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Morphoanatomy and Phenoplasticity of *Cardamine hupingshanensis* (Brassicaceae) Under Cadmium Stress

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Using a soil culture method, this study investigated the morphoanatomical responses and phenotypic plasticity of *Cardamine hupingshanensis* (Brassicaceae) under different cadmium (Cd) concentrations. Root morphological indices initially increased at low Cd levels but decreased as Cd concentrations rose, exhibiting a low-promoting and high-inhibiting effect. Leaf morphology and biomass peaked at 5 or 10 mg kg^{-1} Cd before decreasing at higher concentrations but were still significantly higher than the control ($P < 0.05$). Anatomical structure analysis revealed no significant difference at 5 mg kg^{-1} Cd; however, at ≥ 100 mg kg^{-1} Cd, epidermal and spongy tissue thickness differed significantly from the control. Correlation analysis indicated a significant positive correlation between Cd concentration and epidermal and spongy tissue thickness but a negative correlation with palisade tissue thickness. The mean phenotypic plasticity index of *C. hupingshanensis* under Cd stress was 0.73, with root morphology exhibiting greater plasticity. These findings show the adaptability of *C. hupingshanensis* in cadmium-contaminated environments and its resilience under heavy metal stress.

Keywords: anatomical structure, cadmium stress, *Cardamine hupingshanensis*, morphological index, phenotypic plasticity

Introduction

Remediating soil contaminated with heavy metals has become an increasingly significant environmental concern worldwide (Hu et al. 2014; Pons-Branchu et al. 2015). In a study on differential expression and cloning of heavy metal accumulation traits and cadmium resistance genes in Brassica, cadmium was noted as one of the toxic elements that widely exist in various soils and wastes (Wang 2004). Cadmium has since become a major focus of environmental research because of its high pollution toxicity and fast transformation speed (Wu 2005; Yang 2009). Consequently, developing effective methods to remove heavy metals from soils without causing pollution remains to be a challenge. Physicochemical methods and phytoremediation technology are typically used in the treatment of soil heavy metal pollution, of which phytoremediation technology is the most ideal (Zhou and Deng 2003; Bing 2015).

Cardamine hupingshanensis is a unique plant in China belonging to the family Brassicaceae which has been proven to be a cadmium and a selenium hyperaccumulator (Bai and Li 2012; Shao-Kai et al. 2018). The morphological structure of plants such as *C. hupingshanensis* is affected by changes in different habitats and ecological factors (Liao et al. 2012; Li 2014). They intuitively respond to environmental pollutants through the appearance of visible injury symptoms brought about by changes in plant tissue and cell structure and function. There have been several reports on changes in plants' anatomical structures in heterogeneous environments. For instance, Meng and Wei (2018) investigated the effect of spraying uniconazole to regulate the anatomical structure of soybean root and alleviate salt stress damage. A similar study by Li and Bao (2005) examined the response and adaptation of leaf morphological and anatomical structures to environmental changes. In cold environments, the protrusion degree of leaf veins and stomatal density of plants increased as reported by Cui and Ma (2016). Sun et al. (2016) also found that plant stems in long-term drought conditions undergo changes to adapt to the environment. The effect of copper on the

morphological and anatomical characteristics and cell ultrastructure of Malayan cabbage was also previously analyzed (Gao et al. 2021). Moreover, in an evaluation of castor resistance to lead-zinc stress, a thickened outer bark layer of castor root and cork accumulation were observed (Yi 2017). However, changes in the morphological and anatomical characteristics of *C. hupingshanensis* under cadmium stress need further investigation. Hence, this study analyzed the morphological and anatomical characteristics of the roots and leaves of *C. hupingshanensis* and compared the plasticity of the plant's anatomical structure under different levels of cadmium stress to provide references for cultivating plants resistant to heavy metal pollution.

Materials and Methods

Experimental design

Preparation of soil containing cadmium

The pot experiment was conducted in the laboratory of Suzhou University, Jiangsu, China from January 2022 to May 2023. Commercially available organic nutrient soil was used as the substrate, with different concentrations of cadmium chloride ($CdCl_2$) (0, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 mg L-1) added to the screened soil. To ensure even distribution of $CdCl_2$, the soil was stirred daily, and distilled water was added. The soil was then covered with plastic film to keep moisture content at 60% of field capacity. After 1 mo, the soil was transferred into a seedling basin measuring 10 × 10 × 10 cm. Each treatment was then applied to 10 basins containing 80 g of soil (Li et al. 2019).

Seedling culture

Seeds of *C. hupingshanensis* were surface-sterilized by dipping in 0.1% mercuric chloride solution for 5 min and 75% ethanol solution for 30 s, followed by washing with sterilized water 5 times. The sterilized seeds were evenly placed on 3 sheets of sterilized filter paper moistened with 4 mL sterilized water in Petri dishes (90 mm in diameter) and germinated in an incubator at a temperature of 22.5 °C. Subsequently, the seedlings with consistent growth traits were transferred to the pots containing nutrient soils with different Cd concentrations. Seedlings were cultured in a phytotron with a 12-h light photoperiod, a stable temperature (22.5/17.5 °C, day/night), and 60% relative humidity. After a cultivation period of 2 mo, all plants were harvested, and their morphological and anatomical structure were analyzed (Han et al. 2022).

Cadmium enrichment in seedlings

Fresh 0.05-g shoot or root samples were transferred into a Teflon tube, to which 1 mL $HClO_4$ and 9 mL concentrated HNO_3 were added. The soaking solution was placed in a thermostatic drying oven and held at 80 °C for 1 to 2 h, 120 °C for 1 to 2 h, and then raised to 160 °C for 4 h. The oven was allowed to cool naturally to room temperature, opened, and heated to drive the acid to near-dryness. The digestion solution was transferred into a 50-mL volumetric flask while the reagent blank test was conducted. The elemental content of cadmium was determined using an atomic absorption spectrophotometer (Feng et al. 2020).

Test indices

Determination of morphological indices

The plants were divided into above-ground and below-ground sections. The root was rinsed with deionized water and soaked in 10 mmol L^{-1} Na_2-EDTA for 20 min and dried with absorbent paper. Total root length (TRL), root surface area (SA), root projection unit area (PA), root volume (Vol), root mean diameter (AvgD), root tips (RT), and branch number (BT) of the *C. hupingshanensis* root system were measured using the WinRHIZO image analysis software (version 2013e, Regent Instruments, Canada) (Yi 2018). The plant samples were dried at 75 °C to a constant weight, and the weights of the above-ground and below-ground parts were recorded.

Determination of anatomical structure

Five to ten pieces of A4 paper were stacked and placed on a flat test bench. Fresh root, stem, or leaf samples were evenly spread on the paper. Two new double-sided razor blades were aligned in parallel and pinched together. While cutting, the edges were carefully aligned and pressed down simultaneously with both hands to cut the tissue. After cutting off 1 piece at a time, each cut section was gently removed with a brush and transferred to a Petri dish containing transparent liquid. The dish was covered and placed in a 70 °C water bath for 1h. The materials were then rinsed thrice with distilled water. The cleaned section was selected and transferred to a glass slide with water. Excess water was absorbed using absorbent paper, and a drop of staining solution was applied for 15 s. After the stain was absorbed and washed, the sample was observed and photographed under a DN-107T microscope (Zhu 2021). Leaf palisade and spongy tissue thickness as well as upper and lower epidermis thickness were also measured.

Plasticity Number

Phenotypic plasticity refers to the ability of species with the same genotype to change phenotypic traits in response to heterogeneous habitats (Xiong and Zhao 2020). The plasticity index of *C. hupingshanensis* was computed using the formula below (Wei et al. 2021):

$$Plasticity\ index\ (PI) = (Max\ (MI) - Min\ (MI) / Max\ (MI)$$

where Max (MI) is the maximum value of the plant morphological index and Min (MI) is the minimum value of the plant morphological index.

Absorption and transport factors

The absorption and transport factors were computed using the formula below (Yan et al. 2018):

$$\text{Absorption factor (AF)} = CA / CS$$

$$\text{Transport factor (TF)} = CA / CU$$

where AF is the absorption factor; TF is the transport factor; CA is the heavy metal concentration in the above-ground part of the plant; CU is the heavy metal concentration in the below-ground part of the plant; and CS is the heavy metal concentration in the soil.

Data analysis

Microsoft Excel 2013 was used for data statistics and chart generation. Tukey's test ($P < 0.05$) was applied for significant difference analysis (Han 2023). The *P*-trend value was calculated using a mixed ANOVA model, with $P < 0.05$ considered statistically significant. Pearson correlation analysis was performed, and SPSS 22.0 was used for cluster analysis.

Results

Effects of cadmium (Cd) stress on the morphology of *C. hupingshanensis*

Under Cd stress, root morphology indices of *C. hupingshanensis* initially increased before decreasing (Table 1, Fig. 1). A significant promoting effect was observed when Cd concentration was ≤ 25 mg kg^{-1}, particularly at 10 mg kg^{-1}, where root morphological indices peaked and were significantly different compared to the control ($P < 0.05$). The total root length, root area, root volume, and average root diameter were 1.79, 3.47, 6.90, and 1.95 times, respectively. However, when Cd concentration was ≥ 50 mg kg^{-1}, all root indices showed slight inhibition. When Cd concentration reached 200 mg kg^{-1}, total root length, number of root tips, and branch number differed significantly from the control. Root surface area, root projection unit area, root volume, and average root diameter showed no significant changes between the groups with a cadmium concentration of 200 mg kg kg^{-1} and the control group ($P < 0.05$).

Leaf morphological indices and biomass increased with lower Cd concentrations but decreased as Cd concentrations rose (Table 2, Fig. 1). Growth was notably promoted at ≤ 25 mg kg^{-1} Cd and especially at 2.5 to 10.0 mg kg^{-1} Cd compared to the control; however, plant height was inhibited at ≥ 50 mg kg^{-1} Cd. Leaf number, biomass dry weight, and fresh weight showed a significant promoting effect at low Cd concentrations (5 to 25 mg kg^{-1}) compared to the control ($P < 0.05$). Specifically, at 10 mg kg^{-1} Cd, leaf number, fresh weight, and dry weight were 1.18, 6.25, and 6.72 times higher than the control, respectively. When Cd concentration reached ≥ 100 mg kg^{-1}, leaf number, dry weight, and fresh weight showed slight inhibition compared to the control, albeit not statistically significant.

Effects of cadmium (Cd) stress on the anatomical structure of *C. hupingshanensis*

Root morphological analysis revealed that Cd stress had a low-promoting and high-inhibiting effect on the root length of *C. hupingshanensis*. The root tip tissues in the control and under low Cd stress were arranged in a regular, orderly manner and with a close meristem arrangement (Fig. 2). Root tip structure was more dense and complete at 5 mg kg^{-1} Cd. However, at 100 mg kg^{-1} Cd, the root tip turned black and the meristem became irregularly arranged.

Table 1 Root morphological indices of *Cardamine hupingshanensis* under different cadmium concentrations

Sample	Total root length (TRL, cm)	Root surface area (SA, cm^2)	Root projection unit area (PA, cm^2)	Root volume (Vol, cm^3)	Root mean diameter (AvgD, mm)	Root tips (RT)	Branch number (BT)
CK	90.39 ± 4.74 d	15.59 ± 0.10 de	4.96 ± 0.03 de	0.21 ± 0.01 cd	0.55 ± 0.02 d	192 ± 18.38 d	379.67 ± 7.93 cd
1.0	94.54 ± 8.90 cd	15.85 ± 1.68 de	5.04 ± 0.54 de	0.21 ± 0.03 cd	0.53 ± 0.03 d	243.17 ± 23.97 cd	355.50 ± 52.78 cd
2.5	123.20 ± 4.35 bc	26.81 ± 0.10 cd	8.53 ± 0.03 cd	0.47 ± 0.02 bcd	0.69 ± 0.03 bcd	276.00 ± 1.41 bc	485.00 ± 18.80 bc
5.0	133.32 ± 6.18 ab	36.46 ± 0.79 bc	11.61 ± 0.25 bc	0.79 ± 0.02 abc	0.87 ± 0.08 abc	330.67 ± 17.44 b	621.00 ± 40.92 b
10.0	161.62 ± 18.26 a	54.12 ± 2.29 a	17.19 ± 0.71 a	1.45 ± 0.07 a	1.07 ± 0.08 a	421.67 ± 24.57 a	1,015.67 ± 138.72 a
25.0	152.38 ± 15.36 ab	46.87 ± 14.44 ab	14.92 ± 4.60 ab	1.20 ± 0.62 ab	0.96 ± 0.20 ab	396.33 ± 13.42 a	849.33 ± 59.11 a
50.0	80.59 ± 11.12 d	12.52 ± 1.19 de	2.65 ± 0.25 e	0.16 ± 0.03 cd	0.60 ± 0.08 cd	132.33 ± 17.44 e	248.83 ± 40.92 de
100.0	34.38 ± 5.08 e	5.15 ± 0.69 e	1.09 ± 0.15 e	0.07 ± 0.02 cd	0.47 ± 0.19 d	59.00 ± 9.11 f	136.83 ± 15.18 e
200.0	23.72 ± 0.20 e	3.45 ± 0.03 e	0.73 ± 0.01 e	0.05 ± 0.01 d	0.56 ± 0.00 d	74.33 ± 3.40 ef	97.00 ± 1.63 e
Trend	Cubic	Linear	Linear	Linear	Linear	Quartic	Linear
Sig.	0	0	0	0	0	0	0

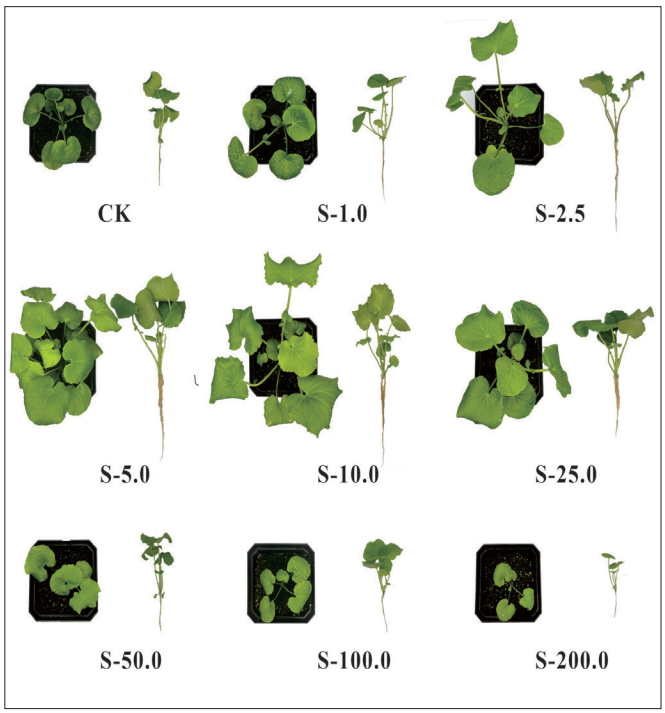

Fig. 1 Growth of *Cardamine hupingshanensis* under different cadmium concentrations (CK = control; S = sample)

The cross-section of the stem included the epidermis, cortex, vascular bundle, and pith. Different Cd concentrations had varying effects on different tissues; for example, the epidermal cells of the control stem showed a close, orderly arrangement and were uniform in size (Fig. 3).

At a Cd concentration of 5 mg kg^{-1}, the epidermis and cortex cells of the stem became smaller with a more compact and orderly arrangement similar to the control. However, at 100 mg kg^{-1} Cd, the epidermis and cortex cells displayed a loose, irregular arrangement. In the control group, the xylem conduits were neatly arranged, with uniform apertures and large cell size. At 5mg kg^{-1} Cd, the xylem cells were denser, with a smaller pore diameter but an orderly arrangement. However, at 100 mg kg^{-1} Cd, the xylem cells were larger, with a significantly larger conduit diameter than the control and at 5mg kg^{-1} Cd. The arrangement became loose and irregular, the cell boundary was not clear, and there were signs of degeneration.

Under Cd stress, structural changes occurred in the upper and lower epidermis, as well as in the palisade and spongy tissues of the leaves (Fig. 4). In the control group, the upper and lower epidermal cells were closely and neatly arranged in a single layer, with the upper epidermal cells being larger

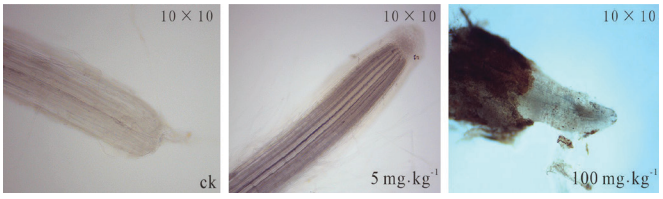

Fig. 2 Differences in the root tip structure of *Cardamine hupingshanensis* under varying cadmium concentrations (at 10 x 10 magnification; CK = control)
Note: 10 x 10 represents magnification times

Fig. 3 Differences in the stem anatomical structure of *Cardamine hupingshanensis* under varying cadmium concentrations (at 10 x 10 magnification; CK = control)
Note: 10 x 10 represents magnification times

Table 2 Leaf morphological indices of *Cardamine hupingshanensis* under different cadmium concentrations

Sample	Plant height (PH, cm)	Shoot height (SH, cm)	Number of leaves (NL)	Plant weight (PFW, g)	Plant weight (PDW, g)	Shoot weight (SFW, g)	Shoot weight (SDW, g)
CK	18.87 ± 0.65 bcd	9.83 ± 1.03 bc	5.67 ± 0.47 b	1.39 ± 0.02 b	0.093 ± 0.004 c	1.25 ± 0.06 bc	0.078 ± 0.001 d
1.0	18.27 ± 1.79 bcd	8.67 ± 1.70 bc	6.67 ± 1.70 ab	1.26 ± 0.21 b	0.077 ± 0.015 c	1.19 ± 0.19bc	0.071 ± 0.013 d
2.5	24.60 ± 3.20 ab	11.93 ± 0.70 abc	6.00 ± 0.82 b	2.78 ± 0.08 b	0.183 ± 0.014 c	2.57 ± 0.03 b	0.161 ± 0.012 cd
5.0	31.60 ± 0.54 a	17.03 ± 1.10 a	9.33 ± 0.47 a	6.38 ± 1.08 a	0.434 ± 0.113 b	5.54 ± 0.63 a	0.345 ± 0.061 ab
10.0	24.30 ± 5.48 ab	14.53 ± 4.67 ab	6.67 ± 0.94 ab	8.70 ± 1.42 a	0.625 ± 0.066 a	7.34 ± 0.64 a	0.487 ± 0.108 a
25.0	23.23 ± 1.99 abc	11.13 ± 1.01 abc	7.00 ± 0.82 ab	6.58 ± 1.07 a	0.383 ± 0.035 b	5.80 ± 1.21 a	0.305 ± 0.049 bc
50.0	18.27 ± 1.32 bcd	8.93 ± 0.49 bc	5.67 ± 0.47b	1.80 ± 0.40 b	0.103 ± 0.022 c	1.66 ± 0.42 bc	0.088 ± 0.024 d
100.0	14.50 ± 2.12 cd	6.83 ± 1.18 c	5.67 ± 0.47 b	1.03 ± 0.22 b	0.062 ± 0.013 c	1.01 ± 0.22 bc	0.059 ± 0.012 d
200.0	12.43 ± 1.74d	6.10 ± 1.51 c	6.00 ± 0.82 b	0.53 ± 0.20 b	0.032 ± 0.013 c	0.49 ± 0.19 c	0.028 ± 0.012 d
Trend	Linear	Linear	/	Linear	Linear	Linear	Linear
Sig.	0	0	0.09	0	0	0	0

than the lower epidermal cells. At 5 mg kg^{-1} Cd, the upper and lower epidermal cells remained neatly arranged in a single layer but were less compact than in the control, and did not change significantly. However, at 100 mg kg^{-1} Cd, the upper and lower epidermal cells became mostly multilayered, smaller, and loosely arranged, with a thickened upper epidermis. The palisade and spongy tissues did not differ significantly from the control at 5 mg kg^{-1} Cd; the palisade mesophyll consisted of a layer of long and closely arranged columnar cells, while the spongy mesophyll cells were oblong or round, loosely arranged, and irregular. At 100 mg kg^{-1} Cd, the palisade tissue became sparse and irregular with localized signs of degeneration, while the spongy mesophyll showed a loose and uneven arrangement than the palisade tissue.

Lower epidermis cell thickness did not significantly change with increasing Cd concentrations, while upper epidermis cell thickness became significantly higher than the control when Cd concentration reached 100 mg kg^{-1} and was 1.9 times higher than the control at 200 mg kg^{-1} Cd (Table 3). Under Cd stress, palisade and spongy tissue thickness did not differ significantly from the control; however, at ≥ 100 mg kg^{-1}, epidermis and spongy tissue thickness were significantly higher than the control ($P < 0.05$). Correlation analysis also showed that cadmium concentration in both above-ground and below-ground parts of *C. hupingshanensis* showed a significant positive correlation with upper epidermal cell and spongy mesophyll thickness, while it showed a significant negative correlation with palisade mesophyll thickness, indicating that Cd stress produced changes in the leaf morphological structure of *C. hupingshanensis* (Table 4).

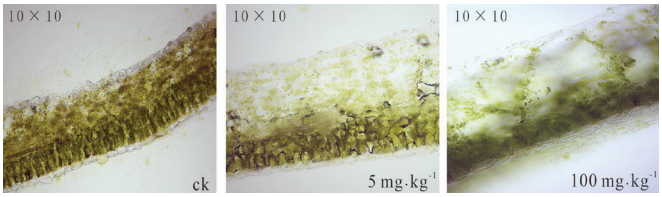

Fig. 4 Differences in the leaf cross-section of *Cardamine hupingshanensis* under varying cadmium concentrations (at 10 x 10 magnification; CK = control)
Note: 10 x 10 represents magnification times

Table 3. Effects of varying cadmium concentrations on the anatomical structure of *Cardamine hupingshanensis* leaves

Cadmium concentration	Epidermis thickness (ET, μm)	Underskin thickness (UT, μm)	Palisade tissue thickness (PTT, μm)	Spongy tissue thickness (STT, μm)
CK	31.03 ± 2.78 c	31.28 ± 3.71 ab	140.92 ± 5.55 ab	236.38 ± 2.98 cd
1.0	34.09 ± 4.12 c	27.96 ± 1.23 ab	132.86 ± 4.36 ab	256.93 ± 10.84 bc
2.5	28.86 ± 5.28 c	20.82 ± 5.82 b	141.10 ± 4.75 ab	234.07 ± 14.22 cd
5.0	29.28 ± 1.94 c	20.93 ± 0.63 b	158.45 ± 15.95 a	235.24 ± 9.75 cd
10.0	28.47 ± 3.63 c	22.55 ± 2.05 b	158.32 ± 16.83 a	207.80 ± 8.23 d
25.0	28.81 ± 3.10 c	20.33 ± 3.81 b	162.72 ± 8.94 a	203.75 ± 7.04 d
50.0	30.56 ± 0.82 c	21.35 ± 1.76 b	128.43 ± 7.50 ab	235.48 ± 14.26 cd
100.0	46.79 ± 3.52 b	25.62 ± 2.80 ab	116.66 ± 4.86 b	338.19 ± 13.25 a
200.0	58.97 ± 2.50 a	33.92 ± 3.27 a	109.89 ± 11.88 b	295.18 ± 17.93 b
Trend	Linear	Cubic	Linear	Cubic
Sig.	0	0.03	0	0

Phenotypic plasticity analysis of *C. hupingshanensis* under cadmium stress

The phenotypic plasticity indices of *C. hupingshanensis* morphological indicators were all above the average values (Fig. 5). Notably, the plasticity indices of root morphology, except for average diameter, were consistently higher, suggesting a strong adaptability of *C. hupingshanensis* roots to Cd stress. In contrast, the plasticity of plant height, above-ground part height, and leaf anatomical structure were lower than average, indicating a weaker adaptability of the leaves to Cd stress compared to the roots.

Table 4 Correlation between changes leaf anatomical structure changes and cadmium absorption and transport

	CAG	CUG	TF	AF	ET	UT	PTT	STT
CAG	1							
CUG	0.998**	1						
TF	-0.238	-0.273	1					
AF	-0.831**	-0.833**	0.386	1				
ET	0.933**	0.933**	-0.286	-0.631	1			
UT	0.481	0.473	-0.289	-0.187	0.709*	1		
PTT	-0.728*	-0.736*	0.426	0.589	-0.824**	-0.642	1	
STT	0.703*	0.722*	-0.23	-0.494	0.701*	0.113	-0.66	1

Note: Cadmium concentration above ground (CAG), cadmium concentration underground (CUG), absorption factor (AF), transport factor (TF), epidermis thickness (ET), underskin thickness (UT), palisade tissue thickness (PTT), spongy tissue thickness (STT), **means significant at the level of 0.01, and * means significant at the level of 0.05.

The different growth-promoting concentrations of *C. hupingshanensis* were clustered according to 22 phenotypic traits and varying Cd concentrations to obtain a dendrogram (Fig. 6). The first group included Cd concentrations of 5, 10, and 25 mg kg^{-1} and was classified as the promotion group, which exhibited longer rootstocks and leaves, higher plant weight, more leaves, better anatomical structure, and significantly better plant phenotypic traits than the control group. The second group included Cd concentrations of 100 and 200 mg kg^{-1} and was classified as the suppression group. The third group included the control and Cd concentrations of 1.0, 2.5, and 50 mg kg^{-1}. Based on the results of Tukey's test, the phenotypic characteristics of the plants were basically not significantly different from those of the control.

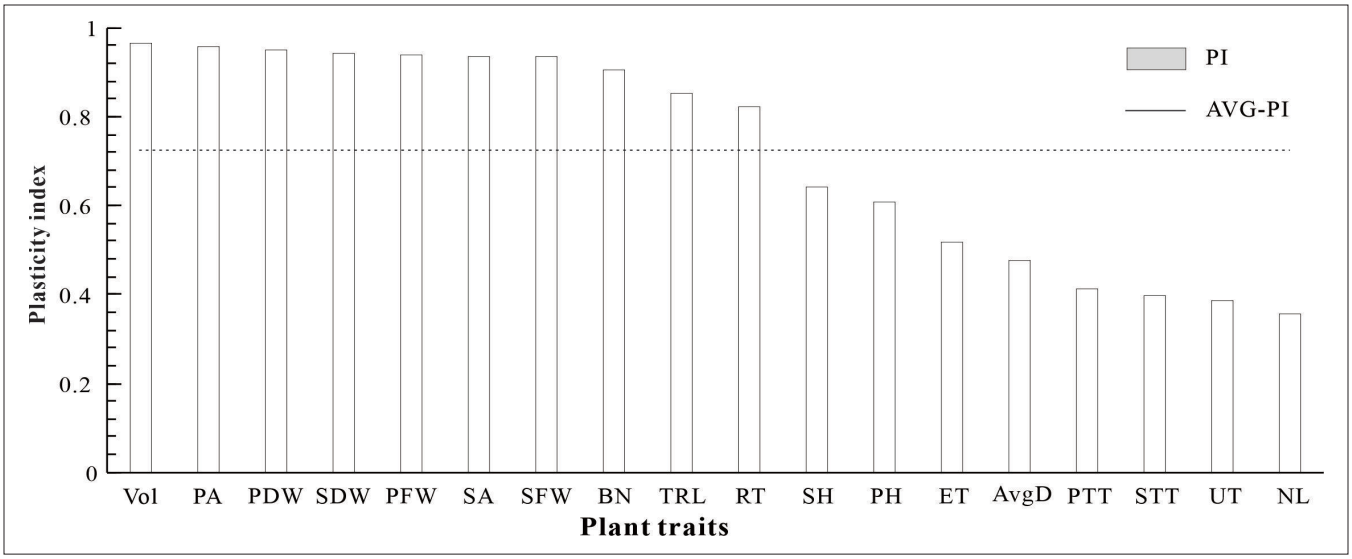

Fig. 5Phenotypic plasticity of Cardamine hupingshanensis under cadmium stress (PI – plasticity index, AVG-PI – average plasticity index, Vol – root volume, PA – root projection unit area, PDW - plant dry weight, SDW - shoot dry weight, PFW - plant fresh weight, SA – root surface area, SFW - shoot fresh weight, BN – branch number, TRL – total root length, RT – root tips, SH - shoot height, PH - plant height, ET - epidermis thickness, AvgD – root mean diameter, PTT - palisade tissue thickness, STT - spongy tissue thickness, UT - underskin thickness, NL - number of leaves)

Discussion

Adverse conditions such as heavy metal contamination and drought limit water and nutrient availability in plants. However, due to their plasticity, roots can adapt by changing their length, volume, and surface area to absorb more water and nutrients from the soil. These changes are the result of its continuous environmental accumulation (Zhan 2016) and reflect a plant's ability to absorb and utilize nutrients. Root length is a key indicator of a plant's ability to absorb water and nutrients from the soil for growth (Ru et al. 2022), and represents the vertical contact area between roots and soil to some extent (Huang et al. 2009). However, the most extensive index of root-soil contact is root surface area — the larger the root surface area, the larger the contact area with soil, and the wider the distribution range of root hair. Root volume is another significant factor that reflects root distribution and the quality of root development (Zhou et al. 2008). Additionally, the number of root tips indicates root system development — more root tips lead to more branching and, consequently, a more developed root system (Wang et al. 2013). This study found that Cd stress influenced root growth and exhibited a low-promoting and high-inhibiting effect in *C. hupingshanensis* — ≤ 25mg kg^{-1} Cd promoted root growth, while ≥ 50 mg kg^{-1} inhibited root growth, indicating that high Cd concentration inhibits root activity, destroys normal physiological metabolism, and reduces the ability of plants to absorb nutrients. The differences in root morphological indices observed through this study are also the result of plant adaptation to adverse environmental conditions.

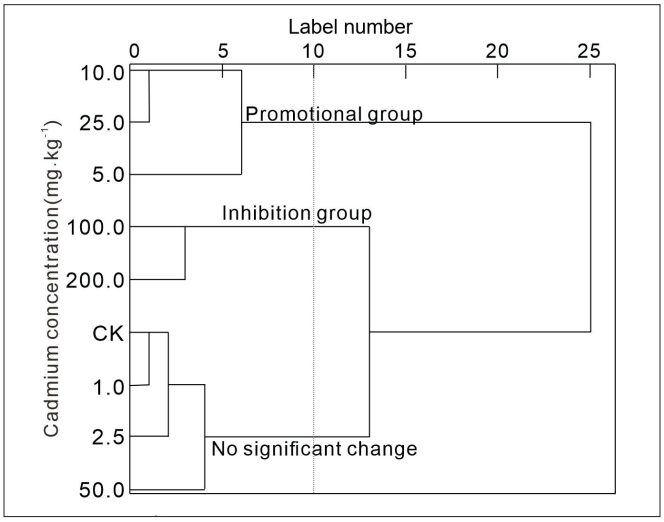

Fig. 6 Dendrogram of *Cardamine hupingshanensis* phenotypic traits under varying cadmium concentrations

The results showed that Cd concentrations below 25 mg kg^{-1} promoted the growth of *C. hupingshanensis*, with the highest growth observed at 5 to 10 mg kg^{-1}, which was a significant increase compared to the control ($P < 0.05$). With increasing Cd concentration, leaf morphology indices gradually decreased, and biomass followed a similar trend. However, the difference was not significant compared to the control, indicating that the inhibition effect of high Cd concentrations on the above-ground parts of the plant was not evident; this finding is in line with the results of previous studies (Xia et al. 2010).

This study also found that the root apical meristem changed significantly under varying Cd concentrations. At low Cd concentrations, no significant difference was observed between the root apical meristem and the control group. However, at high Cd concentrations (> 100 mg kg^{-1}), the root apical meristem became significantly shorter, blackened, and displayed a disorderly arrangement. The leaf is sensitive to environmental changes, and its morphology and anatomical structure can reflect the adaptability of plants to heavy metals and other stresses. This study showed that under Cd stress, leaf epidermal cells became small and irregular the volume of epidermal cells on the leaves was small and irregular. The palisade layer was loosely arranged and the spongy tissue was degraded. When Cd concentration was higher than 100 mg kg^{-1}, spongy tissue thickness significantly increased, while palisade tissue thickness significantly decreased. This would make the plant maintain a lower transpiration rate and a higher net photosynthetic rate, indicating that the plant could not adapt to the growth environment under this concentration. Using a mixed ANOVA model with significance testing, trend analysis was conducted for root morphology indicators (Table 1), leaf morphological indices (Table 2), and anatomical structure (Table 3). Most of the response variables (SA, PA, Vol, AvgD, BT, plant height [PH], shoot height [SH], plant fresh weight [PFW], plant dry weight [PDW], shoot fresh weight [SFW], shoot dry weight [SDW], epidermis thickness [ET], and palisade tissue thickness [PTT]) showed a significant linear trend, indicating obvious increases or decreases with exogenous Cd stress. A cubic regression model was more suitable for fitting TRL, underskin thickness (UT), and spongy tissue thickness (STT), while RT followed a quartic trend. An exception was observed in the trend analysis of the relationship between number of leaves (NL) and increasing Cd concentrations, where all polynomial tests (linear, quadratic, cubic, and quartic) were insignificant, suggesting no definitive trend.

The plasticity index of *C. hupingshanensis*, ranked from highest to lowest, followed the order: VOL > PA > SA > PDW > SDW > PFW > SFW > BN > TRL > RT > SH > PH > ET > AvgD > PTT > STT > UT > NL under varying Cd concentrations. It could be seen that most morphological indices exhibited higher plasticity than the average, except for AvgD. In contrast, the plasticity of leaf morphological indices was lower than the average, indicating that roots showed stronger environmental adaptability to Cd stress. This study categorized the different Cd concentrations into proliferation, inhibition, and no significant change groups. The results revealed a correlation between plant phenotypic traits and Cd concentration, confirming the strong plasticity of *C. hupingshanensis* in response to Cd stress.

Conclusion

This study showed that varying Cd concentrations produced a low-promoting and high-inhibiting effect on *C. hupingshanensis*, which was particularly exhibited in the plant's root and leaf morphological indicators and biomass. Low Cd concentrations significantly promoted growth compared to the control ($P < 0.05$), while high Cd concentrations significantly inhibited root morphological indicators ($P < 0.05$). Moreover, high Cd stress caused significant damage to the root, stem, and leaf anatomical structures of *C. hupingshanensis*. The mean phenotypic plasticity index under Cd stress was 0.73, with root morphology exhibiting greater phenotypic plasticity. Cluster analysis of phenotypic traits further highlighted the effects of varying Cd concentrations on plant morphological variation. These findings confirm the high adaptability of *C. hupingshanensis* in response to Cd stress and may serve as a reference for further examination of similar plant responses to adverse environmental conditions such as heavy metal contamination.

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