Pol. J. Environ. Stud. Vol. XX, No. X (XXXX), 1-9
DOI: *10.15244/pjoes/200467* ONLINE PUBLICATION DATE: 2025-03-27

Original Research

Effects of Gamma-Aminobutyric Acid (GABA) on Cadmium Accumulation in Rice Seedlings

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Received: 29 October 2024
Accepted: 24 January 2025

Abstract

To reduce cadmium (Cd) accumulation in rice, the effects of gamma-aminobutyric acid (GABA) on growth and Cd accumulation in rice seedlings were investigated in a pot experiment under Cd stress. The results indicated that treatment with 1 mg/L Cd significantly decreased the biomass, photosynthetic pigment content, photosynthesis rate, and antioxidant enzyme activity of rice seedlings. Consequently, exposure to 1 mg/L Cd-induced stress in rice seedlings, thereby inhibiting the growth of rice seedlings. Under Cd stress, concentrations of 0.1, 0.25, and 0.5 mmol/L GABA enhanced the biomass, photosynthetic pigment content, and photosynthesis of rice seedlings, whereas a concentration of 1 mmol/L GABA did not significantly affect these parameters. Lower concentrations (0.1 and 0.25 mmol/L) of GABA reduced the antioxidant enzyme activity and Cd contents in the roots, stems, and sheaths of rice seedlings under Cd stress, whereas higher concentrations (0.5 and 1 mmol/L) increased these parameters. Specifically, the concentrations of 0.1, 0.25, 0.5, and 1 mmol/L GABA decreased leaf Cd content by 78.79%, 71.74%, 56.77%, and 28.82%, respectively, compared with the Cd treatment. Therefore, the concentrations of 0.1 and 0.25 mmol/L GABA not only promote the growth of rice but also reduce Cd accumulation in rice under Cd stress.

Keywords: growth, heavy metal, physiology, plant growth regulator, rice

Introduction

Cadmium (Cd) is a mobile heavy metal that poses a significant threat to crops when present in soil. It can enter agricultural systems through various pathways, including fertilizer application, sewage irrigation, atmospheric deposition, and industrial emissions [1]. Cd-contaminated agricultural soils are a growing concern [2]. Additionally, increasingly acidic soils can enhance the release of Cd from paddy fields, further increasing the risk of Cd contamination in these areas [3]. Therefore, it is crucial to implement strategies to

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reduce Cd uptake in crops or remediate Cd-contaminated soils.

To effectively alleviate Cd stress in crops, strategies such as water and fertilizer regulation, soil amendments, and the application of antagonistic elements are used to alter the forms of soil Cd and reduce its uptake by plants [4-6]. In addition, several novel plant growth regulators have been introduced to enhance crop resistance to heavy metal stress and decrease heavy metal absorption in plants [7-9]. Gamma-aminobutyric acid (GABA) is a naturally occurring non-protein amino acid that plays a crucial role in regulating plant growth, development, and acclimatization to adverse conditions [10]. Under stress, GABA levels increase in crops, which enhances plant tolerance by regulating photosynthetic activity, stomatal conductance, and reactive oxygen species (ROS) levels [11]. The exogenous application of GABA not only elevates GABA content in crops but also improves their resilience to stress, such as increasing salinity resistance in oat seedlings [12], mitigating heat stress damage in tomato seedlings [13], and protecting the photosynthetic system of maize seedlings under drought stress [14]. In the context of Cd stress, exogenous GABA could alleviate these effects by promoting plant height, biomass, photosynthetic pigment content, and antioxidant enzyme activity [15, 16]. Furthermore, exogenous GABA reduced Cd accumulation in oilseed rape (*Brassica napus*), whereas it increased Cd uptake in the Cd hyperaccumulator plant *Solanum nigrum* var. *humile* [16, 17]. However, although exogenous GABA decreased Cd content in the roots of peach seedlings, it increased Cd levels in shoots [18]. Therefore, the effects of exogenous GABA on Cd accumulation vary among the crops.

Rice (*Oryza sativa*) is a grain crop known for its ability to accumulate Cd, which adversely affects its quality [19]. Furthermore, Cd that accumulates in rice can enter the human body through the food chain, leading to cardiovascular, musculoskeletal, renal dysfunction, and other diseases, thereby posing a serious threat to human health [20]. Therefore, it is essential to reduce the accumulation of Cd in rice plants, and the application of exogenous GABA could decrease Cd accumulation in rice. Therefore, this study aimed to investigate the effects of exogenous GABA on growth and Cd accumulation in rice seedlings. This study aimed to identify the optimal GABA concentration that could enhance growth while reducing Cd uptake in rice, thereby providing a reference for the safe production of rice in Cd-contaminated areas.

Materials and Methods

Materials

The rice variety used in this study was Mianhui 6139, a hybrid rice restorer line developed by the Mianyang Academy of Agricultural Sciences, China. In May 2023, rice seeds were sown in trays filled with perlite and placed in a greenhouse under the conditions described by Li et al. [21]. After germination, the trays were irrigated with Hoagland solution every three days. When the rice seedlings reached an approximate height of 10 cm (three-leaf stage), they were transplanted into square pots.

GABA was obtained from Beijing Solarbio Science and Technology Co. Ltd. (Beijing, China).

Experimental Design

The experiment was conducted in a rain shelter between June and July 2023. Uniform rice seedlings at the three-leaf stage were transplanted into square pots (height, 6.5 cm; length, 11 cm; and width, 8 cm) filled with perlite, with four seedlings evenly distributed in the corners of each pot. Six treatments were applied in this experiment: (1) control (CK), without Cd treatment; (2) 1 mg/L Cd treatment (Cd); (3) 1 mg/L Cd treatment + 0.1 mmol/L GABA (GABA0.1); (4) 1 mg/L Cd treatment + 0.25 mmol/L GABA (GABA0.25); (5) 1 mg/L Cd treatment + 0.5 mmol/L GABA (GABA0.5); and (6) 1 mg/L Cd treatment + 1 mmol/L GABA (GABA1) [22]. Hoagland nutrient solution containing 1 mg/L Cd (in the form of $CdCl_2 \cdot 2.5H_2O$) and GABA was applied to the pots for the Cd, GABA0.1, GABA0.5, GABA1, and GABA2 treatments, whereas Hoagland nutrient solution without Cd and GABA was applied for the CK treatment. Each treatment was replicated thrice, with one pot serving as the replicate. All Hoagland nutrient solutions were replaced every three days. After one month of growth, all plants were harvested to determine various parameters.

Determination of Parameters

In July 2023, the third mature leaf from the top of the main stem was selected to measure the photosynthetic characteristics using a Li-6400 photosynthetic system (LI-COR, Lincoln, NE, USA). The parameters assessed included the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr). The conditions for determining these photosynthetic parameters were established according to a previous report [21]. The same leaves were collected to evaluate the levels of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) and the activities of antioxidant enzymes, such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD). The levels of photosynthetic pigments were measured using the acetone-ethanol mixed extraction method [23]. Antioxidant enzyme activity was determined using previously described methods [23, 24]. The whole plants were harvested, washed, and separated into roots, stems and sheaths, and leaves. The tissue samples were dried at 80ºC until they reached a constant weight to record their dry weight (biomass). Dried tissue samples

were finely ground and digested in a mixture of nitric and perchloric acids. The resulting solution was analyzed for Cd content using an iCAP 6300 ICP-MS spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

SPSS software version 20.0.0 (IBM, Inc., Armonk, NY, USA) was used for all statistical analyses. Data were analyzed using a one-way analysis of variance and Duncan's multiple range test ($p<0.05$). Pearson's correlation analysis was used to assess correlations among all parameters under Cd stress.

Results and Discussion

Biomass of Rice Seedlings Under Cd Stress

Under Cd stress, rice growth was inhibited because of the toxic effects of Cd [25]. In this study, the Cd treatment decreased rice seedlings' biomass compared with CK (Table 1). Compared with CK, the Cd treatment decreased the root, stem and sheath, and leaf biomass by 14.35%, 14.48%, and 13.54%, respectively, indicating that treatment with 1 mg/L Cd inhibited rice growth, which aligns with the findings of a previous study [25].

Under Cd stress, GABA could enhance biomass and promote maize growth [26]. GABA, at appropriate concentrations, can mitigate the inhibitory effects of Cd stress on plant height and stimulate growth [17, 18, 22]. In this study, the concentrations of 0.1, 0.25, and 0.5 mmol/L GABA increased the root, stem and sheath, and leaf biomass of rice seedlings under Cd stress, whereas GABA at a concentration of 1 mmol/L only increased the stem and sheath biomass (Table 1). Compared with the Cd treatment, the concentrations of 0.1, 0.25, and 0.5 mmol/L GABA increased the root biomass by 65.64%, 48.88%, and 38.83%, respectively; stem and sheath biomass by 21.43%, 17.99%, and 15.08%, respectively; and leaf biomass by 32.21%, 29.70%, and 19.55%, respectively. The concentration of 0.1 mmol/L GABA was the best dose, which increased the root and leaf biomass by 41.87% and 14.30%, respectively, compared with CK. These results suggest that GABA can promote rice growth under Cd stress, consistent with previous studies [17, 18, 22, 26]. This effect may be attributed to GABA acting as a signaling molecule that enhances plant growth by influencing the expression and activity of key enzymes (e.g., photosynthetic enzymes) and ion channel-related proteins, thereby facilitating the uptake of essential mineral elements and improving plant resistance [27].

Photosynthetic Pigment Content in Rice Seedlings Under Cd Stress

Photosynthetic pigments are the core components of the photosynthetic system in higher plants and significantly influence the photosynthesis rate, reflecting plants' physiological evolution [28]. Cd stress inhibits the biosynthesis of photosynthetic pigments in plants [15, 16]. In this study, compared with CK, the Cd treatment decreased photosynthetic pigment content in rice seedlings (Table 2). Compared with CK, the Cd treatment decreased the contents of chlorophyll *a*, chlorophyll *b*, and total chlorophyll by 20.28%, 22.60%, and 20.77%, respectively. Treatment with 1 mg/L Cd inhibited the biosynthesis of photosynthetic pigments in rice, consistent with previous studies [15, 16].

Under Cd stress, GABA increased the content of photosynthetic pigments in oilseed rape, indicating that GABA promotes the biosynthesis of these pigments [16]. GABA also enhances chlorophyll *a*, chlorophyll *b*, and carotenoid levels in *Camptotheca acuminata* seedlings under Cd stress [15]. In this study, the concentrations of 0.1, 0.25, and 0.5 mmol/L GABA increased the contents of chlorophyll *a*, chlorophyll *b*, and total chlorophyll in rice seedlings under Cd stress, whereas the concentration of 1 mmol/L GABA did not affect these parameters (Table 2). Compared with the Cd treatment,

Table 1. Effects of GABA on the biomass of rice seedlings under Cd stress.

Treatment	Root (g/plant)	Stem and sheath (g/plant)	Leaf (g/plant)
CK	0.418±0.013d	0.884±0.022ab	0.923±0.025b
Cd	0.358±0.006e	0.756±0.023d	0.798±0.021c
GABA0.1	0.593±0.009a	0.918±0.029a	1.055±0.046a
GABA0.25	0.533±0.020b	0.892±0.027ab	1.035±0.058a
GABA0.5	0.497±0.012c	0.870±0.031b	0.954±0.045b
GABA1	0.366±0.014e	0.803±0.029c	0.854±0.028c

Values represent the mean±SE of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan's multiple range test, $p<0.05$). CK = control, Cd = Cd treatment (1 mg/L), GABA0.1 = 1 mg/L Cd + 0.1 mmol/L GABA, GABA0.25 = 1 mg/L Cd + 0.25 mmol/L ABA, GABA0.5 = 1 mg/L Cd + 0.5 mmol/L GABA, GABA1 = 1 mg/L Cd + 1 mmol/L GABA.

the concentrations of 0.1, 0.25, and 0.5 mmol/L GABA increased the chlorophyll *a* by 34.04%, 30.16%, and 20.81%, respectively; chlorophyll *b* content by 36.00%, 22.80%, and 20.80%, respectively; and total chlorophyll by 34.41%, 28.78%, and 20.81%, respectively. For carotenoid content, only the concentrations of 0.1 and 0.25 mmol/L GABA resulted in significant increases in the carotenoid content, whereas the concentrations of 0.5 and 1 mmol/L GABA did not have any effect. The concentration of 0.1 mmol/L GABA was the best dose, which increased the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid by 6.86%, 5.26%, 6.49%, and 11.43%, respectively, compared with CK. Therefore, GABA may promote the biosynthesis of photosynthetic pigments in rice under Cd stress to some extent, which aligns with previous findings [15, 16]. This effect may be attributed to the ability of GABA to mitigate stress-induced cell membrane damage and facilitate membrane recovery [29, 30]. Further investigation is required to elucidate the underlying mechanisms of action.

Photosynthetic Characteristics of Rice Seedlings Under Cd Stress

Under Cd stress, photosynthesis in crops is impaired, leaf stomatal resistance increases, and stomatal density decreases [31]. In this study, the Cd treatment reduced the rice seedlings' Pn, Gs, Ci, and Tr by 13.16%, 27.72%, 11.00%, and 21.02%, respectively, compared with CK (Table 3). This result indicates that treatment with 1 mg/L Cd inhibited photosynthesis in rice.

The application of GABA can enhance the Pn, Tr, and Gs in plant leaves, thereby improving photosynthesis [32]. In this study, 0.1 and 0.25 mmol/L GABA concentrations increased the Pn of rice seedlings under Cd stress, whereas the concentrations of 0.5 and 1 mmol/L GABA had no significant effects (Table 3). The Gs and Ci values treated with GABA at concentrations of 0.1, 0.25, and 0.5 mmol/L were higher than those under Cd treatment, whereas treatment with 1 mmol/L GABA showed no significant effect. The Tr values for all four GABA concentrations were higher than those for the Cd treatment. Overall, treatment with 0.1 mmol/L GABA yielded the most significant improvements in the Pn, Gs, Ci, and Tr, which increased by 14.77%, 32.44%, 11.61%, and 21.37%, respectively, compared with the Cd treatment. These results align with those of previous studies on cucumber [33] and oilseed rape [16], suggesting that GABA can mitigate the inhibitory effects of Cd stress on photosynthesis and likely influences stomatal opening in rice by regulating photosynthetic processes [34].

Antioxidant Enzyme Activity of Rice Seedlings Under Cd Stress

The concentration of ROS in crops increases under Cd stress, leading to the elevated activity of antioxidant enzymes that scavenge excessive ROS [35, 36]. In this study, the Cd treatment enhanced the activities of POD, CAT, and SOD in rice seedlings by 13.21%, 4.03%, and 5.71%, respectively, compared with CK (Table 4). This finding is consistent with those of previous studies [37]. These results indicated that treatment with 1 mg/L Cd caused oxidative damage to rice, prompting an increase in antioxidant enzyme activity to adapt to Cd stress.

GABA plays a crucial role in regulating the antioxidant defense system of crops, thereby alleviating adverse stress conditions [26, 38]. Under salinity stress, foliar application of 4-6 mmol/L GABA significantly increased the activities of SOD, POD, and CAT in oat seedlings [12]. In the presence of Cd stress, GABA reduced root CAT and POD activities in *Festuca elata*, decreased shoot POD activity, and increased shoot CAT activity [39]. In this study, concentrations of 0.5 and 1 mmol/L GABA enhanced the POD activity of rice seedlings by 17.05% and 54.95%, respectively, compared with the Cd treatment (Table 4). The 0.1 mmol/L GABA concentration did not affect the POD activity, whereas the 0.25 mmol/L GABA concentration resulted in a decrease compared with the Cd treatment. Additionally,

Table 2. Effects of GABA on photosynthetic pigment content of rice seedlings under Cd stress.

Treatment	Chlorophyll *a* content (mg/g)	Chlorophyll *b* content (mg/g)	Total chlorophyll content (mg/g)	Carotenoid content (mg/g)
CK	1.356±0.039bc	0.323±0.008b	1.680±0.033b	0.140±0.005bc
Cd	1.081±0.024d	0.250±0.004d	1.331±0.021d	0.136±0.004c
GABA0.1	1.449±0.035a	0.340±0.010a	1.789±0.034a	0.156±0.004a
GABA0.25	1.407±0.027ab	0.307±0.007c	1.714±0.020b	0.151±0.008ab
GABA0.5	1.306±0.040c	0.302±0.004c	1.608±0.044c	0.141±0.003bc
GABA1	1.094±0.021d	0.258±0.007d	1.352±0.018d	0.137±0.002c

Values are expressed as mean±SE of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan's multiple range test, $p<0.05$). CK = control, Cd = Cd treatment (1 mg/L), GABA0.1 = 1 mg/L Cd + 0.1 mmol/L GABA, GABA0.25 = 1 mg/L Cd + 0.25 mmol/L ABA, GABA0.5 = 1 mg/L Cd + 0.5 mmol/L GABA, GABA1 = 1 mg/L Cd + 1 mmol/L GABA.

0.1 and 0.25 mmol/L GABA concentrations reduced the CAT activity, whereas the 0.5 and 1 mmol/L GABA concentrations increased the CAT activity compared with the Cd treatment. For the SOD activity, only the 1 mmol/L GABA concentration led to an increase, whereas the other GABA concentrations resulted in a decrease compared with the Cd treatment. These findings are not entirely consistent with those observed

Table 3. Effects of GABA on the contents of different photosynthetic pigments in rice seedlings under Cd stress.

Treatment	Pn (μmol $CO_2/m^2/s$)	Gs (mol $H_2O/m^2/s$)	Ci (μmol CO_2/mol)	Tr (mmol $H_2O/m^2/s$)
CK	23.70±0.63a	0.772±0.027a	317.99±7.00a	7.855±0.176a
Cd	20.58±0.80d	0.558±0.012d	283.01±4.19d	6.204±0.265e
GABA0.1	23.62±0.87ab	0.739±0.024b	315.88±4.03a	7.530±0.211ab
GABA0.25	22.48±0.47bc	0.727±0.017b	297.79±2.46b	7.402±0.169bc
GABA0.5	21.36±0.88cd	0.614±0.008c	291.85±8.45bc	7.115±0.178c
GABA1	20.79±0.16d	0.580±0.017d	283.68±2.85cd	6.632±0.179d

Values are expressed as the mean±SE of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan's multiple range test, $p<0.05$). Pn = net photosynthetic rate, Gs = stomatal conductance, Ci = intercellular CO_2 concentration, Tr = transpiration rate, CK = control, Cd = Cd treatment (1 mg/L), GABA0.1 = 1 mg/L Cd + 0.1 mmol/L GABA, GABA0.25 = 1 mg/L Cd + 0.25 mmol/L ABA, GABA0.5 = 1 mg/L Cd + 0.5 mmol/L GABA, GABA1 = 1 mg/L Cd + 1 mmol/L GABA.

Table 4. Effects of GABA on the antioxidant enzyme activity of rice seedlings under Cd stress.

Treatment	POD activity (U/g/min)	CAT activity (mg/g/min)	SOD activity (U/g)
CK	2543±72.63d	12.91±0.08c	128.47±1.611b
Cd	2879±30.27c	13.43±0.13b	135.81±4.316a
GABA0.1	2777±21.80c	12.87±0.05c	122.35±2.604cd
GABA0.25	2136±86.80e	12.84±0.03c	120.89±2.422d
GABA0.5	3370±61.22b	13.52±0.12b	125.44±0.712bc
GABA1	4461±125.27a	14.57±0.39a	135.21±0.816a

Values are expressed as the mean±SE of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan's multiple range test, $p<0.05$). CK = control, Cd = Cd treatment (1 mg/L), GABA0.1 = 1 mg/L Cd + 0.1 mmol/L GABA, GABA0.25 = 1 mg/L Cd + 0.25 mmol/L ABA, GABA0.5 = 1 mg/L Cd + 0.5 mmol/L GABA, GABA1 = 1 mg/L Cd + 1 mmol/L GABA.

Table 5. Effects of GABA on the Cd contents in different tissues of rice seedlings.

Treatment	Root (mg/kg)	Stem and sheath (mg/kg)	Leaf (mg/kg)
CK	–	–	–
Cd	96.65±0.87c	27.31±1.28c	15.89±0.31a
GABA0.1	89.03±1.32d	19.25±0.41e	3.37±0.13e
GABA0.25	91.89±0.92c	22.66±0.62d	4.49±0.11d
GABA0.5	124.82±1.13b	43.64±0.88b	6.87±0.45c
GABA1	154.63±6.86a	47.55±0.93a	11.31±0.51b

Values are expressed as mean±SE of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan's multiple range test, $p<0.05$). Cd content was not detected in the CK. CK = control, Cd = Cd treatment (1 mg/L), GABA 0.1 = 1 mg/L Cd + 0.1 mmol/L GABA, GABA0.25 = 1 mg/L Cd + 0.25 mmol/L ABA, GABA0.5 = 1 mg/L Cd + 0.5 mmol/L GABA, GABA1 = 1 mg/L Cd + 1 mmol/L GABA.

Table 6. Correlations among the different parameters under Cd stress.

Parameter	RB	SB	LB	Cha	Chb	Tch	Car	Pn	Gs	Ci	Tr	POD	CAT	SOD	RCd	SCd	LCd
RB	1.000																
SB	0.915**	1.000															
LB	0.834**	0.876**	1.000														
Cha	0.976**	0.928**	0.845**	1.000													
Chb	0.976**	0.899**	0.870**	0.939**	1.000												
Tch	0.985**	0.931**	0.857**	0.998**	0.958**	1.000											
Car	0.859**	0.828**	0.718**	0.846**	0.823**	0.850**	1.000										
Pn	0.845**	0.765**	0.785**	0.835**	0.836**	0.843**	0.707**	1.000									
Gs	0.907**	0.842**	0.884**	0.917**	0.889**	0.921**	0.861**	0.880**	1.000								
Ci	0.880**	0.722**	0.691**	0.812**	0.873**	0.830**	0.817**	0.826**	0.813**	1.000							
Tr	0.899**	0.875**	0.891**	0.907**	0.886**	0.911**	0.796**	0.787**	0.841**	0.797**	1.000						
POD	-0.577*	-0.383	-0.305	-0.619*	-0.487	-0.600*	-0.542*	-0.506	-0.634*	-0.471	-0.417	1.000					
CAT	-0.725**	-0.539*	-0.423	-0.752**	-0.656**	-0.742**	-0.685**	-0.615*	-0.732**	-0.636*	-0.526*	0.931**	1.000				
SOD	-0.898**	-0.806**	-0.790**	-0.926**	-0.865**	-0.923**	-0.680**	-0.693**	-0.844**	-0.723**	-0.826**	0.621*	0.708**	1.000			
RCd	-0.544*	-0.352	-0.248	-0.563*	-0.489	-0.555*	-0.560*	-0.532*	-0.581*	-0.541*	-0.355	0.930**	0.915**	0.508	1.000		
SCd	-0.551*	-0.357	-0.323	-0.563*	-0.504	-0.558*	-0.629*	-0.615*	-0.673**	-0.614*	-0.365	0.868**	0.854**	0.495	0.945**	1.000	
LCd	-0.944**	-0.930**	-0.921**	-0.941**	-0.934**	-0.948**	-0.808**	-0.782**	-0.899**	-0.787**	-0.934**	0.404	0.546*	0.900**	0.310	0.352	1.000

$N = 15$. **Correlation is significant at the 0.01 level (2-tailed test). *Correlation is significant at the 0.05 level (2-tailed test). RB = root biomass, SB = stem and sheath biomass, LB = leaf biomass, Cha = chlorophyll *a* content, Chb = chlorophyll *b* content, Tch = total chlorophyll content, Car = carotenoid content, Pn = net photosynthetic rate, Gs = stomatal conductance, Ci = intercellular CO_2 concentration, Tr = transpiration rate, POD = POD activity, CAT = CAT activity, SOD = SOD activity, RCd = root Cd content, SCd = stem and sheath Cd content, LCd = leaf Cd content.

in oats and *F. elata* [12, 29], suggesting that GABA may enhance antioxidant enzyme activity in rice to a certain extent. This effect may be attributed to the ability of GABA to upregulate the expression of antioxidant enzyme genes in plants [40], which helps scavenge ROS and mitigate the toxic effects of Cd on plants [29].

Cd Content in Rice Seedlings Under Cd Stress

Under chromium (Cr) stress, GABA decreased the Cr content in the roots and shoots of mustard, alleviated the toxicity of Cr to mustard, and promoted mustard growth [41]. Similarly, under Cd stress, GABA decreased Cd content in oilseed rape and limited the accumulation of Cd in its organelles, thereby alleviating the toxicity associated with Cd stress [16]. In hyperaccumulator plants, GABA increased Cd content in *S. nigrum* var. *humile* [33]. In this study, the root Cd content in rice seedlings ranged from 91.89 to 154.63 mg/kg, whereas the Cd content in the leaves was between 19.25 and 47.55 mg/kg, and the leaf content ranged from 3.37 to 15.89 mg/kg under Cd stress (Table 5). Compared with the Cd treatment, the concentrations of 0.1 and 0.25 mmol/L GABA did not significantly affect or even decrease the root Cd content, whereas the concentrations of 0.5 and 1 mmol/L GABA increased it. The concentrations of 0.1 and 0.25 mmol/L GABA decreased the Cd content in the stem and sheath by 29.51% and 17.03%, respectively, whereas the higher concentrations of 0.5 and 1 mmol/L GABA led to an increase in these values compared with the Cd treatment. Additionally, concentrations of 0.1, 0.25, 0.5, and 1 mmol/L GABA reduced leaf Cd content by 78.79%, 71.74%, 56.77%, and 28.82%, respectively, compared with the Cd treatment. These results suggest that low GABA concentrations may inhibit Cd accumulation in rice, whereas high concentrations can promote Cd accumulation. This effect may be attributed to the ability of GABA to alter the subcellular distribution of elements and mitigate ROS induced by Cd stress [42-44].

Correlation Analysis

Correlation analysis was used to assess correlations among all parameters under Cd stress. The root, stem and sheath, and leaf biomass were positively correlated with one another (Table 6) and with chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content, carotenoid content, Pn, Gs, Ci, and Tr. Conversely, root, stem, sheath, and leaf biomass were negatively correlated with SOD activity and leaf Cd content. Additionally, root biomass was negatively correlated with POD activity, CAT activity, root Cd content, and Cd content in both the stems and sheaths. Similarly, stem and sheath biomasses were negatively correlated with CAT activity. The contents of root Cd and stem and sheath Cd were positively correlated with each other, negatively correlated with chlorophyll *a* content, total chlorophyll content, carotenoid content, Pn, Gs, and Ci, and positively correlated with POD and CAT activities. Furthermore, leaf Cd content was negatively correlated with chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content, carotenoid content, Pn, Gs, Ci, and Tr but positively correlated with CAT and SOD activities.

Conclusions

Treatment with 1 mg/L Cd inhibited the growth of rice seedlings. Under Cd stress, GABA increased biomass, photosynthetic pigment content, and photosynthesis in rice seedlings, thereby promoting their growth. Low GABA concentrations reduced the antioxidant enzyme activity of rice seedlings under Cd stress, whereas high concentrations enhanced the antioxidant enzyme activity. Additionally, low concentrations of GABA decreased Cd accumulation in various tissues of rice seedlings, whereas high concentrations of GABA reduced Cd accumulation only in leaves. Future studies should investigate the mechanisms underlying the effects of low GABA concentration on Cd uptake in rice.

Acknowledgments

This work was financially supported by the Crop Characteristic Resources Creation and Utilization Key Laboratory of Sichuan Province Program (myzdsys23-04), Sichuan Science and Technology Program (2024YFHZ0253; 2025YFHZ0119), Sichuan Rice Innovation Team of National Modern Agricultural Industry Technology System (SCCXTD-2024-SD-13), Key Research and Development Program of Sichuan Province (2022YFYZ0016), and Mianyang Experiment Station of National Rice Industry Technology System (CARS-01-69).

Conflict of Interest

The authors declare no conflict of interest.

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