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The tolerance of *Sphagneticola trilobata* and its hybrid to cadmium stress

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Abstract

During the invasion process, *Sphagneticola trilobata* produced a hybrid with native species. In this study, the adaptability of *S. trilobata* and its hybrid was investigated under cadmium (Cd) stress. Results showed that the net photosynthetic rate and maximum quantum yield of PSII photochemistry of *S. trilobata* leaves were significantly lower than those in the hybrid, while malondialdehyde and electrolyte leakage were significantly higher than those in the hybrid. The accumulation of Cd in the hybrid was significantly higher than that in *S. trilobata*, and more active detoxification strategies were shown in the hybrid. The antioxidant enzyme activity in the hybrid was significantly higher than that in *S. trilobata*. Compared with *S. trilobata*, more Cd was accumulated in the cell walls and transformed into less toxic chemical forms in the hybrid. The results indicate that hybridization of *S. trilobata* with native species during invasion enhances the adaptability of the hybrid to Cd stress.

Keywords: adaptability; antioxidant; biological invasion; heavy metals; photosynthesis.

Introduction

With the development of industry, soil and water pollution caused by heavy metals has become an increasingly severe problem worldwide (Rao and Parsai 2025). Cadmium (Cd), as a common heavy metal, is one of the main pollutants in the environment. It is not only toxic to plants but also absorbed by plant roots and transported to various organs, causing harm to animals and humans through the food chain (Zulfiqar *et al.* 2022, Meng *et al.* 2025). Under Cd stress, plants accumulate a large amount of reactive oxygen species (ROS), leading to oxidative stress and damage to membrane lipids, cellular components, proteins, and genetic material (Zulfiqar *et al.* 2024). Photosynthesis is the basis of plant organic matter accumulation and growth, and Cd stress significantly affects photosynthesis, thereby inhibiting plant growth (Al-Obaidi *et al.* 2024). Studies have shown that Cd stress damages the thylakoid membrane structure of chloroplasts, inhibits chloroplast proliferation, and hinders chlorophyll (Chl) synthesis. Cd stress leads to a decrease in the maximum quantum yield of PSII photochemistry (F_v/F_m) in plant leaves, inhibits Rubisco enzyme activity, and causes a decrease in stomatal conductance (g_s) (Al-Obaidi *et al.* 2024, Zhang *et al.* 2024a,b).

Plants have evolved various strategies to adapt to heavy metal stress. The adaptability of plants to Cd stress can be achieved through avoidance and tolerance (Zhang

Highlights

- Photosynthetic ability of the hybrid was stronger than *Sphagneticola trilobata* under Cd stress
- Antioxidant ability of the hybrid was stronger than *S. trilobata* under Cd stress
- Hybridization improved tolerance of the hybrid to Cd stress

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Abbreviations: CAT – catalase; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; ETR – electron transfer rate; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net photosynthetic rate; POD – peroxidase; q_N – nonphotochemical quenching; q_P – photochemical quenching; ROS – reactive oxygen species; SOD – superoxide dismutase.

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et al. 2024c, Panda *et al.* 2025). The tolerance of plants to Cd stress includes metal enrichment. Avoidance refers to plants reducing the absorption of Cd in the environment to avoid harm. Metal enrichment refers to the ability of plants to reduce the toxicity of Cd in the body through detoxification, mainly through the following strategies: (*1*) cell wall precipitation – by altering the subcellular distribution of Cd and utilizing compartmentalization to reduce toxicity, organelles can be protected (Jia *et al.* 2022); (*2*) converting Cd into low-toxicity chemical forms is an important detoxification mechanism for Cd in plant tissues (Zheng *et al.* 2024); (*3*) enhancing antioxidant capacity. Cd stress is similar to other forms of oxidative stress because it can generate a large amount of ROS, which can damage biomolecules and cause membrane lipid peroxidation. Under Cd stress, plants clear ROS to protect cells from damage through various antioxidant defense systems (Guo *et al.* 2024). In addition, plants synthesize metal-binding proteins such as phytochelatins, which achieve detoxification of Cd through metal chelation (Al-Obaidi *et al.* 2024).

Hybridization can enhance the tolerance of various plants to environmental stress, which is a common phenomenon among plants. During biological invasion, hybridization between invasive plants and native plants is becoming increasingly common, which may result in more adaptive hybrids (Pfeilsticker *et al.* 2023). Hybridization between invasive and native species is considered one of the main driving forces for adaptive evolution (Li *et al.* 2024). Hybridization generates new evolutionary types, fixes hybrid vigor, reduces genetic burden, and leads to genetic variation, which may enhance the invasiveness of hybrids (Pfeilsticker *et al.* 2023). For example, *Spartina anglica* is a hybrid species produced by natural hybridization between *S. alterniflora* and *S. maritima*, with stronger invasion ability than its parents (Li *et al.* 2024). Under constantly changing environmental conditions, hybridization is particularly important, and heterosis may lead to hybrid varieties being superior to invasive parents in survival competition. Hybrids with strong competitiveness may harm native species, leading to a decline in the population of native species (Bock *et al.* 2021).

Sphagneticola trilobata is one of the common invasive plants in South China. It is a perennial creeping herbaceous plant in the Asteraceae family, native to South America. Researchers have discovered a natural hybrid of *S. trilobata* with its native relative (*S. calendulacea*) in the invasion area. The pollen of the hybrid comes from *S. trilobata*, indicating that its male parent is *S. trilobata*. The growth potential of the hybrid varieties is similar to that of *S. trilobata*, and its energy-use efficiency is higher than that of *S. trilobata* (Wu *et al.* 2013). Previous studies found that the hybrid was more sensitive to nitrogen deposition treatment, and the response pattern was similar to that of *S. trilobata* (Ni *et al.* 2014). The hybrid's adaptability under high-temperature stress was lower than that of *S. trilobata* (Cai *et al.* 2021), while its tolerance under low-temperature stress was stronger than that of *S. trilobata* (Sun *et al.* 2015). The hybrid's adaptability under drought stress was lower than that of *S. trilobata* (Zhang *et al.* 2021), while the hybrid's adaptability under flooding stress was stronger than that of *S. trilobata* (Zhang *et al.* 2024d). At present, there is still a lack of research on the tolerance of this hybrid to Cd stress. This study aimed to determine whether the hybrid's adaptability to Cd stress is stronger than that of *S. trilobata* and to explore its adaptive strategies.

Materials and methods

Plant material: *S. trilobata* and its hybrid used in this study were collected from South China Botanical Garden, Chinese Academy of Sciences. Through asexual propagation, stems with leaves removed were cut into about 15-cm stem segments to ensure that each segment contained two stem nodes. The stem segments were placed in a culture bottle with tap water. After the stem segments grew roots and leaves, they were transplanted into a culture basin containing Hoagland nutrient solution, and the nutrient solution was supplemented every 7 d. After 28 d of cultivation, we selected seedlings with consistent growth for the experiment. All experimental materials were cultured under natural light in a glass greenhouse at a temperature of 32/26°C day and night. In the preliminary experiment, eight $CdCl_2$ concentrations were set (30, 60, 90, 120, 150, 180, 210, and 240 μM $CdCl_2$, respectively). We found significant differences in leaf phenotype under 180 μM $CdCl_2$ treatment. The control group was cultured in Hoagland nutrient solution, while the treatment group was cultured in Hoagland nutrient solution containing 180 μM $CdCl_2$.

Gas exchange and chlorophyll (Chl) *a* fluorescence parameters: The leaf gas-exchange parameters were measured using a portable infrared gas analyzer (*Li-6800*, *Li-COR Inc.*, Lincoln, NE, USA). The measurements were conducted from 9:00–11:00 h on a sunny day, with a leaf chamber light intensity of 900 μmol(photon) m^{-2} s^{-1} (red and blue light ratio of 9:1), humidity of 65%, and temperature of 28°C. The values were recorded after the data stabilized. The portable modulated Chl fluorometer (*PAM-2500*, *Walz*, Germany) was used to measure the Chl fluorescence parameters of leaves. According to the method described by Ndhlovu *et al.* (2025), after 30 min of dark adaptation, leaves were measured for minimal (F_o) and maximal (F_m) fluorescence, and then the maximum Chl fluorescence in the light (F_m') and steady-state fluorescence (F_s) were measured. The minimal level of fluorescence (F_o') was measured under far-red light. The F_v/F_m, electron transfer rate (ETR), photochemical quenching (q_P), and nonphotochemical quenching (q_N) were calculated according to the method described by Klughammer and Schreiber (2008) and Tambussi *et al.* (2002).

Chlorophyll content: Fresh leaf samples (0.05 g) were weighed and cut into pieces in a 10-mL centrifuge tube. Then, 4 mL of 80% acetone solution was added, and pigments were extracted in the dark at 4°C for 24 h. The absorbance values of the extract were measured at

663 and 645 nm (*UV-2450*, *Shimadzu*, Kyoto, Japan). The content of Chl *a* and *b* was calculated according to the method described by Wellburn (1994).

Enzyme activities: Fresh leaves (0.2 g) were ground in 2 mL of ice-cold phosphate buffer (0.05 M, pH 7.0) containing 0.1 M EDTA, 0.1% *Triton X-100*, and 2% (w/v) polyvinylpyrrolidone. Homogenates were centrifuged at 12,000 × *g* for 20 min at 4°C, and the supernatant was used for enzyme activity analysis (Xin *et al.* 2019).

The activity of catalase (CAT, EC 1.11.1.6) in the supernatant was measured and adjusted appropriately. Firstly, 0.1 mL of enzyme extract was mixed with 2.9 mL of 30 mM H_2O_2. The absorbance was recorded at 240 nm (*UV-2450*, *Shimadzu*, Kyoto, Japan) for calculating CAT activity. One unit of CAT activity was defined as the absorbance change of 0.01 unit per min.

For peroxidase (POD, EC 1.11.1.7) activity, we mixed 0.1 mL of enzyme extract with 1 mL H_2O_2 (30 mM), 1.875 mL phosphate buffer (50 mM, pH 7.0), and 0.025 mL guaiacol. The POD activity was calculated from the absorbance at 470 nm (*UV-2450*, *Shimadzu*, Kyoto, Japan). One unit of POD activity was defined as the absorbance change of 0.01 unit per min.

For superoxide dismutase (SOD, EC 1.15.1.1) activity, we mixed 0.1 mL of enzyme extract with 0.3 mL NBT (0.75 mM), 1.7 mL phosphate buffer (50 mM, pH 7.8), 0.3 mL EDTA-Na_2 (0.1 mM), 0.3 mL methionine (130 mM), and 0.3 mL riboflavin (0.02 mM). The SOD activity was calculated from the absorbance at 560 nm (*UV-2450*, *Shimadzu*, Kyoto, Japan). The 50% inhibition of NBT photoreduction was taken as one unit of SOD activity.

H_2O_2 and $O_2^{\bullet-}$: 3,3'-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) were used to determine H_2O_2 and $O_2^{\bullet-}$ in leaves, respectively. Fresh leaves were incubated in a 0.5 mg mL^{-1} DAB solution (dissolved in 0.05 M phosphate buffer, pH 7.0). After being vacuum-dried, staining in the dark for 4 h, then decolorized with 95% ethanol. The H_2O_2 in the leaves was stained brown by DAB. Fresh leaves were incubated in 1 mg mL^{-1} NBT solution (dissolved in 0.05 M phosphate buffer, pH 6.4), after vacuuming, stained in the dark for 6 h, then decolorized with 95% ethanol. The $O_2^{\bullet-}$ in the leaves was stained blue by NBT.

Malondialdehyde: Fresh leaves (0.2 g) were weighed and ground with 2 mL of 10% trichloroacetic acid. After grinding, homogenates were centrifuged at 4,000 × *g* for 15 min at 4°C, and then we mixed 1 mL of supernatant with an equal volume of 0.67% thiobarbituric acid solution. After boiling in water for 20 min, samples were centrifuged at 4,000 × *g* for 15 min at 4°C. The absorbance of the sample was measured at 600, 532, and 450 nm (*UV-2450*, *Shimadzu*, Kyoto, Japan), and the content of malondialdehyde (MDA) was calculated according to the method described by Sun *et al.* (2015).

Electrolyte leakage: Three blade circles with a diameter of 0.7 cm were collected from fully expanded leaves and immersed in 10 mL of deionized water for 3 h, and the initial conductivity of the solution was measured using a conductivity meter (*Mettler Toledo*, Zurich, Switzerland). The total conductivity of the sample was measured after boiling the water for 30 min. The electrolyte leakage was calculated according to the method described by Lee *et al.* (2015).

Cd content: The fresh samples of roots, stems, and leaves were washed with deionized water, and heated at 110°C for 30 min, and dried at 65°C to a constant mass. The dried samples were ground to powder, and 0.2 g was weighed in a digestion tank containing 5 mL of concentrated nitric acid. A microwave digestion analyzer (*COOLPEX-E*, *Yiyao*, China) and inductively coupled plasma mass spectrometer (*7800*, *Agilent*, USA) were used to determine Cd content in samples according to the method described by Liu *et al.* (2022). The Cd translocation factor was calculated as the shoot-to-root ratio of Cd concentration.

Chemical forms of Cd: Five extraction solutions were sequentially used to extract the chemical forms of Cd: (*1*) 80% ethanol (F_E), primarily composed of inorganic Cd; (*2*) deionized water (F_W), water-soluble Cd; (*3*) 1 M NaCl (F_{NaCl}), Cd bound to pectates and proteins; (*4*) 2% acetic acid (F_{HAc}), sparingly soluble Cd phosphate compounds; (*5*) 0.6 M HCl (F_{HCl}), Cd oxalate precipitates. Fresh plant tissues were mixed with the extract at a ratio of 1:10 (w/v), ground in a mortar, centrifuged at 4,000 × *g* for 10 min, the supernatant was collected, and repeated twice with the same extract for precipitation. The supernatant of each extraction solution was dried at 70°C to determine the Cd content according to the method described by Liu *et al.* (2022).

Subcellular distribution of Cd: Differential centrifugation was used to extract subcellular components. Fresh samples (1.0 g) were weighed and ground with 10 mL of extraction solution at 4°C. The extraction solution consists of 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), and 1 mM dithiothreitol. After grinding, the extract was centrifuged at 4,000 × *g* for 15 min to obtain the precipitate as the cell wall component (F_C). Then the supernatant was centrifuged at 15,000 × *g* for 45 min. The bottom precipitate was the organelle component (F_O), and the supernatant was the soluble component (F_S). Different subcellular components were dried at 70°C to determine the Cd content according to the method described by Liu *et al.* (2022).

Statistics: The data were analyzed using *SPSS 19.0* software, and one-way analysis of variance (*ANOVA*) was used. *Student*'s *t*-test or *Duncan*'s multiple range test were used to compare the mean values, with significance set at $p<0.05$. *SigmaPlot 12.5* was used for graphic drawing.

Results

Phenotypic changes: Cd stress affected the growth of *S. trilobata* and its hybrid. After 15 d of Cd stress, necrotic spots appeared on the leaves of *S. trilobata*, and the young leaves showed obvious curling (Fig. 1).

Gas-exchange parameters and Chl: Under Cd stress, the net photosynthetic rate (P_N), g_s, and transpiration rate (E) of *S. trilobata* and the hybrid significantly decreased, while the intercellular CO_2 concentration (C_i) significantly increased. After 15 d of Cd stress, the P_N, g_s, and E of the hybrid under Cd stress were significantly higher than those of *S. trilobata*, with no significant difference in C_i. Under Cd stress, the P_N of *S. trilobata* and the hybrid decreased by 67.7 and 25.6%, respectively (Fig. 2*A*). The g_s decreased by 47.7 and 18.9%, respectively (Fig. 2*B*). The E decreased by 49.4 and 32.3%, respectively (Fig. 2*D*). The C_i increased by 17.1 and 14.6% (Fig. 2*C*), respectively. Under Cd stress, the Chl content of *S. trilobata* and the hybrid significantly decreased, while the Chl *a*/*b* increased. The Chl content of *S. trilobata* and the hybrid decreased by 43.2 and 25.2%, respectively (Fig. 2*E*). The Chl *a*/*b* increased by 12.2 and 4.6%, respectively (Fig. 2*F*). Under Cd stress, the Chl content of the hybrid was significantly higher than that of *S. trilobata*, while the Chl *a*/*b* was lower than that of *S. trilobata*.

Fig. 1. Effects of Cd stress on the phenotype of *Sphagneticola trilobata* and hybrid. Control – Hoagland nutrient solution. Cd – Hoagland nutrient solution containing 180 µM Cd.

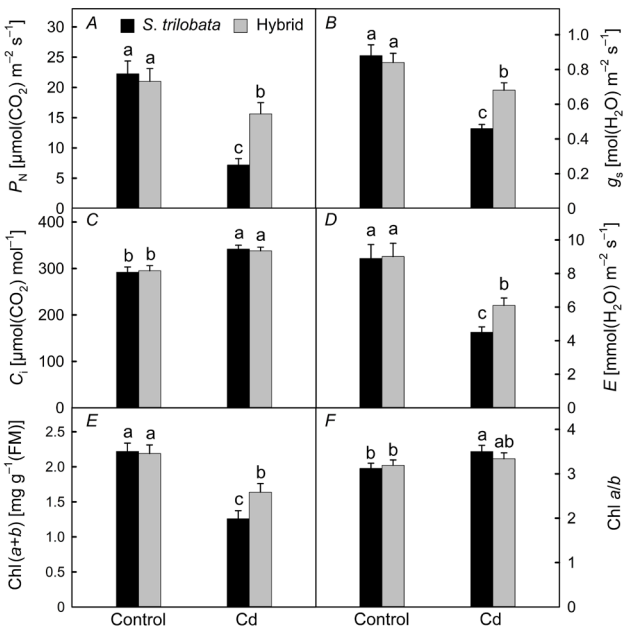

Fig. 2. Effects of Cd stress on gas-exchange parameters and photosynthetic pigments of *Sphagneticola trilobata* and the hybrid. (*A*) Net photosynthetic rate (P_N), (*B*) stomatal conductance (g_s), (*C*) intercellular CO_2 concentration (C_i), (*D*) transpiration rate (E), (*E*) chlorophyll (*a*+*b*) content [Chl (*a*+*b*)], (*F*) chlorophyll *a*/*b* (Chl *a*/*b*). Data are means of five replicates ± SE. Bars with *different letters* indicate significant differences between treatments at $p<0.05$ by *Duncan*'s multiple range test. Control – Hoagland nutrient solution. Cd – Hoagland nutrient solution containing 180 µM Cd.

Chl fluorescence *a* parameters: Cd stress significantly reduced the F_v/F_m, ETR, and q_P, and significantly increased q_N of leaves in *S. trilobata* and the hybrid. After 15 d of Cd stress, the F_v/F_m, ETR, and q_P of the hybrid leaves were significantly higher than those of *S. trilobata*, while q_N was significantly lower. Under Cd stress, the F_v/F_m of *S. trilobata* and the hybrid decreased by 41.9 and 18.6%, respectively (Fig. 3*A*). The ETR decreased by 51.8 and 29.1%, respectively (Fig. 3*B*). The q_P decreased by 39.4 and 18.8%, respectively (Fig. 3*C*). The q_N increased by 52.9 and 26.9%, respectively (Fig. 3*D*).

ROS, MDA, electrolyte leakage, and antioxidant enzyme activity: DAB and NBT staining, respectively, showed the accumulation of H_2O_2 and $O_2^{\bullet-}$. Cd stress led to an increase in the accumulation of ROS in the leaves of *S. trilobata* and the hybrid. After 15 d of Cd stress, the accumulation of H_2O_2 and $O_2^{\bullet-}$ in the leaves of *S. trilobata* was higher than that in the hybrid (Fig. 4*A*,*B*). Cd stress led to a significant increase in the content of MDA in *S. trilobata* and the hybrid, with the MDA content in *S. trilobata* being significantly higher than that in the hybrid. Under Cd stress, the content of MDA in *S. trilobata* and the hybrid increased by 114.9 and 68.8%,

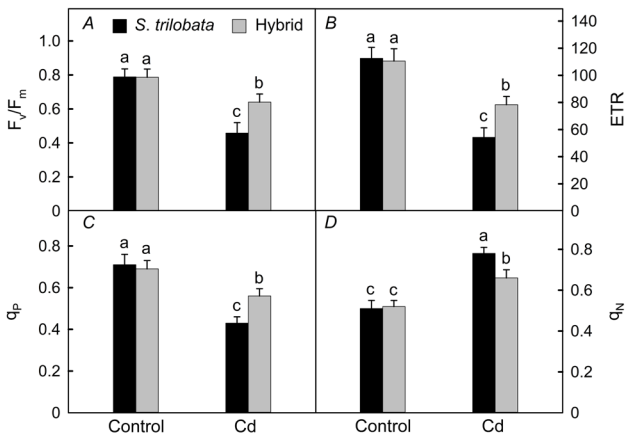

Fig. 3. Effects of Cd stress on Chl fluorescence parameters of *Sphagneticola trilobata* and hybrid. (*A*) Maximal quantum yield of PSII photochemistry (F_v/F_m), (*B*) electron transport rate (ETR), (*C*) photochemical quenching coefficient (q_P), (*D*) nonphotochemical quenching coefficient (q_N). Data are means of five replicates ± SE. Bars with *different letters* indicate significant differences between treatments at $p<0.05$ by *Duncan*'s multiple range test. Control – Hoagland nutrient solution. Cd – Hoagland nutrient solution containing 180 μM Cd.

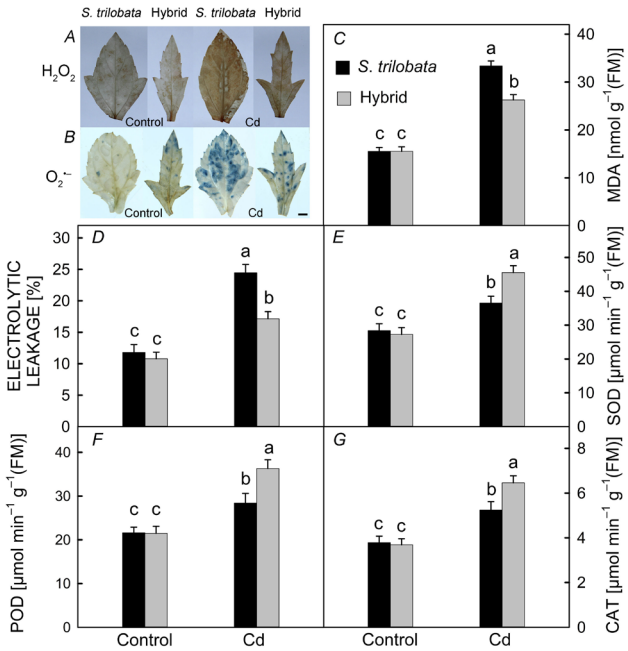

Fig. 4. Effects of Cd stress on ROS, MDA, electrolyte leakage, and antioxidant enzyme activity of *Sphagneticola trilobata* and hybrid. (*A*) H_2O_2, (*B*) $O_2^{\bullet-}$, (*C*) malondialdehyde (MDA), (*D*) electrolyte leakage, (*E*) superoxide dismutase (SOD), (*F*) peroxidase (POD), (*G*) catalase (CAT). Data are the means of five replicates ± SE. Bars with *different letters* indicate significant differences between treatments at $p<0.05$ by *Duncan*'s multiple range test. Scale bar = 0.5 cm. Control – Hoagland nutrient solution. Cd – Hoagland nutrient solution containing 180 μM Cd.

respectively (Fig. 4*C*). Under Cd stress, the electrolyte leakage of *S. trilobata* and the hybrid leaves significantly increased, with an increase of 107.5% in *S. trilobata* and 58.9% in the hybrid leaves. The electrolyte leakage of *S. trilobata* was significantly higher than that of the hybrid (Fig. 4*D*). Under Cd stress, the SOD, POD, and CAT activities of *S. trilobata* and the hybrid significantly increased. The SOD, POD, and CAT activities of the hybrid were significantly higher than those of *S. trilobata*. Under Cd stress, the SOD activity of *S. trilobata* and the hybrid increased by 28.7 and 67.1%, respectively; POD activity increased by 31.5 and 68.8%, respectively; and CAT activity increased by 38.4 and 75.1%, respectively (Fig. 4*E–G*).

Cd accumulation and translocation factor: After 15 d of Cd stress, the Cd concentration in total plant of the hybrid was significantly higher than that of *S. trilobata*. The Cd concentration in the roots of *S. trilobata* was significantly higher than that of the hybrid, and the Cd concentration in the stems and leaves of the hybrid was significantly higher than that of *S. trilobata* (Fig. 5*A*). The Cd translocation factor of the hybrid was significantly higher than that of *S. trilobata* (Fig. 5*B*). Indicating that the hybrid absorbed more Cd and transferred more Cd to the stems and leaves.

Different chemical forms of Cd: The chemical form of Cd in plants determines toxicity of Cd. The chemical form of Cd extracted with 80% ethanol (F_E) is the most toxic to plants, followed by the one extracted with deionized water (F_W). The chemical form of Cd extracted with 1 M NaCl (F_{NaCl}), 2% acetic acid (F_{HAc}), and 0.6 M HCl (F_{HCl}) are considered as detoxifying forms for plants. After 15 d of Cd stress, the proportion of F_E and F_W in the hybrid was relatively lower, at 45.8%, while it was relatively higher in *S. trilobata*, at 57.3%. The proportion of F_{NaCl}, F_{Hac}, and F_{HCl} in hybrid was relatively higher, accounting for 54.2%, while it was relatively lower in *S. trilobata*, accounting for 42.7% (Fig. 5*C*).

Cd in subcellular distributions: Accumulating Cd in the cell wall effectively reduces the toxicity of Cd to cells. After 15 d of Cd stress, the proportion of Cd in the cell wall of *S. trilobata* was 61.4%, which was lower than the 73.8% of the hybrid. The proportion of Cd in the soluble fraction and organelles of *S. trilobata* was 29.1 and 9.5%, respectively. The proportion of Cd in the soluble fraction and organelles of the hybrid was 19.5 and 6.7%, respectively (Fig. 5*D*). The results indicate that the detoxification ability of the hybrid is stronger than that of *S. trilobata*.

Discussion

Cd, as a non-essential element for plants, mainly inhibits their growth and development due to its toxicity. Cd is easily absorbed and transferred by plant roots due to its high mobility. Prolonged Cd stress leads to leaf necrosis, curling, yellowing, and even whole plant death (Al-Obaidi *et al.* 2024). Phenotypic changes in plants are commonly used as indicators of their tolerance to Cd stress (Wu *et al.* 2022). In this study, the growth of both *S. trilobata* and its hybrid was affected by Cd stress, and consistent results

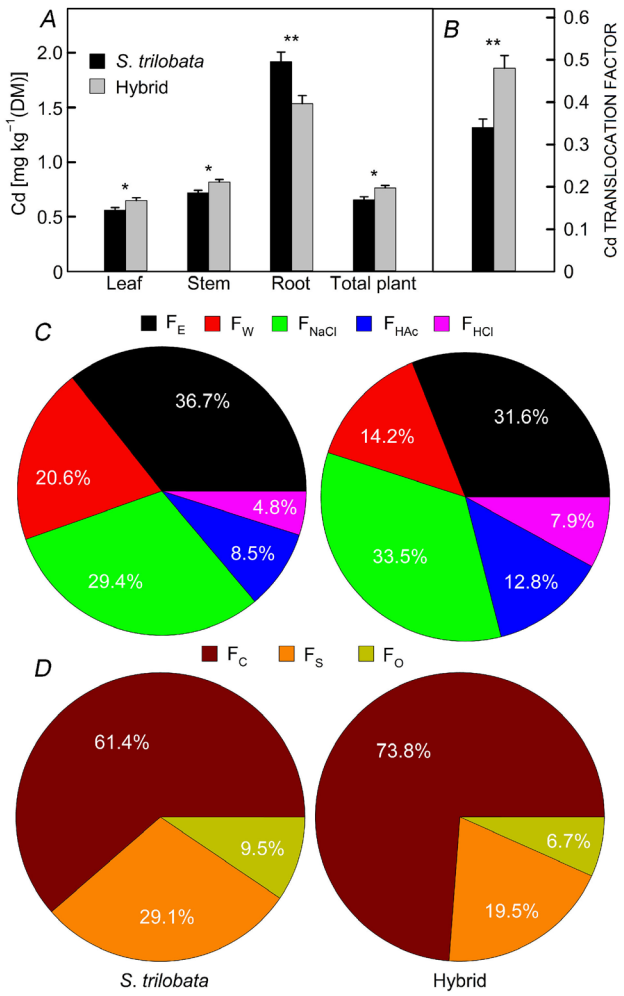

Fig. 5. Accumulation of Cd, different chemical forms of Cd and Cd in subcellular distributions of *Sphagneticola trilobata* and hybrid under Cd stress. (*A*) Cd concentration, (*B*) Cd translocation factor, (*C*) chemical forms of Cd, (*D*) Cd in subcellular distributions. Data are means of five replicates ± SE. * indicate significant difference at $p<0.05$ by *Student*'s *t*-test. *$P<0.05$; **$P<0.01$. F_E – Cd extracted by 80% ethanol. F_W – Cd extracted by deionized water. F_{NaCl} – Cd extracted by 1 M NaCl. F_{HAc} – Cd extracted by 2% acetic acid. F_{HCl} – Cd extracted by 0.6 M HCl. F_C – Cd concentration in cell wall fraction. F_S – Cd concentration in soluble fraction. F_O – Cd concentration in organelle fraction.

were found in *Nicotiana benthamiana* (Su *et al.* 2020). This study found that after 15 d of Cd stress, the leaves of *S. trilobata* curled and necrotic spots appeared. The hybrid showed stronger tolerance to Cd than *S. trilobata*, indicating that the hybridization between the invasive *S. trilobata* and the native species produced a more adaptable hybrid.

Photosynthesis is the foundation of plant growth, and its changes under Cd stress reflect the degree of damage to plants. Cd stress inhibits plant photosynthesis, which is one of the reasons for hindered plant growth (Zulfiqar *et al.* 2024). This study found that under Cd stress, the P_N and Chl content of *S. trilobata* and the hybrid significantly decreased, indicating that Cd stress caused damage to plants (Su *et al.* 2020). This is related to the dissolution of thylakoid membranes in plant leaf chloroplasts under Cd stress, which hinders Chl synthesis (Al-Obaidi *et al.* 2024). The P_N of the hybrid under Cd stress was significantly higher than that of *S. trilobata*. The F_v/F_m reflects the health status of the plant, and its value significantly decreases under stress (Giordano *et al.* 2025). In this study, the trend of changes in the F_v/F_m in the hybrid and *S. trilobata* leaves was consistent with the P_N, indicating that *S. trilobata* was more severely damaged by Cd stress and the hybrid showed stronger resistance to Cd stress. Previous studies found that enhanced Cd adaptability improved the P_N and F_v/F_m of plant leaves (Su *et al.* 2020, Wu and Lu 2024).

Cd, as a harmful element, causes negative effects on various physiological and biochemical processes in plants. Under Cd stress, a large amount of ROS accumulates, leading to oxidative stress (Muhammad *et al.* 2019). MDA, as a product of membrane lipid peroxidation, reflects damage to plant cell membrane lipids. Cell membrane damage leads to an increase in electrolyte leakage. Under Cd stress, the stronger the plant's resistance, the lower the MDA content and electrolyte leakage (Zulfiqar *et al.* 2024). In this study, the MDA content and electrolyte leakage of the hybrid were significantly lower than those of *S. trilobata*, indicating a stronger tolerance of the hybrid to Cd stress. Antioxidant enzymes clear excess ROS in plant cells, and Cd stress causes an increase in antioxidant enzyme activity. Strong antioxidant enzyme activity reduces oxidative stress caused by Cd stress (Zuo *et al.* 2023). In this study, the antioxidant enzyme activity of the hybrid was significantly higher than that of *S. trilobata*, which is consistent with the results of ROS accumulation, MDA content, and electrolyte leakage. Previous research has also found that plants with stronger antioxidant enzyme activity under Cd stress showed stronger adaptability (Zuo *et al.* 2023).

Under Cd stress, the Cd concentration in the leaves and stems of the hybrid was significantly higher than that of *S. trilobata*, and the Cd concentration in the roots was significantly lower than that of *S. trilobata*. Compared with *S. trilobata*, the Cd translocation factor of the hybrid was significantly higher, indicating that the transport capacity of Cd in the hybrid is stronger than that of *S. trilobata* (Al-Obaidi *et al.* 2024). The Cd concentration of the total plant in the hybrid was significantly higher than that of *S. trilobata*, indicating that the ability to absorb Cd of the hybrid is stronger than that of *S. trilobata*, suggesting that the adaptation of the hybrid to Cd stress is not through reducing the Cd content in the body, but through a stronger detoxification ability (Panda *et al.* 2025). The chemical form of Cd is related to its migration ability and toxicity in plant organs. Deionized water- and 80% ethanol-extracted Cd are more toxic, while 1 M NaCl-, 0.6 M HCl-, and 2% acetic acid-extracted Cd are the main detoxification forms in plants (Zhang *et al.* 2019). In this study, the proportion of deionized water- and 80% ethanol-extracted Cd in the leaves of *S. trilobata* was higher than that of the hybrid, while the proportion of 1 M NaCl-, 0.6 M HCl-, and 2% acetic acid-extracted Cd in the leaves of the hybrid was higher than that of *S. trilobata*.

In addition, the deposition of Cd in the cell wall can reduce the toxicity of Cd in cells, and resistance will be enhanced by depositing more Cd in the cell wall (Zhang *et al.* 2019). The results of this study showed that the proportion of Cd in the cell wall of the hybrid was higher than that of *S. trilobata* indicating that the detoxification ability of the hybrid was stronger than that of *S. trilobata* under Cd stress.

Conclusions: The tolerance of the hybrid to Cd stress was stronger than that of the invasive species *S. trilobata*. Under Cd stress, the hybrid accumulated more Cd, and more Cd was transformed into low-toxicity forms and deposited on the cell wall, with stronger detoxification ability and antioxidant enzyme activity than *S. trilobata*. The accumulation of ROS, MDA content, and electrolyte leakage of the hybrid were lower than those of *S. trilobata*, resulting in the photosynthetic capacity of the hybrid being stronger than that of *S. trilobata*. The results in this study suggest that the hybridization of *S. trilobata* with native species enhances the Cd stress adaptability of the hybrid. From a genetic perspective, this may be a genetic invasion method of invasive plants, which involves crossing with native species to form hybrid species, thereby spreading the genes of the invasive species (Xia *et al.* 2015). Further, molecular targets and regulatory pathways underlying the response of the hybrid and *S. trilobata* to Cd stress require investigation. This study found that the Cd translocation factor of the hybrid was significantly higher than that of *S. trilobata*. In Cd-contaminated areas, the hybrid may be used for phytoremediation.

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