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Differences in cadmium accumulation and physiological characteristics of three different ecotypes of the hyperaccumulator *Solanum nigrum* L. survived in northern, central and southern China

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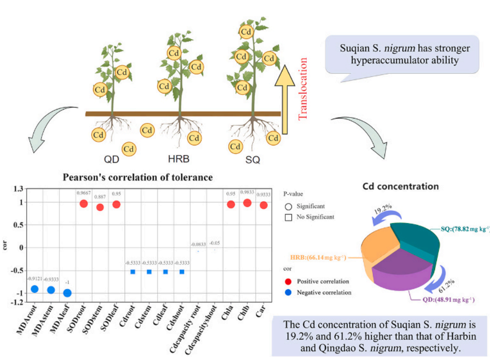
HIGHLIGHTS

- Three ecotype *Solanum nigrum* L. showed Cd hyperaccumulation
- Suqian ecotype *S. nigrum* showed the strongest Cd enrichment capacity
- Biomass of Suqian ecotype was the highest either and with stronger tolerance
- Strong tolerance positively correlated to some physiological traits.

GRAPHICAL ABSTRACT



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ABSTRACT

Solanum nigrum L. has attracted scientific interest due to its capacity to accumulate exceptionally high cadmium (Cd) concentrations. Previous studies, which utilized different *S. nigrum* ecotypes reported varying levels of phytoremediation potentials for Cd pollution. However, due to differing experimental conditions, identifying the ecotype with the highest remediation potential has been challenging. The present article describes experiment involving nutrient solutions with two Cd concentrations [0.2 mg L^{-1} Cd (T1), and 5 mg L^{-1} Cd (T2)] to compare Cd accumulation differences in three *S. nigrum* ecotypes. The results showed that Cd accumulation was highest in the Suqian (SQ) ecotype, followed by Harbin (HRB) and Qingdao (QD) ecotypes. Cd concentrations in the roots, stems, leaves, and shoots of SQ were significantly increased by 31.0 %, 61.4 %, 29.1 %, and 43.1 %, respectively, in T1, and by 54.8 %, 57.6 %, 59.2 %, and 61.2 %, respectively, in T2 compared to QD. Correspondingly, Cd

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accumulation capacity (μg plant^{-1}) in SQ shoots were also the highest, and increased significantly by 95.2 % compared to QD under T2 conditions, respectively, indicating its very high phytoremediation potential. Notably, the biomass of the SQ ecotype was also the highest in all treatments. Moreover, this ecotype demonstrated higher chlorophyll *a* and *b*, carotenoid contents, net photosynthetic rate, transpiration rate, stomatal conductance, SOD activity, and the lowest MDA content. These physiological characteristics may have contributed to the higher stress tolerance of the SQ ecotype. The present findings provide valuable insights for identifying and screening *S. nigrum* ecotypes with hyperaccumulate Cd accumulation potential worldwide.

1. Introduction

Heavy metals have long been a major contributor to soil contamination, posing serious threats to food safety and human health (Zhou et al., 2020). This issue has emerged as a critical environmental concern affecting China's social and economic development. Cadmium (Cd), a non-essential and toxic element for plants and animals, is of particular concern (Chen et al., 2014). According to the National Soil Pollution Survey Bulletin released by the Ministry of Environmental Protection and the Ministry of Land and Resources of China in 2014, the overall soil contamination rate in the country was 16.1 %, with inorganic pollutants being the main contaminants. Among them, Cd reached a point exceedance rate of 7.0 %, making it one of the most common soil pollutants. Consequently, the treatment and reclamation of Cd-contaminated soil is an urgent issue. Land resources are essential to various human activities, and the deterioration of soil quality due to heavy metal contamination can have far-reaching implications for human development (Ma et al., 2020; Liu et al., 2023). Compared to physical and chemical methods of remediating heavy metal-contaminated soil, phytoremediation, primarily utilizing hyperaccumulators, offers several advantages. These include lower remediation costs, minimal environmental disturbance, and suitability for large-scale remediation of heavy metal-contaminated sites (Yang et al., 2019a, 2019b). However, despite these advantages, current phytoremediation methods remain insufficient to achieve optimal results. Therefore, it is necessary to identify hyperaccumulators with enhanced remediation efficiency and higher environmental adaptability.

Solanum nigrum L. is a Cd hyperaccumulator, first identified and described by Wei et al. (2005) in the Shenyang area of China. The most important feature of hyperaccumulators is their accumulation threshold of specific elements, which for Cd is 100 mg kg^{-1} (Reeves et al., 2000). Other defining characteristics include the enrichment factor (EF, the ratio of Cd concentration in shoots compared to soil) and translocation factor (TF, the ratio of Cd concentration in shoots compared to roots) values higher than 1. At the same time, the biomass of a hyperaccumulator should remain unaffected by heavy metal contamination compared to control without Cd pollution (Wei et al., 2005).

Cd extraction capacities of *S. nigrum* ecotypes distributed in different regions worldwide have been determined in many studies. For instance, Fidalgo et al. (2011) conducted a Cd pot experiment using *S. nigrum* seeds collected from the vicinity of the University of Porto campus in Portugal. At Cd concentration in the nutrient solution of 0.84–3.36 mg kg^{-1}, Cd content in *S. nigrum* shoots was approximately 100–370 mg kg^{-1}, with EF values ranging from 101.1 to 220.2. Du and Wang (2012) conducted experiments on *S. nigrum* collected from mountainous areas in Linhai City, Zhejiang Province, China. When Cd was added to the soil at concentrations of 25–100 mg kg^{-1}, the levels of this heavy metal in the roots and shoots of *S. nigrum* under natural light ranged from 8.93 to 28.57 mg kg^{-1} and 18.28 to 58.15 mg kg^{-1}, respectively; the EF and TF values were in the range of 0.58–0.76 and 1.97–2.09, respectively (Du and Wang, 2012). Liu et al. (2013) conducted a pot experiment using *S. nigrum* transplanted from Nanshanlizhi Park in Shenzhen city, Guangdong province to promote Cd enrichment by Proteobacteria. At a Cd soil concentration of 50 mg kg^{-1}, its root and shoot levels amounted to 122.7 and 82.6 mg kg^{-1}, respectively, with EF and TF values of 2.45 and 0.67, respectively. Khan et al. (2014) evaluated the Cd enrichment potential of a Korean ecotype of *S. nigrum* collected from Daegu, South Korea. When Cd concentrations of 10–80 mg kg^{-1} were applied by these authors to the soil, Cd root, stem and leaf contents in *S. nigrum* plants were from 120.49 to 3162.83 mg kg^{1}. The EF values ranged from 347 to 1883, while the TF values varied between 0.49 and 0.92 (Khan et al., 2014). Obviously, there were very huge differences among different *S. nigrum* ecotypes accumulating Cd under different experimental situations.

It is expected that different *S. nigrum* ecotypes from various regions will show similar characteristics in terms of Cd enrichment. However, due to variations in Cd concentrations in individual contaminated media, the differences in Cd accumulation among these ecotypes can vary significantly, sometimes by several or even tens of orders of magnitude. Thus, it is very important to compare the Cd accumulation capacity of different *S. nigrum* ecotypes under the same pollution conditions. This experiment was designed to compare differences in Cd accumulation and corresponding physiological characteristics of three different *S. nigrum* ecotypes collected from different areas of China. We hypothesized that there may be significant differences in Cd accumulation and some physiological indices among three *S. nigrum* ecotypes under consistent experimental situations.

2. Materials and methods

2.1. Hydroponic experiment

The seeds of *S. nigrum* ecotypes used in this experiment were collected from the wild in areas located in northern, central, and southern China, i.e., Harbin (HRB) in Heilongjiang province, Qingdao (QD) in Shandong province and Suqian (SQ) in Jiangsu province. The three ecotypes were selected because of roughly representing the wide geographical situations of China from north to south.

A total of 9 treatments (3 Cd treatments x 3 ecotypes) were established. Each pot was filled with 2.5 l of Hoagland nutrient solution, and three different Cd concentration treatments were tested in triplicate the experiment: a control without Cd addition (CK), 0.2 mg L^{-1} Cd (T1), and 5 mg L^{-1} Cd (T2). T1, the addition of 0.2 mg L^{-1} Cd aimed to show normal growth of *S. nigrum,* especially without decreased biomass. T2, the addition of 5 mg L^{-1} Cd aimed to show inhibited growth of *S. nigrum*, especially with decreased biomass (Dai et al., 2022a). Cd was added in the form of the Guaranteed reagent (G.R.), i.e. $CdCl_2$ 2.5 H_2O. Large, pest-free *S. nigrum* seeds were selected and disinfected with 0.1 % $HgCl_2$ for 10 min. The plants were cultivated under greenhouse conditions. After 15 days of sprouting, 12 evenly growing seedlings were retained in each pot. Following 14 days of hydroponic cultivation, Cd treatments were applied, and all plant samples were collected after additional 14 days of exposure. During this period, the nutrient solution in each pot was changed every two days and continuously aerated. Conditions in the greenhouse were maintained at temperatures of 20–30 °C, relative humidity of 50–70 %, a 10-h photoperiod, and an illumination intensity of 20,000 Lux.

2.2. Hoagland nutrient solution

The hydroponic experiment was conducted using a modified Hoagland nutrient solution with the following composition (per liter of working solution):

Macronutrients: $Ca(NO_3)_2·4H_2O$: 2 mM, KNO_3: 1 mM, $NH_4H_2PO_4$: 0.5 mM, $MgSO_4·7H_2O$: 1 mM.

Micronutrients: H_3BO_3: 2.86 mg L^{-1}, $MnCl_2·4H_2O$: 1.81 mg L^{-1}, $ZnSO_4·7H_2O$: 0.22 mg L^{-1}, $CuSO_4·5H_2O$: 0.08 mg L^{-1}, $Na_2MoO_4·2H_2O$: 0.09 mg L^{-1}.

Iron Chelate: Fe-EDTA: 74.5 mg L^{-1}.

The pH of the nutrient solution was adjusted to 5.8 using 0.1 M NaOH or 0.1 M HCl. All chemicals were of analytical grade, and deionized water was used for preparation.

2.3. Biomass and Cd concentration measurements

Samples of *S. nigrum* were collected and rinsed repeatedly with deionized water, then blotted dry with filter paper. The roots, stems and leaves were separated and initially heated in a drying oven at 105 °C for 5 min. The samples were then dried at 80 °C until constant weight was achieved. The dry weight of the roots and shoots (stems and leaves) was determined using a 0.001 g precision balance. About half stems and leaves were mixed to determine shoot Cd concentration (Dai et al., 2022b).

The concentration of Cd in the roots, stems, leaves and shoots of *S. nigrum* was determined using an atomic absorption spectrophotometer (Hitachi 180–80). For quality assurance and quality control (QA/QC), the standard reference material GBW07405 (GSS-5) was used to verify the measured values. The EF was calculated as the ratio of Cd concentration in the shoot to that in the solution, while the TF was the ratio of Cd concentration in the shoot to that in the root (Dai et al., 2021).

2.4. Determination of physiological indices

The contents of Chl *a*, Chl *b* and Car in the 5th fully unfolded leaf of *S. nigrum* were determined using the methods described by Niu et al. (2015).

The net photosynthetic rate, transpiration rate and stomatal conductance were measured in the 5th fully unfolded *S. nigrum* leaves using a CI-340 portable photosynthetic instrument (CID, USA).

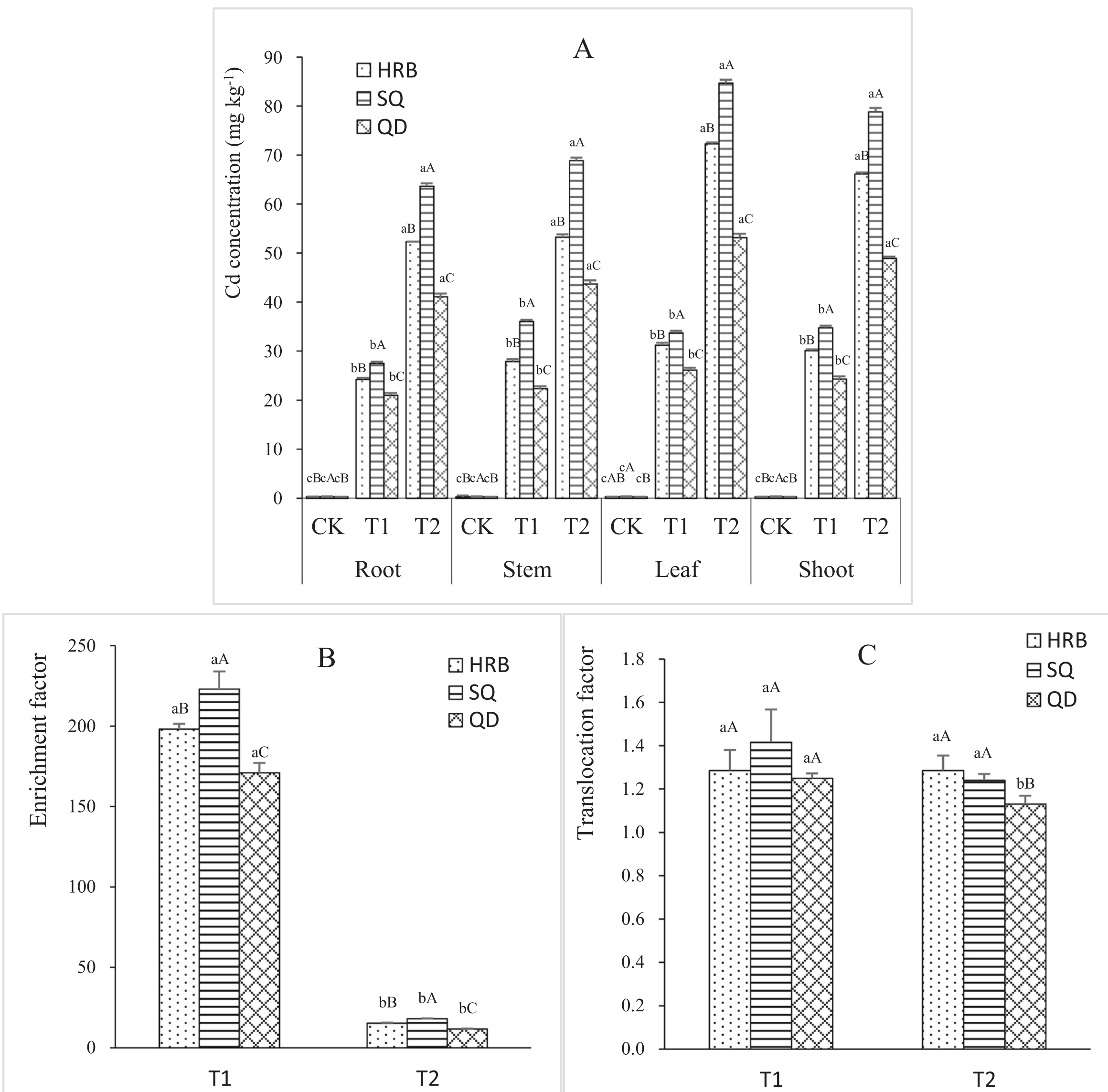

Fig. 1. Cd concentration, enrichment factor and translocation factor of three ecotypes
(HRB:Harbin, SQ: SuQian, QD: QingDao, CK = control, T1 = 0.2 mg L^{-1}, T2 = 5 mg L^{-1}. Capital letters over the bars indicated significant differences ($P < 0.05$) between different ecotypes of *S. nigrum* in same treatment and small letters indicated significant differences ($P < 0.05$) between different treatments of same ecotype.)

Measurements were conducted from 8:00 to 10:00 am, following the method described by Krause and Winter (2015).

Superoxide dismutase (SOD) determination was performed according to the method of Gao et al. (Niu et al., 2015).

MDA content determination was carried out according to the methods provided by Dai et al. (2017).

2.5. Data processing and statistical analysis

Microsoft Excel 2021 and SPSS 12.0 were used to process the data and analyze the significance of differences, and SPSS 12.0 and Origin 2021 were used to analyze the data for correlation. Origin 2021 was used for Pearson correlation analysis to assess the relationship between Cd accumulation and physiological indices (Qiu et al., 2025). Means of different treatments were compared using one-way ANOVA. For post hoc test, Duncan's multiple comparison was performed and the significance level was $P < 0.05$ based on the assumption of normal distribution and homogeneity of variance (Ma, 1990; Yang et al., 2019a, 2019b).

3. Results

3.1. Differences in Cd accumulation characteristics of three *S. nigrum* ecotypes

As shown in Fig. 1A, Cd concentrations ranged from 21.00 mg kg^{-1} to 84.64 mg kg^{-1} in the roots and leaves under T1 and T2 conditions. Overall, Cd contents in the roots, stems, leaves and shoots were highest in the SQ ecotype, followed by HRB, with QD showing the lowest concentrations. In treatment T1, Cd concentrations in the roots, stems, leaves and shoots of SQ were significantly higher by 13.5 % and 31.0 %, 29.4 % and 61.4 %, 8.0 % and 29.1 %, 15.6 %, and 43.1 %, respectively, compared to HRB and QD ($P < 0.05$). In treatment T2, Cd concentrations in the roots, stems, leaves and shoots of SQ increased significantly by 21.7 % and 54.8 %, 29.4 % and 57.6 %, 17.1 % and 59.2 %, and 19.2 % and 61.2 %, respectively ($P < 0.05$). Generally, the differences in Cd concentrations were more pronounced with higher Cd concentrations in nutrient solutions (T1, T2).

Fig. 1B illustrates the EF values of the three ecotypes. The trends were similar to those observed for Cd concentrations in the shoots, with the EF values ranging from 170.1 to 223.0 in T1, and 11.7 to 18.1 in T2. As for the TF values of the three ecotypes, although all of them were >1 and no significant differences were observed among them in T1 treatment, the TF of QD *S. nigrum* was significantly lower than that of HRB and SQ ecotypes in T2 treatment (Fig. 1C).

Fig. 2A presents the biomass of the three ecotypes. Compared to the CKs, root and shoot biomasses in treatment T1 were not significantly reduced ($P < 0.05$), while significant decreases were recorded in treatment T2 ($P < 0.05$). Among the three ecotypes, the biomass of SQ was the highest, followed by HRB and QD.

Fig. 2B shows the Cd accumulation capacity of the three ecotypes, which were calculated as the product of Cd concentration and biomass. In treatment T1, Cd accumulation capacity was the highest for SQ shoots, significantly increasing by 47.7 % and 161.2 % compared to HRB and QD, respectively ($P < 0.05$). In treatment T2, Cd content in SQ shoots was also the highest, and increased significantly by 39.5 % and 95.2 % compared to HRB and QD, respectively ($P < 0.05$). Overall, similar trends in root Cd accumulation capacity were observed under both treatments T1 and T2.

3.2. Differences in physiological indices between three ecotypes

Table 1 shows the chlorophyll content in the leaves of individual *S. nigrum* ecotypes. Generally, compared to the CKs, Chl *a*, Chl *b* and Car contents in the three ecotypes were not markedly reduced under treatment T1, but were significantly reduced in treatment T2 ($P < 0.05$). Among the different ecotypes, SQ showed the highest chlorophyll concentrations. Under T1 treatment conditions, SQ had 13.2 % and 14.2 %

Table 1
Chlorophyll contents in the leaves of individual *S. nigrum* ecotypes.

	Treatment	HRB	SQ	QD
	CK	1.30 ± 0.02aAB	1.41 ± 0.12aA	1.26 ± 0.02aB
Chlorophyll a	T1	1.21 ± 0.04aB	1.37 ± 0.06aA	1.20 ± 0.05aB
(mg kg^{-1})	T2	0.99 ± 0.08bB	1.14 ± 0.03bA	0.77 ± 0.05bC
	CK	1.60 ± 0.14aB	1.91 ± 0.06aA	1.36 ± 0.08aC
Chlorophyll b	T1	1.45 ± 0.04aB	1.75 ± 0.16aA	1.10 ± 0.05bC
(mg kg^{-1})	T2	0.84 ± 0.08bB	1.05 ± 0.04bA	0.62 ± 0.11cC
	CK	3.41 ± 0.14aB	3.73 ± 0.07aA	3.50 ± 0.03aB
Carotenoid	T1	3.23 ± 0.07aAB	3.53 ± 0.03aA	2.93 ± 0.27bB
(mg kg^{-1})	T2	2.37 ± 0.13bA	2.73 ± 0.41bA	1.70 ± 0.26cB

Note:HRB:Harbin, SQ: SuQian, QD: QingDao, CK = control, T1 = 0.2 mg L^{-1}, T2 = 5 mg L^{-1}. Capital letters indicated significant differences ($P < 0.05$) between different ecotypes of *S. nigrum* in same treatment and small letters indicated significant differences ($P < 0.05$) between different treatments of same ecotype.

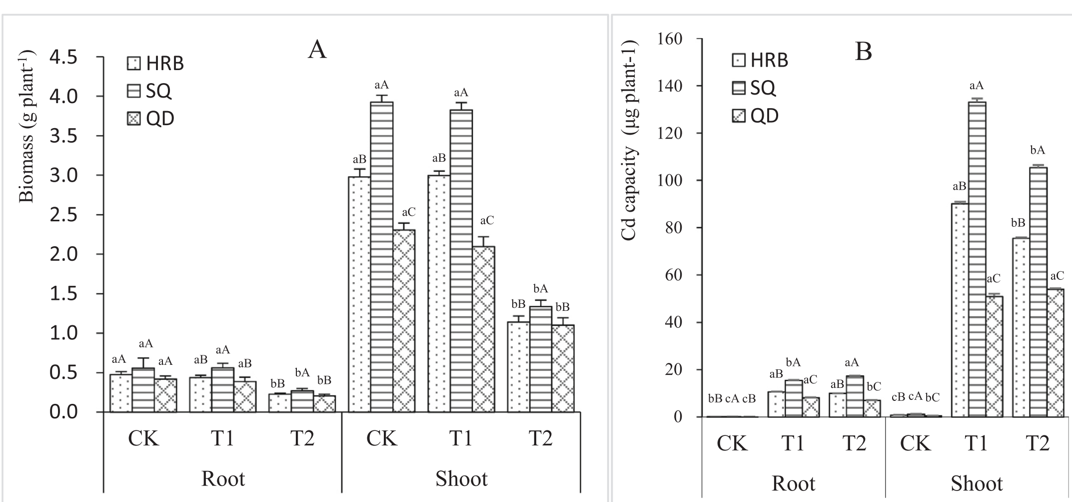

Fig. 2. Biomass and Cd accumulation capacity of three *S. nigrum* ecotypes
(HRB:Harbin, SQ: SuQian, QD: QingDao, CK = control, T1 = 0.2 mg L^{-1}, T2 = 5 mg L^{-1}.Capital letters over the bars indicated significant differences ($P < 0.05$) between different ecotypes of *S. nigrum* in same treatment and small letters indicated significant differences ($P < 0.05$) between different treatments of same ecotype.)

higher Chl *a* content, 20.7 % and 51.9 % higher Chl *b* content, and 9.3 % and 20.5 % higher Car content than HRB and QD, respectively. The advantage of SQ was further extended under T2 treatment conditions: its Chl *a* content was 15.2 % and 48.1 % higher than that of HRB and QD, respectively, and its Chl *b* content was elevated by 25.0 % and 69.4 %, while a significant increase of 15.2 % and 60.6 % in the Car content was also realized.

Table 2 summarizes the photosynthetic performance, including net photosynthetic rate (NR), transpiration rate (TR) and stomatal conductance (SC) in the leaves of the three ecotypes. Compared to the CKs, the NR, TR and SC of the three ecotypes were not significantly decreased as a result of treatment T1, but were significantly reduced under T2 conditions ($P < 0.05$). Among the ecotypes, SQ had the highest NR, TR, and SC, followed by HRB and QD.

Table 3 shows SOD antioxidant activity of in the leaves of the three ecotypes. Overall, SOD activity of the three ecotypes was not significantly decreased in treatment T1 but was markedly reduced in treatment T2 ($P < 0.05$) compared to the CKs. The highest levels were observed in the SQ ecotype, while the lowest were recorded for QD, with HRB displaying intermediate values.

The MDA content in the stems and leaves of the three *S. nigrum* ecotypes is presented in Fig. 3. Compared to the CKs, the MDA content of all three ecotypes did not significantly decrease in treatment T1, while it was significantly elevated under T2 conditions ($P < 0.05$). Generally, SQ had the lowest MDA content, QD the highest, and HRB showed intermediate levels.

Table 3
SOD activity in the leaves of individual *S. nigrum* ecotypes (U mg^{-1}).

Organ	Treatment	HRB	SQ	QD
Root	CK	4.60 ± 0.02aB	5.13 ± 0.02aA	3.58 ± 0.02aC
	T1	4.40 ± 0.57aB	5.61 ± 0.27aA	3.51 ± 0.64aB
	T2	2.66 ± 0.28bB	2.98 ± 0.02bA	1.94 ± 0.02bC
Stem	CK	4.35 ± 0.54aAB	4.64 ± 0.06aA	3.97 ± 0.01aB
	T1	3.42 ± 0.01aAB	4.35 ± 0.59aA	3.82 ± 0.17aB
	T2	2.71 ± 0.01bB	2.91 ± 0.01bA	1.94 ± 0.01bC
Leaf	CK	5.73 ± 0.02aA	5.85 ± 0.07aA	4.70 ± 0.16aB
	T1	5.38 ± 0.58aAB	6.10 ± 0.27aA	4.77 ± 0.01aB
	T2	2.99 ± 0.01bB	3.22 ± 0.06bA	2.74 ± 0.10bC

Note:HRB:Harbin, SQ: SuQian, QD: QingDao, CK = control, T1 = 0.2 mg L^{-1}, T2 = 5 mg L^{-1}. Capital letters indicated significant differences ($P < 0.05$) between different ecotypes of *S. nigrum* in same treatment and small letters indicated significant differences ($P < 0.05$) between different treatments of same ecotype.

3.3. Correlation analysis between Cd accumulation, biomass and physiological indices

In generally, the results of Pearson's correlation analysis (Fig. 4) revealed that Cd concentrations in the roots, stems, leaves and shoots were significantly and negatively correlated with biomass and SOD activity ($P < 0.05$), while showing significantly positive correlations with MDA content ($P < 0.05$ and $P < 0.01$, respectively). Cd accumulation capacity showed a significantly positive correlation with Cd concentrations ($P < 0.05$ and $P < 0.001$). Biomass was significantly and positively correlated with SOD activity, Chl *a*, *b* and Car contents, as well as net photosynthetic rate, transpiration rate and stomatal conductance ($P < 0.01$ or $P < 0.001$), while it showed a significantly negative correlation with MDA contents ($P < 0.01$ or $P < 0.001$).

4. Discussion

In this experiment, Cd concentrations in the stems, leaves and shoots of the HRB, QD and SQ ecotypes under T1 and T2 conditions did not exceed 100 mg kg^{-1}, the threshold value for Cd hyperaccumulators (Fig. 1A). This was primarily due to the relatively low Cd concentrations in the nutrient solutions, which were set at 0.2 and 5 mg L^{-1}. These findings are consistent with results reported for *S. nigrum* ecotypes from Shenyang and Hanzhong in China, Australia and Japan under different Cd concentration gradients in contaminated soils (Wei et al., 2013; Dai et al., 2022b). Although aboveground partial Cd levels did not reach the hyperaccumulator plant threshold for all three ecotypes, EF values >1 were found for the HRB, QD and SQ ecotypes (Fig. 1B) (Wei et al., 2013; Dai et al., 2022b). The TF values obtained for the HRB, QD and SQ ecotypes were also all higher than 1 (Fig. 1C), suggests that it still has some Cd transport capacity. In treatment T1, the root and shoot biomass of the HRB, QD and SQ ecotypes were not significantly reduced compared to the CKs (Fig. 2A), indicating strong Cd tolerance under these conditions. All three ecotypes showed the fundamental characteristics of Cd hyperaccumulators (Wei et al., 2005; Wei et al., 2013; Dai et al., 2022b). In particular, its higher EF value indicated that the SQ ecotype was able to effectively absorb Cd from the soil, while the TF value >1 indicated that it was able to transport Cd from the roots to the aboveground part. Therefore, the SQ ecotype still has some potential for application in the remediation of Cd pollution. As shown in Fig. 2B, Cd accumulation capacity in the shoots of the SQ ecotype were the highest and increased by 161.2 % in T1 and 95.2 % in T2 compared to the lowest values observed in the QD ecotype. This demonstrated that the phytoremediation potential of the SQ ecotype was higher, indicating the necessity of expanding the screening for ecotypes with even greater Cd accumulation potential in China. Previous studies have demonstrated significant differences in Cd accumulation among Chinese ecotypes, such as those from Shenyang and Hanzhong, as well as ecotypes from Australia and Japan (Wei et al., 2013; Dai et al., 2022b). Therefore, identifying *S. nigrum* ecotypes with higher Cd accumulation capacity on a global scale seems feasible.

Photosynthesis is an important metabolic process in plants, in which chlorophyll *a* and chlorophyll *b* are responsible for the absorption, transfer and conversion of light energy, and their content indicates the intensity of photosynthetic activity. Carotenoids, present in photosynthetic tissues, absorb excess light energy to protect chlorophyll *a*nd maintain photosynthesis. Panković et al. (2000) showed that chlorophyll content of sunflower leaves decreased significantly under high Cd stress, whereas lower Cd levels did not cause a significant reduction,

Table 2
Net photosynthetic rate, transpiration rate and stomatal conductance in *S. nigrum* leaves.

	Treatment	HRB	SQ	QD
Net photosynthetic rate (μmol s^{-1} m^{-2})	CK	4.01 ± 0.65aB	8.01 ± 0.57aA	3.22 ± 0.16aB
	T1	3.77 ± 0.24aB	7.55 ± 0.43aA	2.99 ± 0.09 aC
	T2	2.49 ± 0.30bB	4.03 ± 0.34bA	1.78 ± 0.66bC
Transpiration rate (μmol s^{-1} m^{-2})	CK	2.68 ± 0.07aB	3.38 ± 0.14aA	1.27 ± 0.19aC
	T1	2.49 ± 0.14aB	3.04 ± 0.22aA	1.13 ± 0.05aC
	T2	1.27 ± 0.11bA	1.44 ± 0.29bA	0.77 ± 0.05bC
Stomatal conductance (μmol s^{-1} m^{-2})	CK	32.37 ± 1.90aA	33.55 ± 3.31aA	27.46 ± 1.64aB
	T1	26.54 ± 1.05bB	29.79 ± 0.91aA	22.26 ± 0.94bC
	T2	17.69 ± 2.63cA	18.89 ± 0.95bA	13.98 ± 0.22cC

Note:HRB:Harbin, SQ: SuQian, QD: QingDao, CK = control, T1 = 0.2 mg L^{-1}, T2 = 5 mg L^{-1}. Capital letters indicated significant differences ($P < 0.05$) between different ecotypes of *S. nigrum* in same treatment and small letters indicated significant differences ($P < 0.05$) between different treatments of same ecotype.

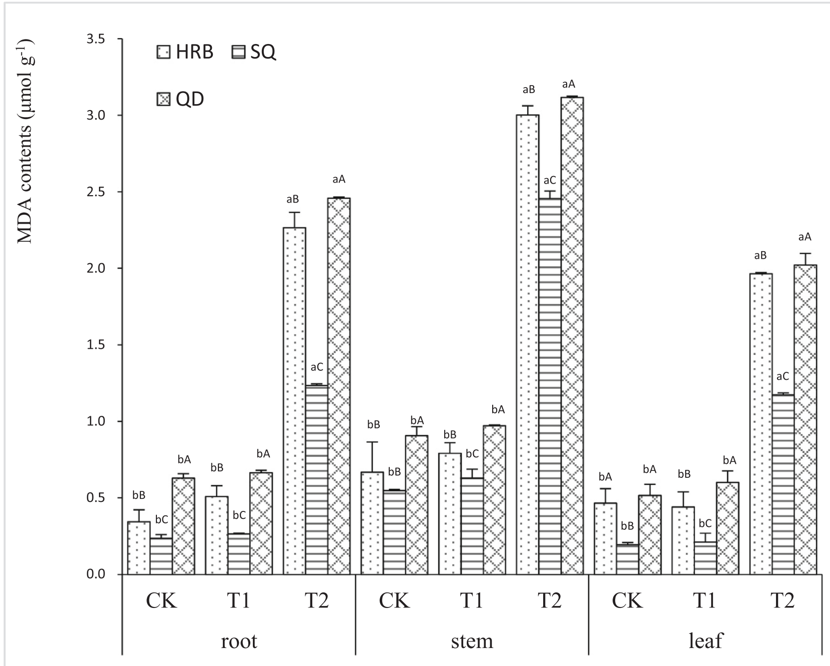

Fig. 3. MDA contents in stems a leaves of three *S. nigrum* ecotypes
(HRB:Harbin, SQ: SuQian, QD: QingDao, CK = control, T1 = 0.2 mg L^{-1}, T2 = 5 mg L^{-1}.Capital letters over the bars indicated significant differences (P < 0.05) between different ecotypes of *S. nigrum* in same treatment and small letters indicated significant differences (P < 0.05) between different treatments of same ecotype.)

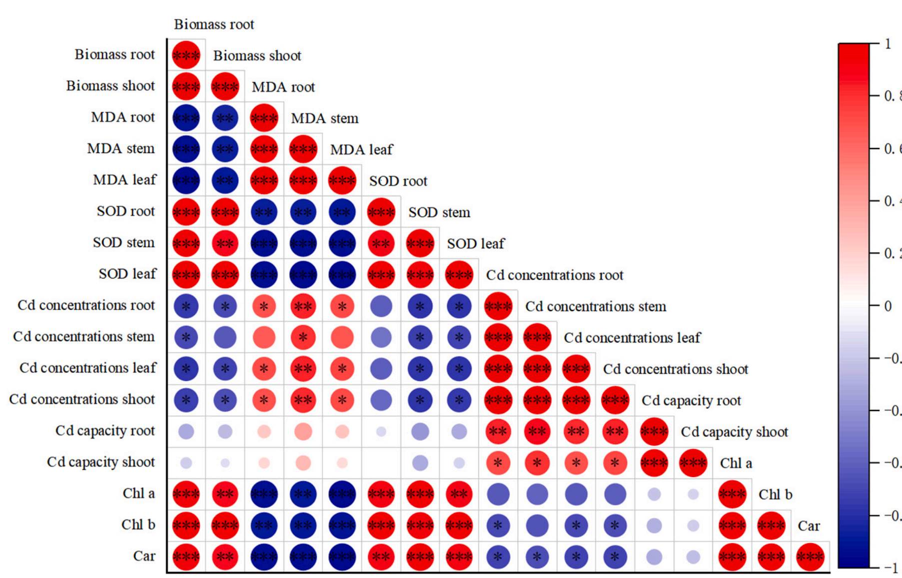

Fig. 4. Pearson's correlation analysis of three ecotypes of *S. nigrum*
(Red indicated positive correlation and blue indicated negative correlation. *, ** and *** were significant differences at P < 0.05, P < 0.01 or P < 0.001, respectively).

indicating some level of tolerance. The changes in chlorophyll content in this study were similar, and the SQ ecotype contained relatively the highest content of chlorophyll pigments (Table 1). The effect of heavy metals on photosynthesis was also reflected in such parameters as net photosynthetic rate, which was reduced, possibly due to RuBP carboxylation, photochemical activity, or inorganic phosphorus deficiency. He et al. (2016) showed that under different Cd concentrations, the net photosynthetic rate, transpiration rate and stomatal conductance of tobacco leaves remained unchanged at low Cd concentrations but were significantly lower than the control under high Cd stress. Similar trends were observed in this study, with the SQ ecotype showing the highest values for these parameters (Table 2). In general, heavy metal stress induces the production of reactive oxygen species (ROS) in plant cells, and excessive ROS accumulation can result in protein denaturation, enzyme inactivation, membrane lipid peroxidation, and even cell death. The antioxidant enzyme system in plant cells, especially SOD, may play a role in scavenging ROS, thereby mitigating the toxic effects of heavy metals on plants (AbdElgawad et al., 2019). The SQ ecotype of *S. nigrum* may mitigate Cd toxicity by enhancing antioxidant enzyme activity (SOD). It has been shown that hyperaccumulating plants are able to alleviate heavy metal stress by enhancing antioxidant capacity. It has been shown that hyperaccumulated plants are able to mitigate heavy metal stress by improving antioxidant capacity, and Cd toxicity induces excessive production of ROS, including H_2O_2, and leads to oxidative stress in plants, which is a common phenomenon under Cd stress (Sterckeman and Thomine, 2020). Cellular antioxidant enzymes are usually stimulated to help plants resist oxidative damage by eliminating ROS (Fasih et al., 2021). At the same time, their gene expression levels and patterns are changed. Wang et al. (2024) found significant alterations in 20 DEGs related to antioxidant enzyme genes, including SOD, CAT and POD, by studying antioxidant enzyme activities and corresponding genes in Lobelia under Cd stress.

In addition, heavy metal stress impairs cell membrane function and permeability, and the oxidation of unsaturated fatty acids can lead to MDA production (AbdElgawad et al., 2019). Cd exposure triggers the generation of highly reactive free radicals in plants, resulting in membrane lipid peroxidation. The extent of cellular damage and plant resistance can be determined by measuring SOD activity and MDA content. Peng (2019) reported that SOD activity of *Amaranthus caudatus* seedlings was significantly reduced, while MDA content significantly increased under high Cd conditions. Conversely, under low Cd levels, the opposite trends were observed. The observed changes in SOD activity and MDA content in the current study were consistent with previous findings, with the SQ ecotype showing the highest and lowest values of these indicators, respectively (Table 2). These physiological indicators appear to correlate with the plant's tolerance to Cd stress and may reflect its overall ability to withstand heavy metal toxicity. Thus, *S. nigrum* ecotypes with high Cd accumulation capacity may show enhanced values of these physiological indicators. However, as this study examined only three ecotypes of *S. nigrum*, further, more comprehensive research is required.

The significant negative correlations were observed between Cd concentrations (in root, stem, leaf and shoot) and biomass and SOD activity (Fig. 4). The significant positive correlation was shown between Cd concentrations and MDA contents either (Fig. 4). However, there were seldom direct evidences in physiological or molecular mechanisms fully explaining these relationships. In particular, the SQ ecotype reached the highest biomass among the three ecotypes of *S. nigrum* (Fig. 2A), while also showing the highest Cd concentrations in roots, stems, leaves and shoots (Fig. 1A). These findings suggest that differences in Cd accumulation between individual *S. nigrum* ecotypes were not directly related to biomass. Instead, Cd tolerance appears to be associated with physiological indicators such as Chl *a*, *b* and Car contents, net photosynthetic rate, transpiration rate, stomatal conductance, SOD activity and MDA content, which collectively reflect the plants' tolerance to Cd stress just like the discussions above. In another word, these indicators together reflected the tolerance differences of three ecotypes to Cd stress.

In soil pot experiment, plant enrichment of Cd is usually positively correlated with the extractable Cd content and negatively correlated with soil pH (Rehman et al., 2017). However, the present study used a nutrient incubation experiment in which the extractable Cd content and pH were basically maintained at consistent levels for all the experimental treatments. Differences in physiological characteristics of different *S. nigrum* ecotypes may originate from plant evolution and adaptation to the environment resulting from their long-term growth in heterogeneous habitats. In fact, the concept of ecotype mainly refers to individuals or populations of the same plant species that have long-term growth in different growth environments and have ecological differences due to divergent adaptation (Luo, 2001). *S. nigrum* grown in different regions have significant differences in environmental conditions such as climate, soil and light just like in Haerbin, Qingdao and Suqian cities, and thus named different *S. nigrum* ecotypes (HRB, QD and SQ ecotypes) in this experiment. Previous studies have shown that Cd is a phytotoxic metal contaminant that induces ROS release and inhibits plant growth. Plants may improve their tolerance through complex reactions involving antioxidant mechanisms, compartmentalization of Cd within the cell wall or vesicles, which might be part reasons of the Cd accumulation and physiological characteristics were different among HRB, QD and SQ ecotypes (Zhang et al., 2019; Sun et al., 2020). However, high expression of metal transporter proteins such as *SaNRAMP6* and IRT1 were also concerned on Cd hyperaccumulation in different plants (Chen et al., 2017; Ye et al., 2020). Thus, the difference mechanisms of Cd enrichment among different *S. nigrum* ecotypes might be explored from molecular biology levels in the future.

5. Conclusions

The nutrient culture experiment revealed significant differences in Cd accumulation between the three *S. nigrum* ecotypes under uniform conditions. The SQ ecotype had the highest Cd concentrations in the roots, stems, leaves, and shoots, as well as the greatest Cd accumulation capacity in its roots and shoots. In addition, the root and shoot biomasses of the SQ ecotype were the highest. Significant positive and negative correlations were observed regarding *S. nigrum* tolerance to Cd. Overall, the SQ ecotype showed the highest levels of chlorophyll a, chlorophyll *b*, and carotenoid contents, net photosynthetic rate, transpiration rate, stomatal conductance, and SOD activity, while maintaining the lowest MDA content in all treatments. The present findings suggest that these physiological indicators may be key characteristics of its Cd tolerance.

CRediT authorship contribution statement

Yuhan Zhang: Writing – original draft, Resources, Formal analysis. **Jibao Jia:** Writing – original draft, Resources, Formal analysis. **Shuhe Wei:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis. **Jie Zhan:** Writing – review & editing, Validation. **Guannan Kong:** Writing – review & editing, Validation. **Baoyu Wang:** Writing – review & editing, Validation. **Brett H. Robinson:** Writing – review & editing, Validation. **Lidia Skuza:** Writing – review & editing, Methodology. **Jianming Xue:** Writing – review & editing, Validation. **Huiping Dai:** Writing – review & editing, Validation, Supervision, Methodology.

Consent to participate

All authors have agreed for authorship, read and approved the manuscript.

Consent for publication

All authors have given consent for publishing this study.

Ethical approval

Not applicable.

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.

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Data availability

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

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