Physiological and Growth Responses of Solanum nigrum L. var. flavovirens Seedlings to Cadmium Stress: Postprint

Authors: Zhou Jiao, Pan Yuanzhi, Zhao Yin, Liu Sili, Jia Yin, Jiang Beibei, Wu Mengxi, Zhang Lu, Xu Qian, Wang Kailu, Zeng Yong

Date: 2021-04-29T00:00:00+00:00

Abstract

Solanum diphyllum is a small shrub in the Solanaceae family, and existing research on its response to cadmium (Cd) is scarce. This study employed a hydroponic control experiment to investigate the physiological growth responses and tolerance characteristics of Solanum diphyllum seedlings to heavy metal Cd stress by measuring growth, tolerance index (TI), Cd accumulation characteristics, superoxide dismutase (SOD) activity, osmotic adjustment substances, and photosynthetic pigments at different stress time points (0, 0.5, 1, 3, 7, 19 d) under 160 M Cd stress. The results showed that Cd had no significant effect on seedling growth during 0-3 d of stress, but exhibited significant inhibition during 3-19 d, with toxicity intensifying as stress duration prolonged. Compared with the control group, Cd stress reduced seedling biomass by 13.28%-62.40%. The maximum Cd accumulation in seedlings was 60.14 g $\cdot$ plant^{-1}, with aboveground Cd accumulation accounting for 15.46%-35.24% of the whole plant. Malondialdehyde (MDA) content showed an increasing trend, with the maximum increase in root MDA content reaching 5.25 times that of the control. SOD activity, free proline, soluble sugar, chlorophyll a, chlorophyll b, and carotenoid contents all exhibited an initial increase followed by a decrease. The tolerance index (TI) of seedlings decreased with prolonged stress duration, with a minimum value of 0.64. The study indicates that Solanum diphyllum seedlings possess a certain degree of tolerance to high-concentration Cd stress in the short term and represent a potential Cd-tolerant plant species. Solanum diphyllum may alleviate Cd toxicity through osmotic adjustment and root immobilization. This study provides a theoretical basis for identifying suitable phytoremediation materials.

Full Text

Preamble

Physiological and Growth Responses of Solanum diphyllum Seedlings to Cadmium Stress

Jiao Zhou, Yuanzhi Pan*, Yin Zhao, Sili Liu, Yin Jia, Beibei Jiang, Mengxi Wu, Lu Zhang, Qian Xu, Kailu Wang, Yong Zeng
College of Landscape Architecture, Sichuan Agricultural University, Chengdu 611130, China

Abstract

Solanum diphyllum is a small shrub belonging to the Solanaceae family, yet its cadmium (Cd) tolerance remains poorly understood. This study employed a hydroponic control experiment to investigate the physiological and growth responses and tolerance characteristics of S. diphyllum seedlings under 160 M Cd stress. We measured growth parameters, tolerance index (TI), Cd accumulation characteristics, superoxide dismutase (SOD) activity, osmotic adjustment substances, and photosynthetic pigments at different stress time points (0, 0.5, 1, 3, 7, and 19 days). The results showed that Cd had no significant effect on seedling growth during the first 3 days of stress, but caused obvious inhibition from 3 to 19 days, with toxicity intensifying as stress duration increased. Compared with the control group, Cd stress reduced S. diphyllum seedling biomass by 13.28%-62.40%. The maximum Cd accumulation per plant was 60.14 g$\cdot$plant^{-1}, with shoot Cd accumulation accounting for 15.46%-35.24% of the whole plant. Malondialdehyde (MDA) content showed an upward trend, with root MDA content reaching a maximum increase of 5.25 times that of the control. SOD activity, free proline, soluble sugar, chlorophyll a, chlorophyll b, and carotenoid contents all exhibited a trend of initial increase followed by decrease. The tolerance index (TI) of S. diphyllum seedlings decreased with prolonged stress, reaching a minimum value of 0.64. These findings indicate that S. diphyllum seedlings possess a certain degree of tolerance to high-concentration Cd stress in the short term, suggesting it is a potential Cd-tolerant plant. S. diphyllum may alleviate Cd toxicity through osmotic adjustment and root sequestration. This study provides a theoretical basis for identifying suitable plant materials for phytoremediation.

Keywords: Solanum diphyllum, cadmium, physiological growth, osmoregulation, photosynthetic pigment

Introduction

Rapid population growth, chemical fertilizer abuse, and accelerated urbanization have exacerbated soil heavy metal pollution in China, with cadmium (Cd) ranking first among heavy metal pollutants at a detection rate of 7.0% in sampling sites (Ministry of Environmental Protection and Ministry of Land and Resources, 2014). As a non-essential element for organisms, Cd is highly mobile, toxic, and non-degradable. Excessive Cd accumulation causes severe damage to plants, with morphological changes being the most direct manifestation of Cd toxicity (Liu et al., 2015). Studies have shown that roots serve as the first barrier against soil Cd entry into plants, and Cd toxicity affects root elongation and development, hindering nutrient absorption (Bočová et al., 2012). Cd translocation from roots to shoots damages photosynthetic reaction centers, degrades photosynthetic pigments, and reduces photosynthetic efficiency (Parmar et al., 2013), thereby affecting leaf photosynthesis and decreasing plant biomass. Cd stress also induces oxidative stress in plants, causing membrane lipid peroxidation (Luo et al., 2019), altering membrane integrity, and disrupting cellular water balance. Plants employ multiple response mechanisms to alleviate Cd toxicity, among which osmotic adjustment represents a crucial strategy. Research by Majumdar et al. (2018) and Semida et al. (2018) has demonstrated that Cd stress promotes the synthesis of free proline, soluble sugars, and other substances involved in osmotic adjustment to maintain membrane integrity, preserve cellular water potential, and resist toxicity. Moreover, since heavy metal effects on plants are often continuous processes, studying the temporal dynamics of plant responses to heavy metal stress holds significant importance.

Phytoremediation technology is widely recognized as an environmentally friendly approach for treating soil heavy metal pollution, with hyperaccumulator plants playing a vital role (Ali et al., 2013). The classical definition of Cd hyperaccumulator plants (Barker & Brooks, 1989) includes: (1) normal growth under high Cd concentration; (2) shoot (or leaf) Cd content exceeding 100 $mg \cdot kg^{-1}$ DW; (3) enrichment coefficient (shoot Cd content/soil Cd content) > 1; and (4) translocation coefficient (shoot Cd content/root Cd content) $>$ 1. Although plant remediation capacity depends on both biomass and heavy metal content, heavy metal content often receives more attention while biomass accumulation tends to be overlooked. Currently discovered hyperaccumulators generally suffer from low biomass and slow growth (Singh et al., 2016), which severely hinders the development of phytoremediation technology. In practical applications, plants with low heavy metal content but high biomass may achieve greater phytoremediation efficiency than those with high heavy metal content but low biomass (Wu et al., 2010). Therefore, identifying suitable plant materials represents a key challenge for phytoremediation applications.

Both Solanum nigrum (Xu et al., 2012) and S. photeinocarpum (Zhang et al., 2011), belonging to Solanaceae, have been confirmed as Cd hyperaccumulators, though neither possesses biomass advantages. Solanum diphyllum shares the same family and genus as S. nigrum and S. photeinocarpum (Hariri & Irsyam, 2018) and exhibits strong adaptability. Currently used primarily in clinical applications, the entire plant can be used medicinally for dispersing blood stasis, reducing swelling, and clearing heat and toxins. As a small shrub, S. diphyllum offers advantages in both biomass and growth rate. However, current research on S. diphyllum has focused mainly on pharmacology (El-Sayed et al., 2009), karyotype identification (Xu et al., 2016), and plant invasion (Pandey et al., 2018; Wang et al., 2019), with few studies examining its heavy metal Cd response characteristics (Singh et al., 2019; Wu et al., 2020). To explore whether S. diphyllum has potential for phytoremediation applications, this study investigated the physiological and growth responses and Cd tolerance of S. diphyllum under high-concentration Cd stress using hydroponic methods, aiming to provide new insights and theoretical basis for selecting plant materials for soil Cd pollution remediation.

1.1 Experimental Materials

Solanum diphyllum seeds were purchased from a horticultural seed company. Uniform, plump seeds were selected and surface-sterilized by soaking in 1.0% NaClO solution for 20 minutes, then rinsed with deionized water. The sterilized seeds were placed in an illumination incubator in mid-February 2019 for germination, with a photoperiod of 14 h light/10 h dark. After developing four true leaves, uniformly growing seedlings were selected as experimental materials and transplanted in late March 2019.

1.2 Experimental Design

The hydroponic experiment was conducted from March to May 2019 in a greenhouse at the Chengdu Campus of Sichuan Agricultural University. Continuous aeration was maintained in the hydroponic tanks throughout the experiment to prevent root rot. The average temperature in the greenhouse was (29.02 ± 8.16) °C, and the average relative humidity was 52.87% ± 10.04%. Seedlings were transplanted into full-strength Hoagland nutrient solution (macronutrients: $Ca(NO_3)_2 \cdot 4H_2O$ 945 $mg \cdot L^{-1}$, KNO_3 607 $mg \cdot L^{-1}$, $NH_4H_2PO_4$ 115 $mg \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$ 493 $mg \cdot L^{-1}$; micronutrients: Na_2Fe-EDTA 30-40 $mg \cdot L^{-1}$, H_3BO_3 2.86 $mg \cdot L^{-1}$, $MnSO_4$ 2.13 $mg \cdot L^{-1}$, $ZnSO_4$ 0.22 $mg \cdot L^{-1}$, $CuSO_4$ 0.08 $mg \cdot L^{-1}$, $(NH_4)_6Mo_7O_{24}$ 0.02 $mg \cdot L^{-1}$) and cultured for 16 days, with nutrient solution replaced every 3 days. Healthy, uniformly growing S. diphyllum plants were selected for Cd stress treatment. Based on preliminary experiments, the Cd stress concentration was set at 160 μM (added as $CdCl_2 \cdot 2.5H_2O$), with no Cd addition as the control (CK). Both stress and control groups had three replicates. During Cd treatment, the Cd concentration in the hydroponic tanks was maintained constant, with plants arranged in a randomized block design. Plants were randomly sampled from both stress and control groups at different time points (0, 0.5, 1, 3, 7, and 19 days). After harvest, plants were immersed in 20 mM Na_2-EDTA solution for 20 minutes to remove surface-adsorbed Cd, then rinsed with deionized water.

1.3.1 Growth Indices and Tolerance Coefficient Determination

At each sampling time point, three S. diphyllum plants were randomly selected from both the stress (Cd) and control (CK) groups to measure main root length, plant height, stem diameter, and leaf number. Each plant sample was separated into roots, stems, and leaves (shoots included stems and leaves), then ovendried at 105 °C for 30 minutes and at 80 °C to constant weight for biomass measurement.

The tolerance index (TI) of S. diphyllum was calculated according to Feng et al. (2018) and Wu et al. (2018):

$$TI = (LH + WR + WS + WL)/4$$

where TI is the Cd tolerance coefficient of S. diphyllum; LH, WR, WS, and WL are the ratios of plant height, root biomass, stem biomass, and leaf biomass between Cd-stressed and control groups, respectively.

1.3.2 Cd Content and Accumulation Determination

Dried plant samples were ground and sieved through a 1 mm mesh. Approximately 0.2000 g of sample was placed in a digestion vessel and digested using HCl-HNO_3 (v:v = 5:4). After overnight storage, samples were microwave-digested (CEM MARS-5) and Cd content was determined by ICP. Cd accumulation was calculated as the product of Cd content and biomass.

1.3.3 Physiological and Biochemical Indices Determination

At each sampling time point, one plant component sample was collected from both stress and control groups. Leaf and root samples were rinsed with deionized water, with each sample consisting of three randomly selected S. diphyllum plants and three replicates. Samples were immediately frozen in liquid nitrogen. Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium reduction method. Malondialdehyde (MDA) content was determined by the thiobarbituric acid method. Free proline content was measured using the sulfosalicylic acid method. Soluble sugar content was determined by the anthrone colorimetric method. Chlorophyll a, chlorophyll b, and carotenoid contents were measured using the ethanol-acetone extraction method (Wang, 2014).

1.3.4 Material Disposal

After sampling for all indices, remaining plant materials were collected at the laboratory heavy metal waste disposal point and sent to a professional company for centralized treatment.

1.3.5 Data Processing

Data processing and statistical analysis were performed using SPSS 20.0. One-way ANOVA and Duncan' s multiple comparison tests were used to examine the effects of Cd stress duration on S. diphyllum morphology, Cd content, Cd accumulation, SOD activity, MDA content, free proline content, soluble sugar content, chlorophyll a content, chlorophyll b content, and carotenoid content. Independent-samples t-tests were used to compare differences between stress and control groups. Pearson correlation analysis (two-tailed test) was conducted. The significance level was set at $P < 0.05$. Graphs were prepared using Microsoft Excel 2019.

Results

2.1 Effects of Cd Stress on Morphology and Tolerance Index of S. diphyllum Seedlings

Compared with the control (CK), main root length, plant height, stem diameter, and leaf number of Cd-stressed S. diphyllum seedlings showed slight increases during the first day of stress. As stress duration extended, these parameters became lower than the control, though only plant height differences were significant ($P > 0.05$ for other indices). The tolerance index (TI) gradually decreased with increasing Cd stress duration, reaching a minimum value of 0.64 at 19 days . Additionally, Cd stress significantly reduced biomass accumulation in roots and stems during 7-19 days and in leaves at 19 days ($P < 0.05$). Compared with the control, root, stem, and leaf biomass decreased by 44.75%-53.81%, 26.94%-46.58%, and 3.37%-26.10%, respectively, with roots suffering the most severe damage. However, Cd stress did not significantly alter the proportion of each component' s biomass in the whole plant; both stress and control groups showed the same biomass ranking of leaf > stem > root [Figure 1: see original paper]. One-way analysis of root/shoot ratio revealed stable values between stress and control groups during 0–3 days, but a significant decreasing trend in the stress group during 3–19 days ($P < 0.05$), while the control group showed an increasing trend without significant differences ($P > 0.05$). Intergroup comparison showed that Cd stress reduced the root/shoot ratio during 3-19 days ($P < 0.05$) [Figure 1: see original paper]. These results indicate that high-concentration Cd stress inhibits the morphological growth of S. diphyllum seedlings over extended periods.

2.2 Effects of Cd Stress on Cd Content and Accumulation in S. diphyllum Seedlings

One-way analysis showed that Cd content in roots and shoots of Cd-stressed S. diphyllum seedlings increased significantly with stress duration ($P < 0.05$). Cd content ranged from 4.04-61.29 $\mathrm{mg \cdot kg^{-1}}$ in roots and 0.44-3.75 $\mathrm{mg \cdot kg^{-1}}$ in shoots [Figure 2: see original paper]. Cd accumulation also increased significantly with stress duration ($P < 0.05$), with root and shoot Cd accumulation accounting for 64.76%-84.54% and 15.46%-35.24% of whole-plant accumulation, respectively. At 19 days, root and shoot Cd accumulation increased by 29.39-fold and 23.45-fold compared with 0.5 days ($P < 0.05$) . These results demonstrate that substantial Cd was sequestered in the roots of S. diphyllum seedlings.

2.3 Effects of Cd Stress on SOD Activity in S. diphyllum Seedlings

Cd stress caused significant differences in root and leaf SOD activity between stress and control groups ($P < 0.05$), with both root and leaf SOD activity lower than the control at 19 days. Compared with the control, Cd significantly increased root SOD activity during the first 7 days of stress ($P < 0.05$), with increases ranging from 13.93% to 64.60%, and root SOD was significantly higher than the control at 0.5 days ($P < 0.05$). Leaf SOD activity increased more slowly than root SOD, with significant differences from the control appearing after 3 days [Figure 3: see original paper]. These results indicate that S. diphyllum seedlings can respond to Cd stress by rapidly increasing SOD activity under high-concentration Cd stress.

2.4 Effects of Cd Stress on Osmotic Adjustment Substances in S. diphyllum Seedlings

Cd treatment caused significant differences in osmotic adjustment substances between stress and control groups in both roots and leaves ($P < 0.05$). Compared with the control, Cd stress significantly increased root MDA content ($P < 0.05$) by 26.90%-525.62%, with the maximum difference occurring at 19 days [FIGURE:4 A]. Leaf MDA content showed a significant increasing trend after 7 days of stress ($P < 0.05$) [FIGURE:4 B]. Cd stress also significantly increased free proline content ($P < 0.05$), with increases of 37.49%-252.47% in roots and 12.05%-103.26% in leaves. Leaf free proline content first fell below the control level at 19 days [FIGURE:4 C-D]. Similarly, Cd stress significantly increased soluble sugar content ($P < 0.05$), with the maximum difference between stress and control groups occurring at day 19 in roots and day 7 in leaves [FIGURE:4 E-F]. These results demonstrate that osmotic adjustment substances in S. diphyllum seedlings can actively respond to high-concentration Cd stress through moderate increases.

2.5 Effects of Cd Stress on Photosynthetic Pigment Content in S. diphyllum Seedlings

Chlorophyll a, chlorophyll b, and carotenoid contents in S. diphyllum seedlings showed a trend of initial increase followed by decrease. However, intergroup analysis revealed no significant differences in chlorophyll a between stress and control groups ($P > 0.05$), significant differences in chlorophyll b only at day 3 ($P < 0.05$), highly significant differences in carotenoids at day 7 ($P < 0.05$), and highly significant differences in chlorophyll a/b ratio at day 19 ($P < 0.05$) [Figure 5: see original paper]. These results indicate that high-concentration Cd stress disrupts photosynthetic pigment synthesis in S. diphyllum seedlings.

2.6 Pearson Correlation Analysis

Pearson correlation analysis revealed that Cd content in S. diphyllum seedlings was extremely significantly positively correlated with total biomass ($P < 0.01$), extremely significantly negatively correlated with total chlorophyll content and TI ($P < 0.01$), but not significantly correlated with SOD, MDA, soluble sugar, or free proline ($P > 0.05$) . These findings suggest that total biomass, total chlorophyll content, and TI strongly influence Cd accumulation characteristics in S. diphyllum seedlings.

Discussion

3.1 Effects of Cd Stress on Physiological Growth of S. diphyllum Seedlings

Cd toxicity in plants depends on species and stress concentration, exhibiting temporal effects (Liu et al., 2020). Studies have shown that Cd stress exceeding plant tolerance thresholds significantly affects plant growth and can cause plant death, making morphological growth a key indicator for evaluating stress tolerance (Jia et al., 2020). In this experiment, Cd toxicity symptoms in S. diphyllum seedlings appeared after 3 days of stress, with only plant height showing significant reduction ($P < 0.05$). Root and leaf biomass decreased substantially [Figure 1: see original paper], consistent with findings on S. nigrum (Liu et al., 2015) and Primula forbesii (Jia et al., 2020). This may occur because proteins, polysaccharides, hydroxyl (-OH), and carboxyl (-COOH) groups in root cell walls readily coordinate with Cd. The high Cd distribution in roots inhibits root enzyme activity, cell division, and nutrient absorption, leading to root cell death (Bočová et al., 2012) and resulting in severe root damage such as stunted growth, yellowing, and rot. Subsequently, Cd translocates from roots to shoots, binding to sulfhydryl (-SH) groups of chloroplast enzymes, destroying chloroplast structure, and causing leaf yellowing and abscission . The substantial reduction in normally photosynthesizing leaves directly affects plant biomass accumulation. Interestingly, during the first day of stress, main root length, plant height, stem diameter, leaf number, and biomass in the stress group increased slightly compared with the control [TABLE:1, FIGURE:1], though not significantly ($P > 0.05$). This may result from two mechanisms: first, a “protective mechanism” in S. diphyllum that enhances various physiological reactions within a certain range to resist Cd toxicity, as demonstrated in Atractylodes lancea (Sun et al., 2018) and P. forbesii (Jia et al., 2020) where short-term Cd stress enhanced photosynthesis to alleviate toxicity; second, the “hormesis” effect where limited Cd absorption in short timeframes may promote plant growth (Carvalho et al., 2020).

Cd stress significantly reduced the root/shoot ratio of S. diphyllum seedlings during 3-19 days [FIGURE:1 D], indicating that root biomass decreased more substantially than shoot biomass after prolonged high-concentration Cd stress, suggesting roots are more sensitive to Cd. This result aligns with studies on the hyperaccumulator S. nigrum (Guo et al., 2009) but contradicts findings on Trifolium repens (Liu et al., 2015), which suggested that increased root/shoot ratio under Cd stress benefits nutrient acquisition for growth maintenance. Additionally, we determined the tolerance index (TI) of S. diphyllum, with a minimum value of 0.64 at 19 days . Lux et al. (2004) defined plants with TI > 0.60 under Cd stress as highly tolerant, indicating that S. diphyllum seedlings exhibit high tolerance to high-concentration Cd stress throughout the experimental period.

3.2 Effects of Cd Stress on Cd Uptake and Distribution in S. diphyllum Seedlings

Our results demonstrate that stress duration significantly affects Cd uptake in S. diphyllum seedlings, with both Cd content and accumulation increasing over time ($P < 0.05$). Cd distribution differed markedly between roots and shoots, with maximum Cd contents of 11.69 $\text{mg} \cdot \text{kg}^{-1}$ in whole plants and 3.75 $\text{mg} \cdot \text{kg}^{-1}$ in shoots. Due to experimental variations, Cd accumulation differs among plant species. Compared with other plants, S. diphyllum Cd content is lower than that of Cd hyperaccumulators such as Lonicera japonica, which reached 300 $\text{mg} \cdot \text{kg}^{-1}$ in shoots under 25 $\text{mg} \cdot \text{kg}^{-1}$ Cd stress (Liu et al., 2013), and Picris divaricata, which reached 270 $\text{mg} \cdot \text{kg}^{-1}$ in shoots under 10 M Cd stress (Tang et al., 2010). However, it is higher than that of other solanaceous plants, such as high-Cd-accumulating tomato cultivars which reached 3.06 $\text{mg} \cdot \text{kg}^{-1}$ under 3 $\text{mg} \cdot \text{kg}^{-1}$ Cd stress (Yang et al., 2020).

Plant heavy metal accumulation depends on both tissue heavy metal content and biomass, representing an important indicator for evaluating plant suitability for phytoremediation applications and reflecting heavy metal extraction capacity. During the experimental period, Cd accumulation in S. diphyllum shoots gradually increased , which benefits pollutant removal in phytoremediation. The maximum Cd accumulation in S. diphyllum seedlings was 60.14 $\text{g} \cdot \text{plant}^{-1}$, higher than that of the solanaceous plant eggplant (S. melongena) (11 $\text{g} \cdot \text{plant}^{-1}$) and the hyperaccumulator S. nigrum (46 $\text{g} \cdot \text{plant}^{-1}$) reported by Sun et al. (2007), indicating strong Cd accumulation capacity in S. diphyllum. This results from substantial biomass conferring high Cd accumulation, supported by Pearson correlation analysis showing an extremely significant positive correlation between Cd content and total biomass ($P < 0.01$) . As the primary organ directly contacting soil, roots can sequester heavy metals through chelation with metallothioneins (MTs), phytochelatins (PCs), or glutathione (GSH) to alleviate toxicity. In this experiment, roots represented the largest Cd accumulation site in S. diphyllum seedlings, similar to findings in P. forbesii (Jia et al., 2020) and rice (Srivastava et al., 2014), but contrasting with hyperaccumulators such as S. nigrum and S. photeinocarpum. This suggests that S. diphyllum cannot efficiently translocate large amounts of Cd to shoots but can sequester substantial Cd in roots, indicating that root sequestration may be one mechanism for alleviating Cd toxicity.

3.3 Effects of Cd Stress on SOD and Osmotic Adjustment Substances in S. diphyllum Seedlings

Generally, severe Cd stress damages plant cell structure. When cell membrane composition, structure, and permeability change, toxic substances outside the membrane can enter cells. Plants produce osmotic adjustment substances to resist stress, and when the antioxidant system balance is disrupted, excessive reactive oxygen species (ROS) accumulation exacerbates membrane lipid peroxidation and can cause cell death (Muradoglu et al., 2015). Malondialdehyde (MDA), a primary product of membrane lipid peroxidation, reflects the degree of peroxidation and can further aggravate membrane system damage (Luo et al., 2019). In this experiment, Cd stress significantly increased MDA content in both roots and leaves of S. diphyllum, indicating intensified membrane lipid peroxidation and membrane system damage that affects carbon metabolism pathways. This response resembles that of Polygonum hydropiper (Ge et al., 2020). The more rapid and significant increase in root MDA content may result from most Cd being sequestered in roots , where high Cd concentrations cause ROS production to exceed scavenging capacity.

SOD represents the first line of defense in plant antioxidant protection systems. Research shows that when plants face environmental stress, SOD catalyzes O_2^- dismutation to O_2 and H_2O_2, maintaining ROS balance within a certain range to adapt to Cd stress. Typically, SOD activity increases with stress intensity but has limited regulatory capacity. Under high-concentration Cd stress, SOD mitigation capacity becomes limited, possibly because Cd similarity to Mn, Cu, and Zn allows it to replace metal cofactors in Mn-SOD, Cu-SOD, and Zn-SOD, thereby reducing ROS scavenging ability. Additionally, Cd can bind to SOD -SH groups, destroying catalytic centers. In this experiment, Cd stress caused SOD activity to initially increase then decrease [Figure 3: see original paper], indicating that short-term Cd stress can induce increased SOD activity in S. diphyllum seedlings, alleviating Cd toxicity to some extent. However, prolonged stress exceeded the normal dismutation capacity of SOD for O_2^-, damaging membrane integrity and enzyme systems.

Free proline and soluble sugars are primary components for maintaining osmotic adjustment in plants (Zhang et al., 2020). Free proline can increase protein hydration and provide energy for stress responses. Soluble sugars form the basis for organic solute synthesis and energy sources. Their accumulation reduces cellular water potential, maintains turgor pressure and membrane integrity, and significantly enhances plant stress resistance. Previous studies have shown that proline seed soaking enhances Cd tolerance in cucumber (Semida et al., 2018), rice proline content increases with Cd stress (Majumdar et al., 2018), and soluble sugar content increases in Robinia pseudoacacia under Cd stress (Jia et al., 2017). However, plant osmotic adjustment capacity is also limited and decreases beyond certain thresholds. In this experiment, Cd stress significantly increased proline and soluble sugar contents in S. diphyllum, suggesting that S. diphyllum may alleviate adverse metabolic effects of Cd stress by increasing osmotic adjustment substance accumulation to maintain protoplasm colloid stability and membrane integrity. Their initial increase followed by decrease with stress duration may occur because severe Cd toxicity and intensified membrane lipid peroxidation in later stress stages cause membrane rupture, reducing proline and soluble sugar synthesis and gradually weakening the role of osmotic adjustment in alleviating Cd toxicity [Figure 4: see original paper].

3.4 Effects of Cd Stress on Photosynthetic Pigments in S. diphyllum Seedlings

Photosynthetic pigments form the basis for plant photosynthesis. Studies have shown that severe Cd stress can destroy chloroplast structure, affect photosynthetic pigment synthesis, and disrupt plant growth (Parmar et al., 2013), while moderate Cd stress may enhance photosynthesis (Sun et al., 2018; Jia et al., 2020). Compared with the control, chlorophyll a, b, and carotenoid contents increased to some extent at most time points except day 1, but only chlorophyll b at day 3 and carotenoids at day 7 showed significant differences [Figure 5: see original paper]. The increase in photosynthetic pigments may result from plant growth and development or from increased synthesis to meet higher energy demands for alleviating Cd toxicity, as photosynthesis directly provides organic matter accumulation. The decrease may occur because Cd stress obstructs calcium messenger-mediated abiotic stress signal transduction. Calmodulin (CAM) can convert Cd stress into plant perception signals, initiating downstream protein phosphorylation/dephosphorylation to regulate photomorphogenesis and other physiological processes. However, Cd^{2+} similarity to Ca^{2+} may allow Cd to compete for Ca carrier proteins and channels, disrupting downstream signal transduction and reducing chlorophyll synthesis. Cd binding to chlorophyllase also accelerates its decomposition. Additionally, the chlorophyll a/b ratio in the stress group increased significantly compared with the control at 19 days, indicating that chlorophyll b became more sensitive to Cd stress in leaves at this stage.

Conclusion

Under 160 M Cd stress, S. diphyllum seedlings showed increasing MDA content, Cd content, and Cd accumulation with prolonged stress duration, with roots being the primary site of Cd absorption. SOD activity, osmotic adjustment substances (free proline and soluble sugars), and photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) exhibited initial increases followed by decreases. Changes in physiological growth indices and TI collectively demonstrated strong Cd tolerance in S. diphyllum, with osmotic adjustment and root sequestration likely representing partial mechanisms for alleviating Cd toxicity. In summary, S. diphyllum can tolerate high-concentration 160 M Cd stress to a certain degree, making it a potential Cd-tolerant plant.

References

ALI H, KHAN E, SAJAD M, 2013. Phytoremediation of heavy metals-concepts and applications[J]. Chemosphere, 91(7): 869-881.

BOCOVA B, HUTTOVA J, LIPTAKOVA L, et al., 2012. Impact of short-term cadmium treatment on catalase and ascorbate peroxidase activities in barley root tips[J]. Biol Plant, 56(4): 724-728.

BARKER A, BROOKS R, 1989. Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and phytochemistry[J]. Biorecovery, 1(2): 81-126.

CARVALHO M, CASTRO P, AZEVEDO R, 2002. Hormesis in plants under Cd exposure: from toxic to beneficial element[J]. J Hazard Mater, 384(2): 121434-121443.

EL-SAYED M, MOHAMED A, HASSAN M, 2009. Cytotoxicity 3-O-(β-D-glucopyranosyl) etioline, a steroidal alkaloid from Solanum diphyllum L.[J]. Z Naturforsch C, 64(9-10): 644-649.

FENG JX, LIN YY, YANG Y, et al., 2018. Tolerance and bioaccumulation of Cd and Cu in Sesuvium portulacastrum[J]. Ecotoxicol Environ Safety, 147(8): 306-312.

GUO Z, WANG T, AO YS, 2009. Physiological responses of Solanmu nigrum L. seedling to cadmium stress[J]. J Agro-Environ Sci, 28(4): 755-760.

HARIRI M, IRSYAM A, 2018. Catatan tentang Solanum diphyllum L. (Solanaceae) ternaturalisasi di Pulau Jawa[J]. Al-Kauniyah, 11(1): 25-32.

JIA X, ZHAO YH, Liu T, et al., 2017. Leaf defense system of Robinia pseudoacacia L. seedlings exposed to 3 years of elevated atmospheric CO2 and Cd-contaminated soils[J]. Sci Total Envir, 605-606(10): 48-57.

JIA Y, LIU CL, LAN XY, et al., 2020. Effect of cadmium stress on the growth and physiological characteristics of Primula forbesii seedlings[J]. Acta Bot Boreal-Occident Sin, 40(3): 454-462.

LIU QQ, ZHANG YX, WANG YJ, et al., 2020. Quantitative proteomic analysis reveals complex regulatory and metabolic response of Iris lactea Pall. var. chinensis to cadmium toxicity[J]. J Hazard Mater, 400(7): 1-13.

LUX A, ANNA S, JANA O, et al., 2004. Differences in structure of adventitious roots in Salix clones with contrasting characteristics of cadmium accumulation and sensitivity[J]. Physiol Plant, 120(4): 537-545.

LIU SL, YANG RJ, MA MD, et al., 2015. Effects of cadmium stress on growth, cadmium content and nutrient distribution in Trifolium repens L. plants[J]. Acta Agric Nucl Sin, 29(3): 595-604.

LIU SL, YANG RJ, MA MD, et al, 2015. Effects of soil cadmium on growth and physiological characteristics of Solanum nigrum L. plants[J]. J Agro-Environ Sci, 34(2): 240-247.

LIU ZL, HE XY, CHEN W, 2013. Lonicera japonica Thunb. —A newly discovered Cd hyper-accumulator [J]. Ecol Environ Sci, 2013, 22(4): 666-670.

LUO Q, WU MX, LONG Y, et al., 2019. Changes in photosynthesis and Cd accumulation characteristic of Malva pusilla Smith under different concentrations of Cd[J]. Acta Agric Nucl Sin, 33(7): 1415-1422.

MAJUMDAR S, CHAKRABORTY B, KUNDU R, 2018. Comparative analysis of cadmium-induced stress responses by the aromatic and non-aromatic rice genotypes of West Bengal[J]. Environ Sci Pollut Res, 25(19): 18451-18461.

MURADOGLU F, GUNDOGDU M, ERCISLI S, et al., 2015. Cadmium toxicity affects chlorophyll a and b content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry[J]. BioRes, 48(1): 1-7.

MINISTRY of Environmental Protection of PRC, MINISTRY of Land and Resources of PRC, 2014. Bulletin of national survey on soil pollution[R]. Beijing: Ministry of Land and Resources of PRC, 2014(5): 10-11.

PANDEY A, PRADHEEP K, GUPTA R, 2018. Two-leaf nightshade (Solanum diphyllum L.) an addition to the flora of Delhi, India and weed risk assessment of the species[J]. Indian J Plant Genet Resour, 31(2): 164-168.

PARMAR P, KUMARI N, SHARMA V, 2013. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress[J]. Bot Stud, 54(1): 45-51.

SEMIDA WM, HEMIDA KA, RADY MM, 2018. Sequenced ascorbate-proline-glutathione seed treatment elevates cadmium tolerance in cucumber transplants[J]. Ecotoxicol Environ Safety, 154(6): 171-179.

SINGH P, SIGNGH PR, SINGH A, et al., 2019. In vitro evaluation of phytochemical and antibacterial activity of wild species of Solanum L[J]. Iosr-Jbb, 5(1): 81-87.

SINGH S, PARIHAR P, SINGH R, et al., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics[J]. Front Plant Sci, 6(2): 1143-1179.

SRIVASTAVA RK, PANDEY P, RAJPOOT R, et al., 2014. Cadmium and lead interactive effects on oxidative stress and antioxidative responses in rice seedlings[J]. Protoplasma, 251(5): 1-13.

SUN RL, ZHOU QX, SUN FH, et al., 2007. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, Solanum nigrum L.[J]. Environ Exp Bot, 60(3): 468-476.

SUN YD, CHAO JG, GU W, et al., 2018. Effect of cadmium stress on physiological and biochemical characteristics of Atractylodes lancea[J]. J Plant Physiol, 54(12): 1857-1864.

TANG YT, GUAN LJ, CHOU RL, et al., 2010. Antioxidative defense to cadmium in hyperaccumulator Picris divaricate V.[J]. Acta Ecologica Sin, 30(2):324-332.

WU CF, HUAN YM, LIU Y, et al., 2020. Effects of intercropping with two Solanum species on the growth and cadmium accumulation of Cyphomandra betacea seedlings[J]. Int J Phytoremediat, 5(12): 1-7.

WU FZ, YANG WQ, ZHANG J, et al., 2010. Cadmium accumulation and growth responses of a poplar (Populus deltoids× Populus nigra) in cadmium contaminated purple soil and alluvial soil[J]. J Hazard Mater, 177(1-3): 268-273.

WU MX, LUO Q, Liu SL, et al., 2018. Screening ornamental plants to identify potential Cd hyperaccumulators for bioremediation[J]. Ecotoxicol Environ Safety, 162(1): 35-41.

WANG QP, SHEN W, ZHANG K, et al., 2019. Hypochaeris albiflora and Solanum diphyllum, two newly naturalized plants in mainland China[J]. Guihaia, 39(12): 1724-1728.

WANG WL, 2014. New plant physiology experiment guide[M]. Beijing: Xinhua Publishing House: 35-136.

XU J, SUN JH, DU LG, et al., 2012. Comparative transcriptome analysis of cadmium responses in Solanum nigrum and Solanum torvum[J]. New Phytol, 196(1): 110-124.

XU XF, ZHANG HY, YUAN QH, et al., 2016. Studies on chromosome of three types of Solanum nigrum in China[J]. Guihaia, 24(6):544-545.

YANG JM, HE XGDL, WAN JY, et al., 2020. Screening of tomato cultivars in cadmium-polluted areas and study on their antioxidant capacity[J/OL]. Chin J Biotech. [2020-12-24] https://doi.org/10.13345/j.cjb.200242.

ZHANG XF, XIA HP, Li ZA, et al., 2011. Identification of a new potential Cd-hyperaccumulator Solanum photeinocarpum by soil seed bank-metal concentration gradient method[J]. J Hazard Mater, 189(1): 414-419.

ZHANG D, LIU Y, ZHANG H, et al., 2020. Response of osmotic regulators and sucrose metabolization-related enzymes to drought stress in Glycyrrhiza uralensis[J]. Acta Bot Bor-Occid Sin, 40(5): 819-827.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.