit aroma, flavor, pigmentation, and nutritional qualities (Li et al., 2023); and are defense metabolites and antioxidants that play important roles in the plant response to abiotic stresses (Khandani et al., 2024b; Liu et al., 2023b; Naikoo et al., 2019).

Plant uptake and transport of Cd^{2+} and its enrichment in different organs vary from species to species. In the present study, Cd accumulation in different organs of 'Sharpblue' plants increased with increasing Cd stress, with the highest Cd content occurring in the roots, followed by the shoots, leaves, and fruits. Similarly, Song et al. (2023) and Guo et al. (2021) reported the same Cd enrichment characteristics in *Vaccinium ashei* R. and *Morus alba* L., respectively. Moreover, the amount of Cd in the fruit, an edible component, has a direct impact on human health. Our results showed that when the substrate Cd concentration exceeded 15 $mg{\cdot}kg^{-1}$, the Cd content in 'Sharpblue' fruits was approximately 0.10 $mg{\cdot}kg^{-1}$, which greatly exceeded the national standard value in fruit (less than 0.05 $mg{\cdot}kg^{-1}$) (Fig. 4a and b). Therefore, there is a serious safety threat to blueberries grown in this Cd condition. However, 'Bluegold' blueberry fruits showed greater tolerance to Cd stress, which may be due to cultivar differences (Chen et al., 2021).

Cd^{2+} interferes with the uptake, utilization, and storage of plant nutrients, resulting in plant nutrient shortages (Hou et al., 2023). A study showed that Cd stress significantly reduced the uptake of Fe, Mn, Cu, and Zn in 'Bluegold' blueberry plants (Chen et al., 2021). Our results also showed that the Cd3 treatment inhibited the uptake of Fe, Mn, Cu, and Zn in various organs of 'Sharpblue'. Relevant studies have confirmed that Cd^{2+} can bind to divalent cation channels for plant uptake and translocation and has antagonistic effects on Fe, Mn, Cu, and Zn (Luo et al., 2023); however, the reduction in Fe content, a key element for chlorophyll synthesis; and in Mn, Cu, and Zn content, important factors involved in the regulation of photosynthesis-related enzyme activities (Hediji et al., 2015), is an important reason for the decline in chlorophyll and biomass in 'Sharpblue' plants under Cd stress. In addition, K, Ca, and Mg are the major elements required for plant growth; among these elements, K plays an important role in plant response to environmental stress, stomatal regulation, osmoprotection, and activation of enzyme activities; the increase in K content in Cd-stressed 'Sharpblue' leaves may be a potential reason for stomatal enlargement (Mostofa et al., 2022). As a second messenger in plants, Ca plays a signaling role in plant resistance to adverse conditions. In the present study, the Ca content of 'Sharpblue' organs increased under low Cd stress and decreased under high Cd stress, which indicated that

within a certain range of Cd concentrations, plants could cope with Cd toxicity by increasing the Ca content of tissues (Luo et al., 2023). Tekdal and Cetiner (2018) reported that Cd treatment increased the Mg content of *Vuralia turcica* tissues (Hediji et al., 2015), and Cd stress also increased the Mg content in tomato fruits. 'Sharpblue' plants exhibited similar changes in Mg content in various organs under Cd stress. The reduction in the Ca, K, and Cu content of 'Sharpblue' fruits under Cd stress were closely related to decreases in fruit weight and quality (Hediji et al., 2015).

Cd stress increases plant ROS levels and the effects of oxidative stress, including ion leakage, increased H_2O_2 production, and lipid peroxidation, which disrupt cellular structures and functions (Wang et al., 2022a). In this study, with increasing substrate Cd concentration, the H_2O_2 and MDA contents of all 'Sharpblue' tissues increased significantly, and overall, the leaves and fruits were more damaged by Cd stress (Fig. 5). Interestingly, although the H_2O_2 content was greater in 'Sharpblue' leaves, the MDA content was relatively low, which might be the result of the combined action of plant osmoregulatory substances and antioxidant defense system (Khandani et al., 2024a; Sikder et al., 2020). As important osmoregulators in plant cells, SP, SS and Pro play important roles in plant response to abiotic stresses and maintenance of the homeostatic balance between cytoplasmic and extracellular environments (Hayat et al., 2012; Szabados and Savouré, 2010). Moreover, Pro enrichment is an effective strategy for alleviating heavy metal stress (Chang et al., 2023). In this study, the content of osmoregulatory substances in all tissues of 'Sharpblue' plants increased to different degrees compared with that in CK plants. These findings indicate that osmoregulatory substances play different roles in different tissues of 'Sharpblue' plants in response to Cd stress. The results of correlation analysis also showed that H_2O_2 was significantly and positively correlated with SP and SS and that MDA was significantly and positively correlated with SS and Pro (Fig. 8b). SOD, CAT, AsA and GSH, which are functional substances in the antioxidant defense system, are essential for maintaining ROS homeostasis in plants in response to abiotic stresses (Chang et al., 2023). SOD acts on superoxide radicals and converts them to H_2O_2, which is subsequently converted to H_2O and O_2 by CAT (Li et al., 2008). Studies have shown that increased SOD and CAT enzyme activities promote blueberry plant adaptation to adverse conditions (Yang et al., 2022). In the present study, Cd stress increased CAT activity and decreased SOD activity in different tissues of 'Sharpblue' plants, but SOD activities in the leaves were significantly greater than those in the CK treatment (Fig. 7a and b). The disruption of enzyme activity centers or the inhibition of enzyme expression by Cd stress was the main reason for the decrease in enzyme activity (Dionisio-Sese and Tobita, 1998). AsA and GSH, important nonenzymatic antioxidants in the Asada-Halliwell pathway, maintain the stability of protein structures and the integrity of biofilm systems by reducing ROS accumulation and scavenging H_2O_2 in plants (Kramarenko et al., 2006). In the present study, the AsA and GSH contents in different tissues of 'Sharpblue' plants under Cd stress tended to decrease but then increase with Cd concentration (Fig. 7c and d), possibly because the activities of related enzymes in the AsA-GSH cycle of 'Sharpblue' in response to Cd stress were affected by Cd dose and the stress duration. In addition, when Cd > 15 $mg \cdot kg^{-1}$, the relevant enzymes of the AsA-GSH cycle were activated, and their levels increased, preventing damage from Cd stress. The results of correlation analysis showed that CAT was significantly and positively correlated with AsA and GSH (Fig. 8b).

5. Conclusion

In summary, we found that Cd stress inhibited the growth of 'Sharpblue' and reduced fruit quality by accelerating tissue ROS accumulation and lipid peroxidation, suppressing chlorophyll synthesis, impairing photosynthesis and antioxidant systems, and disrupting nutrient absorption and transport (Fig. 9). In addition, with increasing substrate Cd concentration, the Cd content in each organ of 'Sharpblue' plants also increased, with roots having the strongest Cd enrichment capacity, followed by shoots, leaves, and fruits. Overall, this study showed that the 50 $mg \cdot kg^{-1}$ Cd treatment was most toxic to 'Sharpblue' plants and fruits; most importantly, when the substrate Cd concentration was >15 $mg \cdot kg^{-1}$, the Cd content of 'Sharpblue' fruits (0.10 $mg \cdot kg^{-1}$) exceeded the food safety threshold of 0.05 $mg \cdot kg^{-1}$, posing a serious safety hazard. In conclusion, 'Sharpblue' blueberry plants are tolerant to Cd stress to a certain extent, but due to the genetic background differences among cultivars, comparisons of Cd stress tolerance among different blueberry cultivars need to be further investigated. Additionally, the molecular mechanisms of plant response, the metabolic mechanisms of fruit quality decline, and effective strategies to mitigate Cd toxicity in blueberries are also important research directions for the future.

CRediT authorship contribution statement

Hao Yang: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Yaqiong Wu:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Jilu Che:** Methodology, Investigation. **Lianfei Lyu:** Formal analysis. **Wenlong Wu:** Supervision, Resources. **Fuliang Cao:** Supervision. **Weilin Li:** Writing – review & editing, Supervision, Funding acquisition.

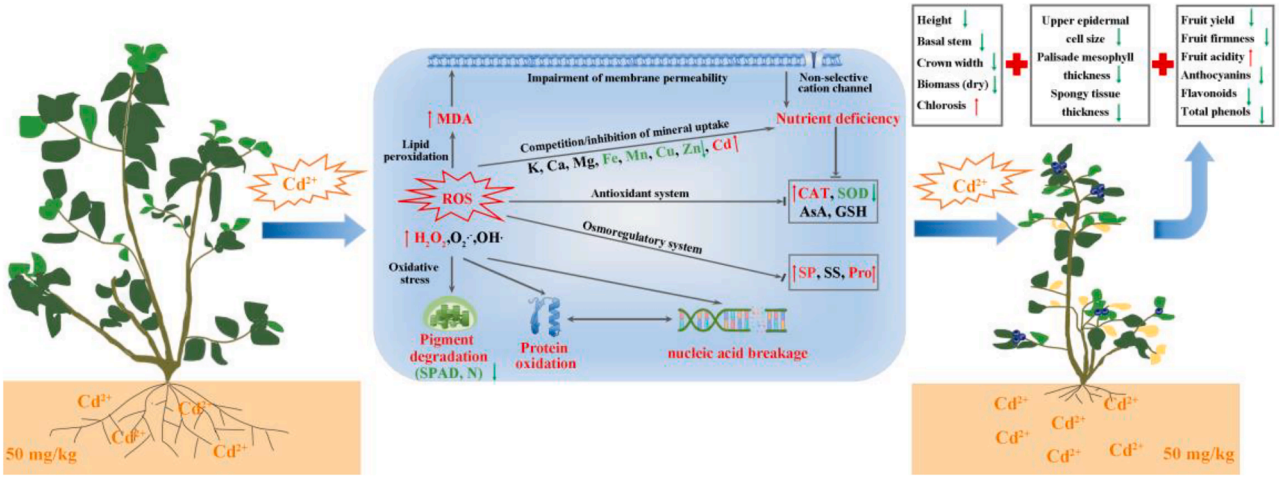

Fig. 9. Potential physiological mechanisms through which cadmium stress affects the growth and quality of 'Sharpblue' blueberry fruits. (→) indicates induced/promoted, (↛) indicates inhibited, red arrows indicate increased, and green arrows indicate decreased.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113593.

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