Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Evaluation of the genes CYP 85 A 2 , BZR1, and CAD1 in the attenuation of cadmium in seedlings of the species Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby (PARICÁ) under different concentrations of 24-epibrassinolide

Erick dos Santos Ribeiro
erick.rib39@gmail.com
Federal Rural University of Amazonia
Cândido Ferreira de Oliveira Neto
Federal Rural University of Amazonia
Ednaldo da Silva Filho
Federal Rural University of Amazonia
Igor Guerreiro Hamoy
Federal Rural University of Amazonia
Ana Ecídia de Araújo Brito
Federal Rural University of Amazonia
Dênmora Gomes de Araújo
Federal Rural University of Amazonia
Juliana Freitas do Nascimento
Federal Rural University of Amazonia
Evelyn Luane Pinheiro de Figueiredo
Federal Rural University of Amazonia
Dayane dos Santos Costa
Federal Rural University of Amazonia
Lilian Tatiana Costa Barros
Federal Rural University of Amazonia
Vinícius Oliveira Amancio
Federal Rural University of Amazonia

Research Article

Keywords: Phytoremediation, molecular biology, plant hormone, 24 epiBL, bioregulation

Posted Date: February 17th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6018382/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

The Amazonian species paricá (*Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby*) accumulates cadmium (Cd) primarily in the roots, but the lack of understanding of gene modulation in response to this metal and the phytohormonal mechanisms complicates its relevance in the rehabilitation of degraded areas. Therefore, the present study aims to examine the expression of the $CYP_{85}A_2$ and *BZR1* genes, precursors of 24-EBL, in the production of chelating proteins by the *CAD1* gene in seedlings of the Paricá species, under various concentrations of $CdCl_2$ and 24-EBL. The experiment was conducted in a growth room at the Laboratory of Studies on Biodiversity of Higher Plants (EBPS) at the Federal Rural University of Amazon (UFRA), Belém-Pará Campus, following a completely randomized experimental design (CRD), in a 4x3 factorial scheme, totaling 60 experimental units with 4 treatments of $CdCl_2$ (0, 50, 100, and 150 µM) and 3 doses of 24-epibrassinolide (0, 20, and 40 nM). The data were subjected to analysis of variance (ANOVA) ($p < 0.05$), and the differences between treatments were analyzed using Tukey's test ($p < 0.05$). The biometric variables indicated a significant reduction in root length due to the harmful effect of $CdCl_2$. Additionally, losses in *Chl a*, *Chl b*, and *Chl a+b* were observed due to the entry of Cd^{2+} into the leaf tissues. Despite this, the $CYP_{85}A_2$, *BZR1*, and *CAD1* genes showed greater expression in the aerial part with varying doses of 24-EBL, while *CAR* and *ACN* were affected by increased $CdCl_2$, indicating a genetic adjustment in the upper parts of the plants to cope with Cd toxicity and maintain biological functions.

Introduction

Anthropic action, resulting from activities such as uncontrolled mining and industrialization, leads to the introduction of heavy metals into the environment (BENDITO, 2017; BENAVENTE, et al., 2020). These metals, including mercury (Hg), arsenic (As), and cadmium (Cd), are released into the soil and water in alarming quantities, polluting terrestrial and aquatic ecosystems (SILVA, 2017; SHI, 2019; ALI, et al., 2019; BALALI-MOOD, 2021).

Cadmium, in particular, is a phytotoxic metal easily absorbed by plants, posing a threat to ecosystems due to its entry into the food chain (FERNANDES, 2014; KUBIER, 2019). Even at low concentrations, its absorption through roots negatively affects mineral nutrition and plant growth (PAN, 2010; LIN, 2015; SUN, 2021). Its high solubility in water facilitates its uptake by plants, compromising their development and homeostasis (SOUSA, 2018; BASTOS, et al., 2021). Visible symptoms include leaf curling and yellowing, impacting water absorption and stomatal function (SOUZA, 2022). It damages the photosynthetic system, reducing chlorophyll and carotenoids, decreasing photosynthetic efficiency, and inhibiting CO2 fixation enzymes (TABELIN, 2018; MORAVCÍKOVA, 2023). In several plant species, cadmium toxicity can lead to genetic anomalies and disturbances in cell division (GU, et al., 2021).

In response to environmental challenges, plants have developed complex cellular communication systems, known as hormones, enabling rapid responses to adverse conditions (BUCKER, et al., 2017; GUO, et al., 2019; HU, 2021). Current studies focus on brassinosteroids (BR) and 24-epibrassinolide (24-

EBL), which influence gene expression and metabolism, affecting growth and cell differentiation (TONG, 2018; ZHENG, et al., 2022; WAHAB, 2022). Genes, essential to genetic inheritance, are DNA segments that define traits and ensure the continuity and diversity of species (MOORE, 2021). This genetic information is crucial for cellular function and phenotype expression (SABELLA, 2021).

The BZR1 and CPY85A2 genes mediate the action of 24-EBL, playing a fundamental role in 24-EBL production in response to plant exposure to Cd (RAJEWSKA, 2016; WEI, 2020). BZR1 is a key regulator in the BR signaling cascade, including 24-EBL, while CPY85A2 is involved in converting plant steroids into bioactive forms like 24-EBL (ZULLO, 2002; RAHMANI, et al., 2021). One of the primary effects of 24-EBL is stimulating the production of phytochelatin proteins derived from the CAD1 gene, which bind to Cd, reducing its toxicity in plant cells (REN, et al., 2023).

Additionally, 24-EBL induces the expression of antioxidant enzymes that help neutralize free radicals produced in response to Cd (LUO, 2021). However, the lack of studies on genetic regulation related to cytological responses to Cd and 24-EBL hinders the understanding of its role in cellular stability in plants (BILAL, et al., 2023). In the Amazon, research seeks pollutant-resistant plants for reforestation projects (ERAS, et al., 2022). The Paricá (*Schizolobium parahyba var. amazonicum*) stands out for its rapid growth, making it a valuable option for reforestation with economic and environmental potential (RODRIGUES, et al., 2018). Its fast growth makes it attractive to cooperatives and timber industries in the short term (OLIVEIRA, 2023). In the presence of Cd, variations occur in the amount accumulated in different organs (leaves and roots), impacting nitrogen (N) uptake and proline production, which acts as an osmotic regulator to reduce biological damage (BASTOS, et al., 2023). Although Cd concentration is more evident in roots, understanding genetic traits is crucial for comprehending this species' resistance to the metal.

Thus, the present study aims to analyze the expression of the cytochrome P450 (*$CYP_{85}A_2$*), brassinazole-resistant 1 (*BZR1*), and phytochelatin synthase 1 (*CAD1*) genes during the exogenous application of the phytohormone 24-epibrassinolide in the species Paricá (*Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby*) subjected to oxidative stress under different dosages of $CdCl_2$.

Material and methods

The experiment was conducted in the growth room at the Laboratory for the Study of Biodiversity in Higher Plants (EBPS), located at the Institute of Agricultural Sciences (ICA) of the Federal Rural University of the Amazon (UFRA), Belém-PA Campus. The biochemical analyses were carried out at the Biochemistry Laboratory of EBPS. The seeds, totaling 600, were provided by the Seed Laboratory (LABSEM) of UFRA, Belém Campus. In the initial stages of seedling production for Paricá (*Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby*), the seeds were scarified using 80-grit sandpaper and soaked in a solution containing 24-EBL (24-epibrassinolide) and deionized water (control) for 24 hours to aid in breaking dormancy. Subsequently, the seeds were sown in pots with washed sand substrate, sterilized in an autoclave, and dried in an oven at 80°C.

Experimental design

The experimental design was completely randomized (CRD) in a 4x3 factorial scheme, with 12 treatments divided among 4 doses of cadmium chloride (0 µM, 50 µM, 100 µM, and 150 µM) and 3 levels of brassinosteroids in the form of 24-epibrassinolide (0, 20, and 40 nM of EBL), with 5 replications, each pot containing 15 plants per pot.

Biometrics

Seedling height and root length were measured at the end of the experiment, on the 14th day, using a centimeter ruler. Biometric measurements were taken from the base of the stem to the apical bud of the plants.

Determination of chlorophyll a, b, total (a + b), carotenoids and anthocyanin levels.

To obtain the total chlorophyll content, the simple calculation was used: Total Chlorophyll = Chlorophyll *a* + Chlorophyll *b*. First, 100 mg of fresh leaf from each treatment was weighed and placed in a crucible with ice for sample maceration, using 3 mL of 80% acetone. After maceration, the samples were centrifuged, and the supernatant was transferred to a 25 mL volumetric flask, with the volume adjusted using 80% acetone. The samples were then read in a spectrophotometer at 663 nm (Chlorophyll *a*), 647 nm (Chlorophyll *b*), 537 nm (Carotenoids), and 470 nm (Anthocyanins), with a blank (80% acetone) used to zero the device beforehand.

Gene expression

To begin the molecular analyses, total RNA extraction was performed from the seedlings in the EBPS Laboratory, located at the UFRA Campus. First, samples of seedlings subjected to the treatments ($CdCl_2$ x 24-EBL) and the control were separated. A total of 5 mg of fresh leaflets and roots were separated and placed in Eppendorf tubes containing 2 ml of a homemade RNAlater solution for subsequent storage in an ultrafreezer at -80°C until RNA extraction could begin.

Quantification and purity of RNA samples.

The assessment of RNA purity and quantification was conducted at the Applied Genetics Laboratory (LGA), located on the UFRA Campus, using the Biodrop Duo UV/Vis spectrophotometer. The purity of the samples was then calculated through the ratio of absorbance measured at 260 and 280 nm (260/280 ratio), with results close to 1.8 considered to have an acceptable degree of purity. Subsequently, the samples were diluted, adjusted to a concentration of 50 ng/µL, and treated with DNase I, RNase-free (Thermo Scientific), following the manufacturer's instructions.

Real-time qPCR molecular analysis

The qRT-PCR analyses were performed using the one-step method at the Serology and Molecular Biology Laboratory, UFRA Campus, Belém-PA. The samples were standardized in duplicate alongside the genes described in Table 1. For single-product amplification, the samples were adjusted to a final volume of 10 µL, containing 1x Power SYBR® Green RNA-to-CT™ One-Step Kit (Applied Biosystems, Foster City, CA, USA), 0.03 µL of reverse transcriptase, 4.4 µL of ultrapure water, 1 µL of RNA, and 0.36 µL of Forward and Reverse primer pairs (Table 1). All reactions were carried out in the CFX96 Touch™ Real-Time Detection System thermocycler (Bio-Rad, Hercules, CA, USA) following the protocol recommended by the kit manufacturer. Gene expression was estimated using the $2^{-\Delta\Delta CT}$ method, with the Actin gene used as a reference to stabilize the target genes in this study.

Table 1
Oligonucleotide sequences for gene expression analysis in the leaves and roots of *S. parahyba var. amazonicum*, using primer sequences with the qRT-PCR technique.

Gene	Primers	GenBank
$CPY_{85}A_2$	F AAGAATGCTCGTCGTCCTCC	ID: 816394
	R ATCTCCTCTGGCACCCATCT	
CAD1	F CAGGGGTAGAGAACTGGGGA	ID: 834430
	R GGATGGTGTGAGACCTAGCG	
BZR1	F TCTCAACTCCGTTCCGTTTC	ID: 843845
	R TGACGAAGAAGCCACAACTG	
ACTIN	F GAAGCACCTCTCAACCCCAA	ID: 14763233
	R GGAAAGGACCGCCTGGATAG	

Caption: CYP85A2 (cytochrome P450), CAD1 (Phytochelatin), BZR1 (Protein of the brassinosteroid signaling positive regulator family), and Actin (Actin).

Experimental statistics

The biometric, biochemical, and molecular variables obtained were subjected to statistical analysis using analysis of variance (ANOVA) in the R software, version 4.2.1. The means were compared using the Tukey test at a 5% probability level. Graphs were created using R Studio software, version 1.3.1093, with the ggplot2 package and the "rcolorbrewer" function.

Results

Influence of $CdCl_2$ and 24-EBL on Biometric Parameters in the Species *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (Paricá)

In Fig. 1.a, regarding the aerial structure of Paricá seedlings under the coordination of phytohormonal attenuators (0, 20, and 40 nM of EBL) at various doses of $CdCl_2$ (0 µM, 50 µM, 100 µM, and 150 µM), no significant difference ($p < 0.05$) was observed only in seedlings treated with 40 nM, which maintained growth stability. The percentages of loss and gain (PRA%) support this statement, showing minimal changes in plant growth when compared to the control (34% > 33% > 33% > 31%). However, the toxic effects of Cd were observed with greater severity in plant groups subjected to 0 and 20 nM of 24-EBL (Fig. 1.a) at varying doses of $CdCl_2$ (0 µM, 50 µM, 100 µM, and 150 µM), showing a statistical difference ($p < 0.05$). The PRA% rates of plants at doses of 0 (0.5% > -21% > -21.6% > -33%) and 20 nM (9.5% > -14.5% > -23.6% > -16%) EBL, corresponding to height variables, decreased as $CdCl_2$ concentrations increased.

Regarding the root structures (Fig. 1.b), a decline in root dimensions was observed. Despite the administration of 24-EBL (0, 20, and 40 nM), there was a reduction in root length at different concentrations of $CdCl_2$ (0 µM, 50 µM, 100 µM, and 150 µM), with a statistically significant variation ($p < 0.05$) compared to the control group. This results in a reduction in root impairment due to the harmful effects of Cd ions.

Influence of $CdCl_2$ and 24-EBL on the photosynthetic pigments of the species *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (Paricá).

The deleterious effects of Cd ions on photosynthetic pigments acted differently among the treatments. For samples treated with 50 µM of $CdCl_2$, a significant negative difference ($p < 0.05$) was observed in chlorophyll a (Fig. 2.a) in the 40 nM 24-EBL segment. However, in chlorophyll b (Fig. 2.b), the effect occurred at 0 nM of EBL, directly affecting the total chlorophyll pigments (Fig. 6.c), with a significant negative impact ($p < 0.05$) under the same $CdCl_2$ conditions (50 µM) between 0 and 40 nM of 24-EBL.

The dosage of 20 nM of 24-EBL under the action of 50 µM of $CdCl_2$ showed a significant difference ($p < 0.05$) when compared to the control in Chl a (Fig. 2.a), Chl b (Fig. 2.b), and Chl a + b (Fig. 2.c). Positive PRA% values were observed in the chlorophyll classes: Chl a (25.1%), Chl b (12.7%), and Chl a + b (9.6%).

The extracts from samples corresponding to the 100 and 150 µM $CdCl_2$ indices reveal a statistically significant negative variation ($p < 0.05$) in the 100 µM class under the interaction with 20 nM EBL in Chl a (Fig. 2.a), b (Fig. 2.b), and a + b (Fig. 2.c). However, the highest degree of hazard was observed in the 150 µM segment, regardless of the 24-EBL phytohormonal class (0, 20, and 40 nM). Statistically significant differences ($p < 0.05$) were detected across all segments of Chl a, b, and a + b, which may have impaired the photosynthetic system of the species *S. parahyba var. amazonicum*.

Despite the changes in chlorophyll indices due to the severe toxic effects of $CdCl_2$, during the experiment, modifications were observed in the metabolism of antioxidant pigments such as carotenoids (CAR) and anthocyanins (ACN). Specifically, CAR pigments (Fig. 2.e) increased significantly ($p < 0.05$) in plants subjected to 0, 20, and 40 nM of 24-EBL compared to variations in $CdCl_2$ (0, 50, 100,

and 150 µM). It is noteworthy that when exposed to 150 µM of $CdCl_2$ and treated with 24-EBL (0, 20, and 40 nM), the plants recorded changes in PRA% of 40%, 15.7%, and 12.6%, respectively. Regarding ACN (Fig. 2.d), a significant difference was observed in the stress response levels induced by the metal, according to the Tukey test ($p < 0.05$), particularly at concentrations of 50 and 150 µM of $CdCl_2$ combined with 0 and 20 nM of 24-EBL. This contributed to the neutralization of reactive oxygen species in plant cells.

Influence of Cd and 24-EBL on the $CYP_{85}A_2$ and *BZR1* genes in the leaves and roots of the species *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (Paricá).

The RT-PCR evaluation of the seedlings' leaflets was conducted after exposure to various concentrations of $CdCl_2$ (0, 50, 100, and 150 µM), influenced by different amounts of 24-EBL (0, 20, and 40 nM). A significant expression of the $CPY_{85}A_2$ (Fig. 3.a) and *BZR1* (Fig. 3.b) genes was observed, showing a notable statistical difference ($p < 0.05$) in the presence of 40 nM of 24-EBL. Interestingly, as the concentrations of $CdCl_2$ (50, 100, and 150 µM) increased, the PRA% of $CPY_{85}A_2$ decreased to 471%, 167%, and 146%, while for *BZR1*, the opposite occurred, with increases of 230%, 446%, and 716%.

Particularly in the root samples, a notable statistical similarity ($p < 0.05$) was observed between the $CPY_{85}A_2$ (Fig. 3.c) and *BZR1* (Fig. 3.d) genes, precursors of EBL, when exposed to 40 nM of 24-EBL in the absence of $CdCl_2$. However, upon introducing 50 µM of $CdCl_2$, the first signs of increased expressivity were observed, with a statistically significant difference ($p < 0.05$) in plants under various doses of 24-EBL (0, 20, and 40 nM) for both $CPY_{85}A_2$ (Fig. 3.c) and *BZR1* (Fig. 3.d) genes.

However, with the increase in concentrations to 100 and 150 µM of $CdCl_2$ in the roots, a low expression of the $CPY_{85}A_2$ (Fig. 3.c) and *BZR1* (Fig. 3.d) genes is observed in the root structures, with no statistical difference ($p < 0.05$) between 0 and 40 nM of EBL. At the 20 nM level of 24-EBL, a significant negative difference ($p < 0.05$) is observed, indicating the initial harmful effects of Cd on root tissues.

In summary, even at high concentrations of 50, 100, and 150 µM of $CdCl_2$, the predominant genetic regulation occurred in the upper parts of the plants. In the roots, there was a reduction in gene stimuli, considering that they are the first organs to come into contact with toxic Cd ions. The application of 40 nM of 24-EBL stood out, emphasizing the importance of the $CPY_{85}A_2$ and *BZR1* genes, which are crucial in the 24-EBL metabolism in the leaves. This pattern suggests a unique response to $CdCl_2$ exposure, highlighting the crucial role of the aerial part in gene regulation under stress conditions. These findings deepen the understanding of the molecular mechanisms related to the plant's response to metal ions and growth-regulating substances.

Influence of $CdCl_2$ and 24-EBL on the *CAD1* gene in the aerial part and root of the species *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ).

The molecular analyses of *CAD1* levels in genetic material extracted from leaves and roots under various doses of phytoregulators (0 nM, 20 nM, and 40 nM) at 0 µM $CdCl_2$ showed a significant difference ($p < 0.05$) only in the roots, with a progressive yield of PRA% (0% > 137% > 278%). However, the addition of the initial doses of Cd showed a statistically significant difference at the 5% level at 50 µM $CdCl_2$ in the fractionation of 24-EBL (0 nM, 20 nM, 40 nM), with positive *CAD1* activity observed in both roots (Fig. 8.b) and leaflets (Fig. 8.a) during the experimental period.

Distinctly, in the root samples (Fig. 8.b) treated with 100 µM and 150 µM of $CdCl_2$, regardless of the attenuator segments used in 24-EBL (0 nM, 20 nM, 40 nM), no significant difference ($p < 0.05$) was observed when compared to the control, revealing early signs of biological instability. This impact was reflected in the leaves (Fig. 8.a), showing no significant difference at the 5% level according to Tukey's test in the 24-EBL applications.

Discusion

Modification in the growth of *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ) seedlings under different concentrations of $CdCl_2$ and 24-EBL.

In the aerial part, high concentrations of Cd trigger a series of events that generally reduce the growth of the main stems, redirecting energy investment toward lateral and root growth (KIM, 2010; ZHU, 2021). This adjustment is mediated by the inhibition of auxin activity, a plant hormone that promotes cell elongation (BAJGUZ et al., 2019; GROSZYK, 2021; QIAO, 2022). Additionally, plants can increase the synthesis of antioxidant compounds, such as glutathione, to mitigate damage caused by Cd-induced oxidative stress, thereby contributing to physiological and biochemical balance (VILLIERS et al., 2012; KARWEL et al., 2020).

In response to energy loss caused by stress from Cd ions, the phytohormone 24-EBL stimulates the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a key enzyme in the photosynthesis process (KAGALE et al., 2007; JIANG et al., 2012; NGUYEN, 2021). This enzyme is responsible for fixing carbon dioxide (CO_2) during the conversion of light energy into carbohydrates (KOCH, 1996; LI, 2005; ZHU, 2016; YIN et al., 2022). This additional regulation enables the plant to accumulate carbohydrate reserves that can later be used to meet energy demands during stress and recovery (GILL, 2010; ZHAO et al., 2017; WANG, 2017; HUQUE, 2021).

The roots of plants are the first line of contact with the environment and are therefore more susceptible to the absorption of heavy metals, such as Cd, present in the soil (BARANDA et al., 2019). Additionally, roots contain a higher concentration of binding sites and ion transporters, which increases Cd uptake compared to leaves (IORI, 2017; SABELLA, 2022). However, in extreme cases of toxicity, plants may strengthen their roots through lignification, converting meristematic cells into xylem cells and depositing lignin, which results from the synthesis and polymerization of phenolic monomers such as coniferaldehyde (WANG et al., 2019; KOBYLETSKA et al., 2020; YAN, 2023).

This process provides mechanical resistance, improves water and nutrient conduction, and seals intercellular spaces, reducing exposure to Cd in the soil and increasing resistance to the metal (MUNEER, 2012; ZHU, 2016; ZULFIQAR et al., 2022). However, despite its importance, lignification does not completely eliminate the stress caused by Cd (XU et al., 2013; BALK, 2023). Plants also employ various intracellular purification strategies, such as using antioxidant enzymes to eliminate reactive oxygen species (ROS) and mitigate oxidative effects in root cells (ARORA, 2010; RAZA et al., 2022).

When it comes to endemic species of the Amazon biome, African mahogany (Khaya grandifoliola) accumulates Cd in its roots, while in the species Ucuúba (Virola surinamensis), under the influence of Cd, an increase in reducing sugars in the roots has been observed, promoting osmotic adjustment and tissue protection (PAIVA et al., 2021; JÚNIOR et al., 2021). Research conducted on S. parahyba var. amazonicum in unfavorable environments, in the presence of zinc (Zn) and with the use of silicon (Si) as a moderating element, demonstrated a reduction in nutritional deficiencies, highlighting the species as a phytostabilizer (ALBUQUERQUE et al., 2020). These characteristics underscore the physiological adaptations of Amazonian species in polluted environments.

Changes in the photosynthetic pigments of *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ) seedlings under different concentrations of $CdCl_2$ and 24-EBL.

At high concentrations, Cd ions can replace Mg in chlorophyll or Fe in ferredoxins (ELLER et al., 2015; ÇIKILI, 2016; CHEN et al., 2019). Magnesium ions (Mg^{2+}) play an essential role in light absorption within the chlorophyll molecule, particularly in its central porphyrin ring (BENAVIDES, 2005; HAJJAOUI et al., 2022). When Cd replaces Mg^{2+}, photosynthesis efficiency is compromised, as Mg^{2+} is crucial for light absorption and electron transfer during this process (SONG et al., 2019; SEREGIN, 2021).

High doses of Cd affect protein complexes that are essential in the electron transport chain during photosynthesis, inhibiting their activity and compromising the efficient transfer of electrons (BAJGUZ, 2011; SACHDEV et al., 2021). This adverse effect results in a decrease in ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate reduced), both crucial for the synthesis of glucose and other vital components of photosynthesis (PARMAR, 2013; GUTSCH, 2018; SABIR et al., 2019). The exogenous application of 24-EBL has shown a significant role in improving tolerance to oxidative stress induced by Cd metal in plants (JAN et al., 2018). When sprayed on leaves, 24-EBL acts as a key modulator, activating intracellular antioxidant systems (YU et al., 2017; HASANUZZAMAN, 2017; STIRK et al., 2018). It induces the synthesis of antioxidant enzymes such as SOD, GPx, and CAT, which help neutralize the free radicals generated by Cd, thereby minimizing oxidative damage to biomolecules (RAZA, 2013; SHAH et al., 2019). Additionally, they promote the physiological maintenance of leaves, stimulating stomatal closure and reducing water loss through transpiration, thus helping preserve cellular membrane integrity and photosynthetic efficiency (PALLIOTTI, 2015; MOHAMMAD et al., 2020; JÚNIOR, 2022; GUO, 2023).

The excessive accumulation of Cd within plant cells promotes the formation of ROS, which are naturally present in plants but in minimal amounts under normal conditions (SIMKIN, 2019).

At the biochemical level, there is also an increase in the concentration of non-enzymatic antioxidant compounds, such as polyphenols and flavonoids, enhancing the plant's ability to combat oxidative stress caused by Cd (ASAMI, 2005; YADAV et al., 2016; EMAMVERDIAN, 2020). To prevent oxidative stress, plants can diversify their strategies to combat reactive oxygen, such as the metabolism of *CAR* and *ACN* (ANDRIANOS et al., 2016; YAO et al., 2022; MULYANINGSIH, 2023). *CAR* and *ACN* play an essential antioxidant role during photosynthesis by neutralizing singlet oxygen activity (MAHALAKSHM, 2017; ENARU et al., 2021; GUPTA, 2023). They interact with lipid peroxides to interrupt the chain production of ROS, eliminate excited chlorophyll molecules to prevent the formation of singlet oxygen, and neutralize excess excited energy during the xanthophyll cycle (BEHRENS et al., 2019; CERQUEIRA et al., 2023).

Fluctuation of $CYP_{85}A_2$ and *BZR1* genes in the aerial part and roots of *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ) subjected to different concentrations of $CdCl_2$ and 24-EBL.

In the presence of high amounts of Cd ions in the aerial part, the formation of reactive oxygen species (ROS) occurs, affecting photosynthesis, stomatal conductance, the RuBisCO enzyme, and transpiration (NOGUEIRA et al., 2019). In situations of physiological stress, *BZR1* orchestrates the expression of genes related to antioxidant enzymes (KHAN, 2023). It operates by neutralizing ROS, such as SOD, which converts superoxide radicals into hydrogen peroxide (H_2O_2), while CAT decomposes peroxide (H_2O_2) into H_2O and O_2 (HUANG et al., 2019). GPx uses reduced glutathione to reduce H_2O_2 into less harmful compounds (SALAMA, 2022). Ascorbate peroxidase (APX) neutralizes H_2O_2 using ascorbic acid as a cofactor (ALAM et al., 2020). Glutathione reductase (GR) regenerates reduced glutathione, maintaining its antioxidant role (CAO, 2013; SANTOS, 2018). The combined action of these enzymes forms a coordinated system that protects cells from reactive oxygen species, preventing oxidative damage (CONSIDINE, 2014; GONCHARUK et al., 2023).

In the case of the species *S. parahyba*, due to its epigeal characteristic, it initially releases the hypocotyl, pushing the cotyledons to the soil surface, followed by the protrusion of the radicle (DUTRA et al., 2017; MOURA, 2019; CUNHA, 2020). This process results in roots, which are the last structures to undergo cellular differentiation and expansion of vascular tissues, resulting in higher levels of brassinosteroids (ARANTES et al., 2020). $CYP_{85}A_2$, by influencing the synthesis of BR, triggers a cascade of events that positively impact cell division and organ formation (GAN et al., 2022; ZHANG et al., 2024). BR, in turn, interacts in the cell nucleus with *BZR1*, regulating the expression of genes related to hormonal response and cell division (BURGER, 2019; LANDI, 2021). Auxin production is strictly related to the interaction of the genes $CYP_{85}A_2$ and *BZR1*, modulating their levels to define plant architecture, determining the spacing and orientation of cells (GRUSZKA et al., 2018; NOSAK et al., 2021).

Plants exposed to high levels of Cd toxicity exhibit changes in their cells, and the $CYP_{85}A_2$ gene is activated, promoting the biosynthesis of phytoesteroids (24-EBL), plant hormones that help reduce the toxic effects of Cd (MUSSIG, 2002; KIM, 2005; CHAKRABORTY et al., 2023). Under stress conditions in the cells, the phytohormone 24-EBL binds to its receptor on the cell membrane (ANWAR et al., 2018; NOLAN et al., 2020). This triggers intracellular signaling of the *BZR1* gene, generating mRNA in the

nucleus (NAM, 2002; KIM, 2010; FANG, 2021; ZUO, 2022). This mRNA is translated into the *BZR1* protein by ribosomes in the cytoplasm (RYU, 2007; EREMINA, 2016; RIVEROLA et al., 2019). The *BZR1* protein acts as a transcription factor, binding to DNA in the nucleus and directing genes associated with Cd tolerance, regulating their expression and the quantification of enzymes and proteins involved in cell homeostasis regulation (LI et al., 2016; BRUNO et al., 2021; KONO, 2020).

In addition, *BZR1* regulates the activity of genes related to the synthesis of phytochelatin proteins (KIM, 2019; XIAN et al., 2020). These proteins have the ability to bind to Cd and other heavy metals, reducing their toxicity in plant cells (DURÁN, 2013). *BZR1* also aids in osmotic regulation by stimulating genes that encode compatible solutes, such as soluble sugars, which help maintain the osmotic balance of cells, preventing dehydration and maintaining cellular equilibrium (OLIVEIRA et al., 2002; FARIDUDDIN, 2014; HEWEDY, 2022).

According to NOGUEIRA et al., 2022, the species S. amazonicum, classified as a phytoextractor, accumulates Cd ions mainly in the roots, despite their presence in the aerial part. Studies indicate that the low activity of $CPY_{85}A_2$ and *BZR1* reduces the production of BR, limiting cell division and allowing the increase of genes related to lignification (YU et al., 2004; ROVERE, 2022; LI et al., 2023). Recent research highlights the essential role of genes related to the lignification of the cell wall in the roots for Cd absorption, transport, and tolerance (LI et al., 2021; LI et al., 2022; SHANGGUAN, 2023). However, this mechanism compromises the root contact surface, resulting in the shortening of the root system (HAN et al., 2022).

Variation of the *CAD1* gene in the aerial part and roots of *S. parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ) subjected to different concentrations of $CdCl_2$ and 24-EBL.

The influence of *CAD1* in the root cells of plants in response to Cd concentration involves a complex genetic regulation mechanism (COUTO, 2016; NOUNURAI, 2022). When the roots detect high concentrations of Cd in their environment, the activation of specific transcription factors occurs, which bind to the *CAD1* promoter (HOLMES, 2021). This triggers the transcription of *CAD1*, resulting in the production of corresponding mRNA (JIANG, 2023). This mRNA is then translated into *CAD1* proteins, which act as Cd transporters in the root cells (HA, 1999; CAZALE, 2001; THEVENIN, 2021). This molecular response helps plants cope with Cd stress by storing it in the vacuole and reducing damage throughout the plant. This is crucial for the survival of plants under exposure to the toxic metal (HOWDEN, 1995; EUDES, 2006; BENEDICTIS et al., 2018).

The application of 24-EBL can maximize the production of phytochelatins (PCs) in the root cells of plants, becoming crucial for the plant's response to stress caused by heavy metals and other environmental pollutants (REEVES, 2018). According to SEREGIN et al. (2023), these small molecules are synthesized through a series of complex biochemical reactions that mainly occur in the cytoplasm of root cells. PCs are activated when the plant detects the presence of toxic metal ions, such as Cd or lead (ANDRESEN et al., 2013; URAGUCHI, 2021). This happens as part of the plant's defense mechanism to

protect against the excessive absorption of these harmful metals (YUAN, 2008; SONG et al., 2017; KOZHEVNIKOVA, 2020).

Firstly, 24-EBL positively influences the production of glutathione in the cells. It regulates the expression of genes involved in glutathione synthesis, such as γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS) (WISZNIEWSKA, 2019). This results in an increase in intracellular glutathione levels, which is a crucial molecule for antioxidant defense and cellular detoxification (CHAUDHURI, 2022).

Moreover, 24-EBL is also involved in the production of PCs. These phytochelatins are formed from glutathione residues and play a critical role in the uptake and sequestration of toxic metal ions in root cells (CORSO, 2018; KOUR, 2021).

In summary, the presence of Cd in plant cells can cause changes in their structure and function, involving morphological, biochemical, and physiological aspects (ANGULO-BEJARANO, 2021). Over time, plants have developed adaptive genetic mechanisms to cope with Cd and maintain their biological stability (HOU et al., 2023). 24-EBL, derived from cycloartenol, plays a crucial role by influencing the gene expression linked to Cd tolerance, affecting the transcription of DNA into mRNA (ANWAR et al., 2018; RIZVI et al., 2020). This gene regulation by 24-EBL can result in changes in the production of antioxidant enzymes, osmotic compounds, and others that help reduce the toxic effects of Cd (SHARMA et al., 2023). The intracellular interaction between RNA, DNA, and phytohormones like 24-EBL is essential for the plant's response to Cd toxicity and its adaptation to contaminated environments (SHU et al., 2016; SHARMA et al., 2018; TIAN, 2022). Although there is little understanding of how this hormone induces molecular tolerance mechanisms in Amazonian plant biodiversity, its role is extremely important for the survival of plants in territories polluted with heavy metals.

Conclusion

In the context of this study, Cd^{2+} concentrated in the root organs, but interfered with chlorophyll (*Chl a*, *Chl b*, and *Chl a + b*) and the height of *S. parahyba var. amazonicum* seedlings, despite the implementation of 24-EBL (0, 20, and 40nM). However, an increase in the variables *CAR* and *ACN* was observed, facilitated by the elimination of ROS. At the same time, the *CAD1* gene acted restrictively up to 50 µM of $CdCl_2$ in both plant organs (leaf and root), assisting in the production of PCs for Cd^{2+} transport to the vacuole in the center of leaf cells. Additionally, molecular analyses revealed the intensification of the $CYP_{85}A_2$ and *BZR1* genes in the leaflets when integrated with 40nM of 24-EBL. These genes may play a significant role in energy conversion and the maintenance of cell stability. These findings suggest the existence of protective mechanisms and genetic management that may extend biological actions in response to the harmful effects of $CdCl_2$.

Abbreviations

ACN
Anthocyanins
APX
Ascorbate Peroxidase
BR
Brassinosteroids
BRZ1
Brassinazole-Resistant 1
CdCl2
Cadmium Chloride
CAD1
Phytochelatin Synthase 1
Chl a
Chlorophyll a
Chl b
Chlorophyll b
Chl (a + b)
Total Chlorophyll
Clo a
Chlorophyll a
Clo b
Chlorophyll b
Clo a + b
Total Chlorophyll
CAR
Carotenoids
CPY85A2
Cytochrome85A2
CAT
Catalase
DNA
Deoxyribonucleic Acid
GR
Glutathione Reductase
H2O2
Hydrogen Peroxide
mRNA
Messenger Ribonucleic Acid
PRA%
Percentage of Loss and Gain

qPCR-TR
Real-Time Polymerase Chain Reaction
RNA
Ribonucleic Acid
SOD
Superoxide Dismutase
24-EBL
24-Epibrassinolide.

Declarations

Author Contribution

Todos os autores revisaram o manuscrito

Acknowledgement

This work was carried out with financial support from Fundação Amazônia de Amparo a Estudos e Pesquisas (FAPESPA), Universidade Federal Rural da Amazônia (UFRA) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and with technical support from the team that worked hard to implement the experiment and collect the data. We thank them all.

References

1. ALI, Hazrat; KHAN, Ezzat; ILAHI, Ikram (2019) Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. Journal of Chemistry, v.19:1-14. https://doi.org/10.1155/2019/6730305
2. Arora, p; Bhardwaj, r; Kanwar, m. k (2010) 24-epibrassinolide regulated diminution of Cr metal toxicity in Brassica juncea L. plants. Braz. J. Plant Physiol., 22(3): 159-165. https://doi.org/10.1590/S1677-04202010000300002
3. ALBUQUERQUE, G. D. P; BATISTA, B. L; SOUZA, A. L. M; BRITO, A. E. A; NASCIMENTO, V. R; NETO, C. F. O; PAIVA, A. P; TEIXEIRA, J. S. S; JÚNIOR, M. L. S (2020) The effect of silicon (Si) on the growth and nutritional status of Schizolobium amazonicum seedlings subjected to zinc toxicity. AJCS 14(02):325-332. doi: 10.21475/ajcs.20.14.02
4. ASAMI, T; NAKANO, T; FUJIOKA S (2005) Plant brassinosteroid hormones. Vitamins and Hormones. 479–504. https://doi.org/10.1016/S0083-6729(05)72014-8
5. ANDRIANOS, V; STOIKOU, V; TSIKRIKA, K; LAMPROU, D; STASINOS, S; PROESTOS, C; ZABETAKIS, I.: Carotenoids and Antioxidant Enzymes as Biomarkers of the Impact of Heavy Metals in food Chain. Curr. Res. Nutr Food Sci Jour. Vol. 4, 15-24p, 2016.

6. ALAM, P; KOHLI, S. K; BALAWI, T. A; ALTALAYAN, F. H; ALAMP; ASHRAF, M; BHARDWAJ, R; AHMAD, P.: Foliar Application of 24-Epibrassinolide Improves Growth, Ascorbate-Glutathione Cycle, and Glyoxalase System in Brown Mustard (Brassica juncea (L.) Czern.) under Cadmium Toxicity. Plants,1-24p, 2020.
7. ARANTES, M. B. S; MARINHO, C. S; GOMES, M. M. A; SANTOS, R. F; GALVÃO, S. P; VAZ, G. P.: Brassinosteroid accelerates the growth of Psidium hybrid during acclimatization of seedlings obtained from minicuttings. Pesq. Agropec. Trop., Goiânia, v. 50, 1-8p, 2020.
8. ANWAR, A; LIU, Y; DONG, R; BAI, L; YU, X; LI, Y.: The physiological and molecular mechanism of brassinosteroid in response to stress: a review. Biological Research, 1-15p, 2018.
9. ANWAR, A; BAI, L; MIAO, L; LIU, Y; LI, S; YU, X; LI, YANSU.: 24-Epibrassinolide Ameliorates Endogenous Hormone Levels to Enhance Low-Temperature Stress Tolerance in Cucumber Seedlings. Int. J. Mol. Sci. 1-17p, 2018.
10. ANDRESEN, E; MATTUSCH, J; WELLENREUTHER, G; THOMAS, G; ARROYO ABAD, U; KUPPER, H.: Different strategies of cadmium detoxification in the submerged macrophyte Ceratophyllum demersum L. Metallomics, 1377–1386p, 2013.
11. ANGULO-BEJARANO, P. I; PUENTE-RIVERA, J; CRUZ-ORTEGA, R.: Metal and Metalloid Toxicity in Plants: An Overview on Molecular Aspects. Plants 1-28p, 2021.
12. BASTOS, A. S. M; NASCIMENTO, C. M; GOMES, L. S; BARROS, L. T. C; ALVES, A. C. B; Ribeiro, D. M; NOGUEIRA, G. A. S; NETO, C. F. O.: A bibliographic review on Paricá (Schizolobium amazonicumHuber ex Ducke) and its relationship with the phytoremediation of environments contaminated by heavy metals. Contribuciones a Las Ciencias Sociales, São José dos Pinhais, v.16, n.7, 7206-7218p, 2023.
13. BAJGUZ, A; ORCZYK, W; GOŁĘBIEWSKA, A; CHMUR, M; NICZYPORUK, A. P.: Occurrence of brassinosteroids and infuence of 24-epibrassinolide with brassinazole on their content in the leaves and roots of Hordeum vulgare L. cv. Golden Promise. Planta 249:123–137p, 2019.
14. BAJGUZ, A.: Suppression of Chlorella vulgaris growth by cadmium, lead and copper stress and its restoration by endogenous brassinolide. Archives of Environmental Contamination and Toxicology, 60: 406–416p, 2011.
15. BALALI-MOOD, M; NASERI, K; TAHERGORABI, Z; KHAZDAIR, M. R; SADEGHI, M.: Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Front. Pharmacol. 1-19p, 2021.
16. BARANDA, Y. H; HERNÁNDEZ, P. R; ICART, M. P; HERNÁNDEZ, Y. H; RUBIO, O. C.: Toxicity of Cadmium in plants and strategies to reduce its effects. Case study: The tomato. Cultivos Tropicales, vol. 40, 1-18p 2019.
17. BASTOS, R. S; ARAÚJO, J. L; AZEVEDO, V. S; FERREIRA, M. L. A; LIMA, L. R; ROCHA, J. A.: Cadmio complexes with biological activity: Scientific and technological prospection. Pesquisa, Sociedade e Desenvolvimento, v. 10, n. 5, 2021.

18. BALK, M; SOFIA, P; NEFFE, A. T; TIRELLI, N.: Lignin, the Lignification Process, and Advanced, Lignin-Based Materials. Int. J. Mol. Sci. 1-47p, 2023.
19. BENDITO, B. P. C; COELHO, C. B; FERREIRA, C. W. R; MARTINS, I. C. M.: QUANTIFICAÇÃO DOS TEORES TOTAIS DE CÁDMIO, CHUMBO E MERCÚRIO, EM ÁREA DE DISPOSIÇÃO INADEQUADA DE RESÍDUOS SÓLIDOS. ENCICLOPÉDIA BIOSFERA, Centro Científico Conhecer - Goiânia, v.14 n.26; 1430-1444p. 2017.
20. BENAVENTE, M. S; ACHATA, L. R; OLIVERA, M; SANCHEZ, V. A; NANO, C. C; QUISPE, J. G.: Riesgos para la salud por metales pesados en productos agrícolas cultivados en áreas abandonadas por la minería aurífera en la Amazonía peruana. Scientia Agropecuaria 11(1): 49 –59, 2020.
21. BENAVIDES, M.P; GALLEGO, S. M; TOMARO, M. L.: Cadmium toxicity in plant. Braz. Plant Physiol, 17 (1): 21-34p, 2005.
22. BILAL, S; SAAD JAN, S; SHAHID, M; ASAF, S; KHAN, A. L; LUBNA; AL-RAWAHI, A; LEE, I. J; AL-HARRASI, A.: Novel Insights into Exogenous Phytohormones: Central Regulators in the Modulation of Physiological, Biochemical, and Molecular Responses in Rice under metal (loid) Stress. Metabolites. 1-28p, 2023.
23. BUCKER, N. L.; PAIVA, A. L. S; MACHADO, R. D; ARENHART, R. A; MARGIS, P. M.: Interactions between plant hormones and heavy metals responses. Genet. Mol. Bio. 40, 373–386, 2017.
24. BURGER, M; CHORY J.: Stressed out about hormones: how plants orchestrate immunity. Cell Host Microbe, 26(2):163–72p, 2019.
25. BEHRENS, C. E; SMITH, K. E, IANCU, C. V, CHOE, J. Y, DEAN, J. V.: Transport of Anthocyanins and other Flavonoids by the Arabidopsis ATP-Binding Cassette Transporter AtABCC2. Sci Rep 9: 437p, 2019.
26. BRUNO, L.; TALARICO, E.; MADEO, M.L.; MUTO, A.; MINERVINO, M.; ARANITI, F.; BITONTI, M.B.; CHIAPPETTA, A. Cadmium Affects Cell Niches Maintenance in Arabidopsis Thaliana Post-Embryonic Shoot and Root Apical Meristem by Altering the Expression of WUS/WOX Homolog Genes and Cytokinin Accumulation. Plant Physiol. Biochem. 167, 785–794p, 2021.
27. BENEDICTIS, M; BRUNETTI, C; BRAUER, E. K; ANDREUCCI, A; POPESCU, S. C; COMMISSO, M; GUZZO, F; SOFO, A; CASTIGLIONE, M. R; VATAMANIUK, O. K.: The Arabidopsis thaliana Knockout Mutant for Phytochelatin Synthase1 (cad1-3) Is Defective in Callose Deposition, Bacterial Pathogen Defense and Auxin Content, But Shows an Increased Stem Lignification. Frontiers in Plant Science. 1-14p, 2018.
28. CORSO, M; SCHVARTZMAN, M. S; GUZZO, F; SOUARD, F; MALKOWSKI, E; HANIKENNE, M; VERBRUGGEN, N.: Contrasting cadmium resistance strategies in two metallicolous populations of Arabidopsis halleri. N. Phytol 283–297p, 2018.
29. COUTO, D.: Regulation of pattern recognition receptor signalling in plants. Nat. Rev. Immunol. 16: 537–552p, 2016.
30. CERQUEIRA, J. V. A; ANDRADE, M. T; RAFAEL, D. D; ZHU, F; MARTINS, S. V. C; NESI, A. N; BENEDITO, V; FERNIE, A. R; ZSOGON, A.: Anthocyanins and reactive oxygen species: a team of rivals regulating

plant development?. Plant Molecular Biology, 213–223p, 2023.

31. CAO, F; LIU, L; IBRAHIM, W; CAI, Y; WU, F.: Alleviating Effects of Exogenous Glutathione, Glycinebetaine, Brassinosteroids and Salicylic Acid on Cadmium Toxicity in Rice Seedlings (Oryza Sativa). Agrotechnol, 1-6p, 2013.
32. CAZALE, A. C; CLEMENS, S.: Arabidopsis thaliana expresses a second functional phytochelatin synthase. FEBS Letters, 215-219p, 2001.
33. CONSIDINE, M, J; FOYER, C. H.: Redox regulation of plant development. Antioxid. Redox Signal. 21: 1305–1326p, 2014.
34. CHAUDHURI, A; KOUSHIK HALDER; K; ABDIN, M. Z; MAJEE, M; DATTA, A.: Abiotic Stress Tolerance in Plants: Brassinosteroids Navigate Competently. Int. J. Mol. Sci., 1-16p, 2022.
35. CHEN, H; SHU, F; YANG, S; LI, Y; WANG, S.: Competitive Inhibitory Effect of Calcium Polypeptides on Cd Enrichment of Brassia campestris L. Int. J. Environ. Res. Public Health, 1-18p. 2019.
36. CHAKRABORTY, P; BISWAS, A; DEY, S; BHATTACHARJEE, T; CHAKRABARTY, S.: Cytochrome P450 Gene Families: Role in Plant Secondary Metabolites Production and Plant Defense. J. Xenobiot, 402–423p. 2023.
37. ÇIKILI, Y; SAMET, H; DURSUN, S.: Cadmium Toxicity and its Effects on Growth and Metal Nutrient Ion Accumulation in Solanaceae Plants. Journal of Agricultural Sciences. 576-587p, 2016.
38. CUNHA, M. C. L; LIMA, T. L; FERREIRA, T. C; SANTOS, Y. M.: Seed, seedling, tirodendro morphology and germination of Anadenanthera macrocarpa (Benth.) Brenan (Fabaceae, Mimosoideae). Special Article on Seed, 1-9p, 2020.
39. DUTRA, F. V; CARDOSO, A. D; SILVA, R. M; LIMA, R. S; MORAIS, O. M; RAMPAZZO, M. C.: Morfobiometria de frutos e sementes de Schizolobium amazonicum Huber ex Ducke. Revista Agropecuária Técnica, Areia-PB, v. 38, n. 2. 58-64p, 2017.
40. DURÁN, R. L; MACHO, A. P; BOUTROT, F; SEGONZAC, C; SOMSSICH, I. E; ZIPFEL, C.: The transcriptional regulator BZR1 mediates trade-off between plant innate immunity and growth. Plant biology, 1-15p, 2013.
41. ERAS, I. C; GRIFFITH, D. M; KALINHOFF, C; RAMÍREZ, J; GÁZQUEZ, M. J.: Native Hyperaccumulator Plants with Differential Phytoremediation Potential in an Artisanal Gold Mine of the Ecuadorian Amazon. Plants, 1-14p, 2022.
42. ELLER, F; BRIXH.: Influence of low calcium availability on cadmium uptake and translocation in a fast-growing shrub and a metalaccumulating herb. AoB PLANTS. 1-9p. 2015.
43. EMAMVERDIAN, A; DING, Y; XIE, Y.: The Role of New Members of Phytohormones in Plant Amelioration under Abiotic Stress with an Emphasis on Heavy Metals. Pol. J. Environ. Stud. Vol. 29, No. 2, 1009-1020p, 2020.
44. ENARU, B; DRETCANU, G; POP, T. D; STANILÃ, A; DIACONEASA, Z.: Anthocyanins: Factors Affecting Their Stability and Degradation. Antioxidants, 1-24p, 2021.

45. EREMINA, M; UNTERHOLZNER, S. J; RATHNAYAKE, AI; CASTELLANOS, M.; KHAN, M.; KUGLER, K.G.; MAIO, ST; MAYERC, KFX; ROZHONA, W.; POPPENBERGER, B. Brassinosteroids participam no controle da tolerância basal e adquirida ao congelamento das plantas. Processo. Nacional. Acad. 1-10p, 2016.
46. EUDES, A; POLLET, B; SIBOUT, R; DO, C. T, SEGUIN, A; LAPIERRE, C; JOUANIN, L.: Evidence for a role of AtCAD 1 in lignification of elongating stems of Arabidopsisthaliana. Planta. 225(1):23–39p, 2006.
47. FARIDUDDIN Q, YUSUF M, AHMAD I, AHMAD, A.: Brassinosteroids and their role in response of plants to abiotic stresses. Biol Plant. 58(1): 9-17p. 2014.
48. FERNANDES, L. H; MAINIER, F. B.: OS RISCOS DA EXPOSIÇÃO OCUPACIONAL AO CÁDMIO. Revista Eletrônica Sistemas & Gestão, Volume 9, Número 2, 194-199p, 2014.
49. FANG, P; WANG, Y; WANG, M; WANG, F; CHI, C; ZHOU, Y; ZHOU, J; SHI, K; XIA, X; FOYE. C. H; YU, J.: Crosstalk between Brassinosteroid and Redox Signaling Contributes to the Activation of CBF Expression during Cold Responses in Tomato. Antioxidants, 1-20p, 2021.
50. GAN, Q; LUAN, M; HU, M; LIU, Z; ZHANG, Z.: Functional study of CYP90A1 and ALDH3F1 gene obtained by transcriptome sequencing analysis of Brassica napus seedlings treated with brassinolide. Front. Plant Sci. 1-17p, 2022.
51. GUTSCH, A; KEUNEN, E; GUERRIERO, G; RENAUT, J; CUYPERS, A; HAUSMAN, J. F; SERGEANT, K.: Long-term cadmium exposure influences the abundance of proteins that impact the cell wall structure in Medicago sativa stems. Plant Biology, 1023–1035p, 2018.
52. GROSZYK, J; HEBDA, M. S.: Effects of 24-Epibrassinolide, Bikinin, and Brassinazole on Barley Growth under Salinity Stress Are Genotype and Dose-Dependent. Agronomy, 1-18p, 2021.
53. GUO, B; LIU, C; LIANG, Y; LI, N; FU, Q.: Salicylic Acid Signals Plant Defence Against Cadmium Toxicity. Int. J. Mol. Sci. 1-19p, 2019.
54. GUO, Z; GAO, Y; YUAN, X; YUAN, M; HUANG, L; WANG, S; LIU, C; DUAN, C.: Effects of Heavy Metals on Stomata in Plants: A Review. Int. J. Mol. Sci. 1-16p, 2023.
55. GU, Q; WANG, C; XIAO, Q; CHEN, Z; HAN, Y.: Melatonin Confers Plant Cadmium Tole-rance: An Update. Int. J. Mol. Sci. 1-18p, 2021.
56. GILL, S. S; TUTEJA, N.: Polyamines and abiotic stress tolerance in plants. Plant Signaling and Behavior 5, 26–33p, 2010.
57. GUPTA, R.: Laboratory Experiments Showing the Results of Carotenoid Content in the Seedlings of Cultivar Pusa Chetki of Radish (Raphanus Sativus L.) Plant after Applying Heavy Metals. International Journal of Research Publication and Reviews, Vol 4, 2977-2980p, 2023.
58. GONCHARUK, E. A; ZAGOSKINA, N. V.: Heavy Metals, Their Phytotoxicity, and the Role of Phenolic Antioxidants in Plant Stress Responses with Focus on Cadmium: Review. Molecules, 1-28p, 2023.
59. GRUSZKA, D.: Crosstalk of the Brassinosteroid Signalosome with Phytohormonal and Stress Signaling Components Maintains a Balance between the Processes of Growth and Stress Tolerance. Int. J. Mol. Sci. 1-47p, 2018.

60. HAJJAOUI, H; ABDENNOURI, M; Q; OURZAL, S; TOUNSADI, H; BARKA, N.: Removal of cadmium ions by magnesium phosphate: Kinetics, isotherm, and mechanism studies. Applied Surface Science Advances. 1-19p, 2022.
61. HASANUZZAMAN, M; NAHAR, K; ANEE, T. I; FUJITA, M.: Exogenous Silicon Attenuates Cadmium-Induced Oxidative Stress in Brassica napus L. by Modulating AsA-GSH Pathway and Glyoxalase System. Frontiers in Plant Science. 1-9p, 2017.
62. HA, S., SMITH, A., HOWDEN, R., DIETRICH, W., BUGG, S., O'CONNELL, M., GOLDSBROUGH, P., & COBBETT, C.: Phytochelatin synthase genes from Arabidopsis and the yeast Schizosaccharomyces pombe. Plant Cell, 11 (6), 1153-1163p. 1999.
63. HU, X; LI, T; XU, W; CHAI, Y.: Distribution of cadmium in subcellular fraction and expression difference of its transport genes among three cultivars of pepper. Ecotoxicology and Environmental Safety. 1-14p, 2021.
64. HUQUE, A. K. M. M; SO, W; NOH, M; YOU, M.K; SHIN, J.S.: Overexpression of Atbbd1, Arabidopsis bifunctional nuclease, confers drought tolerance by enhancing the expression of regulatory genes in aba-mediated drought stress signaling. Int. J. Mol. Sci, 22-29, 2021.
65. HEWEDY, O, A; MAHMOUD, G. A. E; ELSHAFEY, N. F; KHAMIS, G; KARKOUR, A. M; LATEIF, K. S. A; AMIN, B. H; CHIAB, N; TAHER, A. M. E; ELSHEERY, N. I.: Plants take action to mitigate salt stress: Ask microbe for help, phytohormones, and genetic approaches. JOURNAL OF WATER AND LAND DEVELOPMENT. No. 55 (X–XII):1-16p, 2022.
66. HOLMES, D. R; BREDOW, M; THOR, K; PASCETTA, S. A; SEMENTCHOUKOVA, I; SIEGEL, K. R; ZIPFEL, C; MONAGHAN. J.: A novel allele in the Arabidopsis thaliana MCPF protein CAD1 results in deregulated immune signaling. bioRxiv preprint, 1-31p, 2021.
67. HUANG, H; ULLAH, F; ZHOU, D. X; YI, M; ZHAO, Y.: Mechanisms of ROS Regulation of Plant Development and Stress Responses. Front. Plant Sci. 1-10p, 2019.
68. HAN, X; ZHAO, Y; CHEN, Y; XU, J; JIANG, C; WANG, X; ZHUO, R; LU, M. Z; ZHANG, J.: Lignin biosynthesis and acumulation in response to abiotic stresses in woody plants. Forestry Research, 1-10p, 2022.
69. HOWDEN, R; COLDSBROUGH, P. B; ANDERSEN, C. R; COBBETT, C. S.: Cadmium-Sensitive, cad7 Mutants of Arabidopsis thaliana Are Phytochelatin Deficient. Plant Physiol, 1059-1066p, 1995.
70. HOU, L; JI; S; ZHANG, Y; WU, X; ZHANG, LI; LIU, P.: The mechanism of silicon on alleviating cadmium toxicity in plants: A review. Front. Plant Sci.1-16p. 2023.
71. IORI, V; PIETRINI, F; BIANCONI, D; MUGHINI, G; MASSACCI, G; ZACCHINI, M.: Analysis of biometric, physiological, and biochemical traits to evaluate the cadmium phytoremediation ability of eucalypt plants under hydroponics. iForest, 10: 416-421p, 2017.
72. JAN, S. ALYEMENI, M. N; WIJAYA, L; ALAM, P; SIDDIQUE, K. H; AHMAD, P.: Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in Pisum sativum L. seedlings. BMC Plant Biology. 1-18p, 2018.

73. JIANG, Y. P; ZHOU, Y. H; XIA, X. J; MAO, W. H; SHI, K; CHEN, Z; YU, J. Q.: Cellular glutathione redox homeostasis plays an important role in the brassinosteroid-induced increase in CO_2 assimilation in Cucumis sativus. New Phytologist, 194: 932–943p, 2012.
74. JIANG, S; TIAN, X; HUANG, X; XIN, X; XIN, J; YAN, H.: Physcomitrium patens *CAD1* has distinct roles in growth and resistance to biotic stress. BMC Plant Biology, 1-13p, 2023.
75. JÚNIOR, W. V. A; NETO, C. F. O; FILHO, B. G. S; CRUZ, E. D; AMARANTE, C. B; BARBOSA, A. V. C; NOGUEIRA, G. A. S; NASCIMENTO, V. R; SOUSA, D. J. P; TEIXEIRA, J. S. S.: Biochemical metabolism of young plants of Ucuúba (Virola surinamensis) in the presence of cádmium. BMC Plant Biology. 1-8p, 2021.
76. JÚNIOR, S. O. M; ANDRADE, J. R; NASCIMENTO, R; LIMA, R. F; BEZERRA, C. V. C; FERREIRA, V. M.: Brassinosteroid application increases tomato tolerance to salinity by changing the effects of stress on membrane integrity and gas Exchange. Acta Scientiarum. Agronomy, v. 44, 1-12p. 2022.
77. KAGALE, S.; DIVI, R. U; KROCHKO, JE; KELLER, WA; KRISHNA, P.: Brassinosteroid confers tolerance in Arabidopsis thaliana and Brassica napus to a range of abiotic stresse. Planta, 353–364p, 2007.
78. KOCH, K.: Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Moleculer Biology, 509-540p, 1996.
79. KIM, T. W; HWANG, J. Y; KIM, Y. S; JOO, S. H; CHANG, S. C; LEE, J. S; TAKATSUTO, S; KIM, S. K.: Arabidopsis CYP85A2, a Cytochrome P450, Mediates the Baeyer-Villiger Oxidation of Castasterone to Brassinolide in Brassinosteroid Biosynthesis. The Plant Cell, Vol. 17, 2397–2412p, 2005.
80. KIM, S.Y; KIM, B.H; LIM, C.J; LIM, C.O; NAM, K.H.: Constitutive activation of stress inducible genes in a brassinosteroid-insensitive 1 (bri1) mutant results in higher tolerance to cold. Physiol. Plantarum, 191–204p, 2010.
81. KIM, T. W, WANG, Z.Y.: Brassinosteroid Signal Transduction from Receptor Kinases to Transcription Factors. Annu Rev Plant Biol. 681–704p, 2010.
82. KIM, E. J; LEE, S; H; PARK, C. H; KIM, H. S; HSU, C. C; XU, S; WANG, Z. Y; KIM, S. K; KIMA, T. W.: Plant U-Box40 Mediates Degradation of the Brassinosteroid-Responsive Transcription Factor BZR1 in Arabidopsis Roots. The Plant Cell, Vol. 31: 791–808p, 2019.
83. KHAN, T. A; KAPPACHERY, S; KARUMANNIL, S; ALHOSANI, M; ALMANSOORI, N; ALMANSOORI, H; YUSUF, M; TRAN, L. S. P; GURURANI, M. A.: Brassinosteroid Signaling Pathways: Insights into Plant Responses under Abiotic Stress. Int. J. Mol. Sci. 1-17p, 2023.
84. KOZHEVNIKOVA, A. D; SEREGIN, I. V; AARTS, M. G. M; SCHAT, H.: Intra-specific variation in zinc, cadmium and nickel hypertolerance and hyperaccumulation capacities in Noccaea caerulescens. Plant Soil, 479–498p, 2020.
85. KOBYLETSKA, M; KAVULYCH, Y; ROMANYUK, N; TEREK, O.: Exogenous Salicylic Acid Modifies Cell Wall Lignification, Total Phenolic Content, PAL-Activity in Wheat (Triticum aestivum L.) and Buckwheat (Fagopyrum esculentum Moench) Plants under Cadmium Chloride Impact. Biointerface Research in Applied Chemistry. Volume 13, 1-13p, 2020.

86. KARWEL, M. T; BAJGUZ, A; NICZYPORUK, A. P.: 24-Epibrassinolide modulates primary metabolites, antioxidants, and phytochelatins in Acutodesmus obliquus exposed to lead stress. Journal of Applied Phycology, 32:263–276p, 2020.
87. KONO, A; YIN, Y; Updates on BES1/BZR1 Regulatory Networks Coordinating Plant Growth and Stress Responses. Front. Plant Sci. 1-7p. 2020.
88. KOUR, J; KOHLI, S. K; KHANNA, K; BAKSHI, P; SHARMA, P; SINGH, A. D; IBRAHIM, M; DEVI, K; SHARMA, N; OHRI, P; SKALICKY, M; BRESTIC, M; BHARDWAJ, R; LANDI, M; SHARMA, A.: Brassinosteroid Signaling, Crosstalk and, Physiological Functions in Plants Under Heavy Metal Stress. Front. Plant Sci. Vol (12): 1-19p, 2021.
89. KUBIER, A; WILKIN, R. T; PICHLER, T; CADMIUM IN SOILS AND GROUNDWATER: A REVIEW. EPA Public Access. 1-42p, 2019.
90. LANDI, M; SHARMA, A.: Brassinosteroid Signaling, Crosstalk and, Physiological Functions in Plants Under Heavy Metal Stress. Front. Plant Sci. Vol (12): 1-19p, 2021.
91. LI, L; XU, J; XU, Z. H, XUE; H. W.: Brassinosteroids stimulate plant tropisms through modulation of polar auxin transport in Brassica and Arabidopsis. Plant Cell, 2738–275p, 2005.
92. LI, Y; HUA, J; HOU, X; QI, N; LI, C; WANG, C; YAO, Y; HUANG, D; ZHANG, H; LIAO, W.: Brassinosteroids is involved in methane-induced adventitious root formation via inducing cell wall relaxation in marigold. BMC Plant Biology, 1-15p, 2023.
93. LI, D; ZHOU, C; MAI, J; WU, Y; KANG, L; AN, Q; ZHANG, J; DENG, K; QI, J. L; PAN, C.: Nanoselenium transformation and inhibition of cadmium accumulation by regulating the lignin biosynthetic pathway and plant hormone signal transduction in pepper plants. J. Nanobiotechnol, 1-14p, 2021.
94. LI, M; LI, P; WANG, C; XU, H; WANG, M; WANG, Y; NIU, X; XU, M; HONG WANG, H; QIN, Y; TANG, W; BAI, M; WANG, W; WU, S.: Brassinosteroid signaling restricts root lignification by antagonizing SHORT-ROOT function in Arabidopsis. PLANT PHYSIOLOGY 1182–1198p, 2022.
95. LUO, J. S; ZHANG, Z.: Mechanisms of cadmium phytoremediation and detoxification in plants. The Crop Journal, 521–529p, 2021.
96. LIN, L. Q; CONG, L; YUN, W. H; YANG, J; MING, H; WAN, Z. B; KAI, C; LEI, H.: Association of soil cadmium contamination with ceramic industry: a case study in a Chinese town. Sci. Total Environ 514, 26– 32p, 2015.
97. LI, X. J; CHEN, X.J; GUO X; YIN L.L; AHAMMED, G.J; XU C. J.: DWARF overexpression induces alteration in phytohormone homeostasis, development, architecture and carotenoid accumulation in tomato. Plant Biotechnol. J. 14: 1021–1033p, 2016.
98. MORAVCÍKOVA, D; ZIAROVSKÁ, J.: The Effect of Cadmium on Plants in Terms of the Response of Gene Expression Level and Activity. Plants,1-19p, 2023.
99. MULYANINGSIH, R. D; PRATIWI, R; HASANAH, A. N.: An Update on the Use of Natural Pigments and Pigment Nanoparticle Adducts for Metal Detection Based on Colour Response. Biosensors, 1-21p, 2023.

100. MUSSIG, C; SABINE FISCHER, S; ALTMANN, T.: Brassinosteroid-Regulated Gene Expression. Plant Physiology, Vol. 129, 1241–1251p, 2002.
101. MAHALAKSHM, M; MARISAMY, K; RAMASUBRAMANIAN, V.: Cadmium Toxicity Induced Changes on Morphological, Photosynthetic, Biochemical and Antioxidative Enzymes Level in Vigna mungo (L.) Hepper. Environment and Ecology Research 5(6): 422-426p, 2017.
102. MOORE, B. M; LEE, Y. S; WANG, P; AZOD, C; GROTEWOLD, E; SHIU, S. H.: Modeling temporal and hormonal regulation of plant transcriptional response to wounding. THE PLANT CELL, 34: 867–888p, 2021.
103. MOURA, S. S. S; SILVA, R. S; ALVES, E. U; GONÇALVES, E. P; ARAÚJO, L. D. A; ALVES, M. M; ARAUJO, P. C.: Morphology of seeds, seedlings, and young plants of Dimorphndra gardneriana Tul. Ciências Agrárias, Londrina, v. 40, n. 3, p. 1063-1078p, 2019.
104. MUNEER, S; KIM, T. H; QURESHI, M. I.: Fe modulates Cd-induced oxidative stress and the expression of stress responsive proteins in the nodules of Vigna radiata. Plant Growth Regul 68:421–433p, 2012.
105. MOHAMMAD, M; TAVAKOLI, A; POURYOUSEF, M. FARD, E. M.: Study the effect of 24-epibrassinolide application on the Cu/ZnSOD expression and tolerance to drought stress in common bean. Physiol Mol Biol Plants, 26(3):459–474 ,2020.
106. NOGUEIRA, G. A. S; BRITO, A. E. A; NASCIMENTO, V. R; ALBUQUERQUE, G. D. P; BOTELHO, S. A; SOUZA, L. C; FREITAS, J. M. N; AMARANTE, C. B; OKUMURA, R. S; CONCEIÇÃO, H. E. O; VIANA, R. G; NETO, C. F. O.: Physiological and Growth Responses in the (Schizolobium amazonicum Huber ex Ducke) Seedlings Subjected to Cadmium Doses. Journal of Agricultural Science; Vol. 11, No. 8, 217-224p, 2019.
107. NOGUEIRA, G. A. S; BRITO, A. E. A; RESENDE, V. N; ALBUQUERQUE, G. D. P; AMARANTES, C. B; OLIVEIRA, J. T; SILVA, P. A; NETO, C. F. O.: NITROGEN AND CARBON METABOLISM EVALUATION IN PARICÁ PLANTS SUBJECTED TO DIFFERENT CADMIUM CONCENTRATIONS. Bioscience Journal. vol. 38, 1-10p, 2022.
108. NOSAK, S; TERADA, T; NAKAMURA, A; HIRABAYASHI, K; XU, Y; BUI, T. B. C; NAKANO, T; TANOKURA, M; MIYAKAWA, T.: Highlighting the potential utility of MBP crystallization chaperone for Arabidopsis BIL1/BZR1 transcription factor-DNA complex. Scientifc Reports, 1-9p, 2021.
109. NOLAN, T. M; VUKASINOVIC, N; LIU, D. R; RUSSINOVA, E; YIN, Y. H.: Brassinosteroids: multidimensional Regulators of Plant Growth, Development, and Stress Responses. Plant Cell 32, 295–318p, 2020.
110. NAM, K. H; LI, J.: BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110, 203–212p, 2002.
111. NOUNURAI, P; AFIFAH, A; KITTISENACHAI, S; ROYTRAKUL, S.: Phosphorylation of CAD1, PLDdelta, NDT1, RPM1 Proteins Induce Resistance in Tomatoes Infected by Ralstonia solanacearum. Plants, 1-16p, 2022.

112. NGUYEN, T. Q; SESIN, V; KISIALA, A. R. J. N: Phytohormonal Roles in Plant Responses to Heavy Metal Stress: Implications for Using Macrophytes in Phytoremediation of Aquatic Ecosystems. Environmental Toxicology and Chemistry, Volume 40, N (1),7–22p, 2021.
113. OLIVEIRA, L. A. B; RESENDE, C. L. P; CINTRA, P. H. N; CARVALHO, D. D. C; RODRIGUES, F.:GROWTH PROMOTION OF Schizolobium parahyba var. amazonicum SEEDLINGS USING STRAINS OF Trichoderma spp. UNDER PHOSPHORUS RATES. FLORESTA, Curitiba, PR, v. 53, n.2, p. 165 – 173p, 2023.
114. OLIVEIRA, I. C; BREARS, T; KNIGHT, T. J; CLARK, A; CORUZZI, G. M.: Overexpression of cytosolic glutamine synthetase. Relation to nitrogen, light, and photorespiration. Plant Physiol. 129, 1170–1180p, 2002.
115. PALLIOTTI, A; TOMBESI, S; FRIONI T.: Physiological parameters and protective energy dissipation mechanisms expressed in the leaves of two Vitis vinifera L. genotypes under multiple summer stresses. J. Plant Physiol. 84-92p, 2015.
116. PAN, J. L; PLANT, J. A; VOULVOULIS N; OATES, C. J; IHLENFELD, C.: Cadmium levels in Europe: implications for human health. Environ Geochem Hlth 32, 1–12p, 2010.
117. PARMAR, P; KUMARI, N; SHARMA, V.: Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. Botanical Studies, 1-6p, 2013.
118. PAIVA, R. C; MACHADO, L. C; SOUSA, J. C. M; MORAES, V. C; CONCEIÇÃO, S. S; NOGUEIRA, G. A. S; OLIVEIRA, J. T; OKUMURA, R. S; SILVA, P. A; NETO, C. F. O: Influência do cádmio em desenvolvimento inicial de plantas de mogno africano. EUROPEAN ACADEMIC RESEARCH, Vol. IX, 1-16p. 2021.
119. QIAO, J; ZHANG, Y; HAN, S; CHANG, S; GAO, Z; QI, Y; QIAN, Q.: OsARF4 regulates leaf inclination via auxin and brassinosteroid pathways in rice. Front. Plant Sci. 1-16p, 2022.
120. RIVEROLA, A. P; GUPTA, A; PUTZE, I. B; BOSCH, N; IBAÑES, M; DELGADO, C.: Brassinosteroid signaling in plant development and adaptation to stress. Development, 1-11p, 2019.
121. REEVES, R. D; BAKER, A.J; JAFFRÉ, T; ERSKINE, P.D; ECHEVARRIA, G; VAN, D. E. A.: A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytol. 407–411p, 2018.
122. RIZVI, A; ZAIDI, A; AMEEN, F; AHMED, B; ALKAHTANI, M. D. F; KHAN, M. S.: Heavy metal induced stress on wheat: phytotoxicity and microbiological management. RSC Adv., 38379–38403p, 2020.
123. RAHMANI, R. S; SHI, T; ZHANG, D; GOU, X; YI, J; MICLOTTE, G; MARCHAL, K; LI, J.: Genome-wide expression and network analyses of mutants in key brassinosteroid signaling genes. BMC Genomics. 1-17p, 2021.
124. RAJEWSKA, I; TALAREK, M; BAJGUZ, A.: Brassinosteroids and Response of Plants to Heavy Metals Action. Front. Plant Sci. 1-5p, 2016.
125. RAZA, S. H; SHAFIQ, F; TAHIR; M.: Screening of Cadmium Tolerance in Sugarcane Using Antioxidative Enzymes as a Selection Criteria. Pak. j. life soc. Sci.,11(1): 8-13p, 2013.
126. REN, Y; LI, X; LIANG, J; WANG, S; WANG, Z; CHEN, H; TANG, M.: Brassinosteroids and gibberellic acid actively regulate the zinc detoxifcation mechanism of Medicago sativa L. Seedlings. BMC Plant

Biology, 1-13p, 2023.

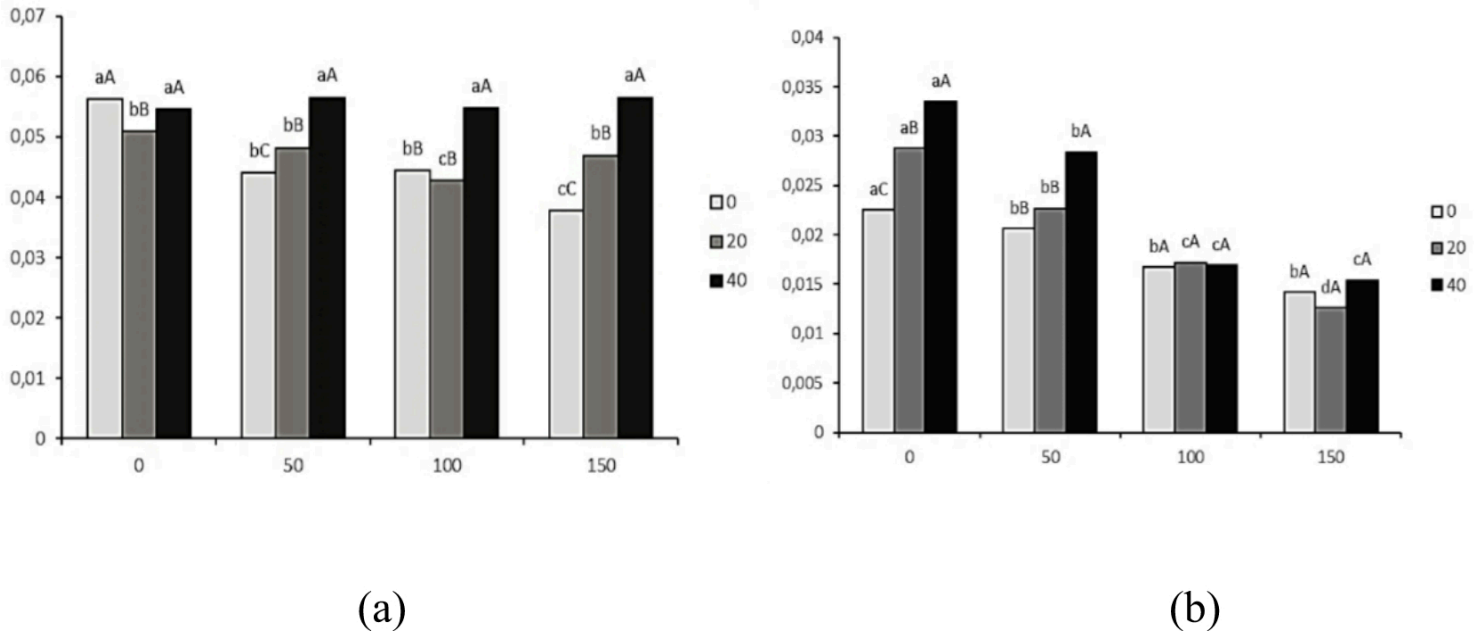
127. RODRIGUES, P. M. M; CONEGLIAN, A; SILVA, M. F; MORAES, M. D. A; JUNIOR, C. R. S.: Characterization of Schizolobium parahyba (Vell.) S.F. Blake and Eucalyptus urophylla S.T. Blake juvenile wood in Brazilian Savanna soil. **Revista de Ciências Agrárias**, 41(2): 539-547p, 2018.
128. RAZA, A; SALEHI, H; RAHMAN, A. M; ZAHID, Z; HAGHJOU, M. M; KAKAVAND, S. N; CHARAGH, S; OSMAN, H. S; ALBAQAMI, M; ALBAQAMI, M; ZHUANG, Y; SIDDIQUE, K. H. M; ZHUANG, W.: Brassinosteroid phytohormones - structure, bioactivity and applications. Braz. J. Plant Physiol., 14(3):143-181, 2022.
129. ROVERE, F. D; PIACENTINI, D; FATTORINI, L; GIRARDI, N; BELLANIMA, D; FALASCA, G; ALTAMURA, M.M; BETTI, C.: Brassinosteroids Mitigate Cadmium Effects in Arabidopsis Root System without Any Cooperation with Nitric Oxide. Int. J. Mol. Sci. 1-20p, 2022.
130. RYU, H; KIM, K; CHO, H; PARK, J; CHOE, S; HWANG, L.: Nucleocytoplasmic Shuttling of BZR1 Mediated by Phosphorylation Is Essential in Arabidopsis Brassinosteroid Signaling. The Plant Cell, Vol. 19: 2749–2762p, 2007.
131. SABELLA, E; LUVISI, A; GENGA, A; BELLIS, L; APRILE, A.: Molecular Responses to Cadmium Exposure in Two Contrasting Durum Wheat Genotypes. Int. J. Mol. Sci. 1-19, 2021.
132. SABELLA, E; APRILE, A; TENUZZO, B. A; CARATA, E; PANZARIN, E; LUVISI, A; BELLIS, L; VERGINE, M.: Effects of Cadmium on Root Morpho-Physiology of Durum Wheat. Frontiers in Plant Science, 1-17p. 2022.
133. SABIR, S; AKASH, M. S. H; FIAYYAZ, F; SALEEM, U; MEHMOOD, M. H; REHMAN, K.: Role of cadmium and arsenic as endocrine disruptors in the metabolism of carbohydrates: Inserting the association into perspectives. Biomedicine & Pharmacotherapy, 1-10p, 2019.
134. SACHDEV, S; ANSARI, S.A; ANSARI, M.I; FUJITA, M; HASANUZZAMAN, M.: Abiotic Stress and Reactive Oxygen Species: Generation, Signaling, and Defense Mechanisms. Antioxidants, 2-27p, 2021.
135. SILVA, J. M. P; LIMA, R. A. P.: Economia mineral e os impactos nos territórios amazônicos do sudeste paraense. Planeta Amazônia: Revista Internacional de Direito Ambiental e Políticas Públicas. Macapá, n. 9, p. 103-116, 2017.
136. SEREGIN, I. V; KOZHEVNIKOVA, A. D.: Phytochelatins: Sulfur-Containing Metal(loid)-Chelating Ligands in Plants. Int. J. Mol. Sci. 1-38p, 2023.
137. SEREGIN, I. V; KOZHEVNIKOVA, A. D; SCHAT, H.: Correlated variation of the Zn accumulation and tolerance capacities among populations and ecotypes of the Zn hyperaccumulator, Noccaea caerulescens. Rus. J. Plant Physiol, 26–36p. 2021.
138. SONG, J; FENG, S. J; CHEN, J; ZHAO, W. T; YANG, Z. M; A cadmium stress-responsive gene AtFC1 confers plant tolerance to cadmium toxicity. BMC Plant Biology, 1-15p, 2017.
139. SONG, X; YUE, X; CHEN, W; JIANG, H; HAN, Y; LI, X.: Detection of Cadmium Risk to the Photosynthetic Performance of Hybrid Pennisetum. Frontiers in Plant Science. 1-14p, 2019.

140. SOUSA, A. K. R; MORASSUTI, C. Y; DEUS, W. B.: POLUIÇÃO DO AMBIENTE POR METAIS PESADOS E UTILIZAÇÃO DE VEGETAIS COMO BIOINDICADORES. Acta Biomedica Brasiliensia. Volume 9. nº 3. 95 – 106p. 2018.
141. SOUZA, N. D; NETO, A. N. A; JUNIOR, A. J. S; OLIVEIRA, A. C. L; SAMPAIO, D. A; CUPERTINO, G. F. M; GONÇALVES, A. N; JÚNIOR, A. F. D.: A Brazilian Amazon Species with High Potential to Phytoextract Potential Toxic Elements. Floresta e Ambiente. 29(1): 1-8p, 2022.
142. SHAH, A. A; AHMED, S; YASIN, N. A.: 24-epibrassinolide triggers cadmium stress mitigation in Cucumis sativus through intonation of antioxidant system. South African Journal of Botany. 349-360p, 2019.
143. SHARMA, D; BHARDWAJ, S; RAZA, A; SINGH, R; KAPOOR, D; SHARMA, N. R; PRASAD, P. V. V.: Alleviatory Effects of Silicon and 24-Epibrassinolide in Modulation of Growth, Osmolytes, Metabolites, Antioxidant Defense System, and Gene Expression in Lead-Exposed Fenugreek (Trigonella foenum-graecum L.) Plants. Agronomy, 1-21p, 2023.
144. SHARMA, I; SHARMA, A; PATI, P; BHARDWAJ, R.: Brassinosteroids Reciprocates Heavy Metals Induced Oxidative Stress in Radish by Regulating the Expression of Key Antioxidant Enzyme Genes. Braz. Arch. Biol. Technol. v.61, 1-9p. 2018.
145. STIRK, W. A; BÁLINT, P; TARKOWSKÁ, D; STRNAD, M; STADEN, J. V; ORDOG.: Endogenous brassinosteroids in microalgae exposed to salt and lowtemperature stress. EUROPEAN JOURNAL OF PHYCOLOGY, VOL. 53, 273–279p, 2018.
146. SHI, G. L.: Accumulation and distribution of arsenic and cadmium in winter wheat (Triticum aestivum L.) at different developmental stages. Science of the Total Environment, v. 667, p. 532-539, 2019.
147. SUN, L; SONG, K; SHI, L; DUAN, D; ZHANG, H; SUN, Y; QIN, Q; XUE, Y.: Infuence of elemental súlfur on cadmium bioavailability, microbial community in paddy soil and Cd accumulation in rice plants. Scientifc Reports. 1-9p, 2021.
148. SIMKIN, A. J; CALCAGNO, P. E; RAINES, C. A.: Feeding the world: improving photosynthetic efficiency for sustainable crop production. J. Exp. Bot, 1119–1140p, 2019.
149. SALAMA, F. M; ALHUQAIL, A. A; ALI, M; ABEED, A. H.: Cd Phytoextraction Potential in Halophyte Salicornia fruticosa: Salinity Impact. Plants, 1-16p, 2022.
150. SANTOS, L. R; BATISTA, B. L; LOBATO, A.K.S; Brassinosteroids mitigate cadmium toxicity in cowpea plants. PHOTOSYNTHETICA 56 (2): 591-605p, 2018.
151. SHU, S; TANG, Y. Y; YUAN, Y. H; SUN, J; ZHONG, M; GUO, S. R.: The role of 24-epibrassinolide in the regulation of photosynthetic characteristics and nitrogen metabolism of tomato seedlings under a combined low temperature and weak light stress. Plant Physiol. Biochem. 107, 344–353p, 2016.
152. SHANGGUAN, X; QI, Y; WANG, A; REN, Y; WANG, Y; XIAO, T; SHEN, Z; WANG, O; XIA, Y.: OsGLP participates in the regulation of lignin synthesis and deposition in rice Against copper and cadmium toxicity. Front. Plant Sci, 1-14p, 2023.

153. TABELIN, C. B; IGARASHI T; VILLACORTE, T. M; PARK I, OPISO, E. M; ITO, M; HIROYOSHI, N.: Arsenic, selenium, boron, lead, cadmium, copper, and zinc in naturally contaminated rocks: a review of their sources, modes of enrichment, mechanisms of release, and mitigation strategies. Sci. Total Environ 645, 1522–1553p, 2018.
154. THEVENIN, J; POLLET, B; LETARNEC, B; SAULNIER, L; GISSOT; MAIA, G. A; LAPIERRE, C; JOUANIN, L.: The simultaneous repression of CCR and CAD, two enzymes of the lignin biosynthetic pathway, results in sterility and dwarfism in Arabidopsis thaliana. Mol Plant. 4(1):70–82, 2021.
155. TONG, H; CHU, C.: Functional specificities of brassinosteroid and potential utilization for crop improvement. Trends Plant Sci. 23, 1016–1028p, 2018.
156. TIAN, F; HAN, C; CHEN, X; WU, X JIAXUAN MI, J WAN, X; QLIU, Q; FANG HE, F; CHEN, L; YANG, H; ZHONG, Y; QIAN, Z; ZHANG, F.: PscCYP716A1-Mediated Brassinolide Biosynthesis Increases Cadmium Tolerance and Enrichment in Poplar. Frontiers in Plant Science. Vol. 13, 1-17p, 2022.
157. URAGUCHI, S; NAGAI, K; NARUSE, F; OTSUKA, Y; OHSHIRO, Y; NAKAMURA, R; TAKANEZAWA, Y; KIYONO, M.: Development of affinity bead–based in vitro metal–ligand binding assay reveals dominant cadmium affinity of thiol-rich small peptides phytochelatins beyond glutathione. Metallomics. 1-13p, 2021.
158. VILLIERS, F; JOURDAIN, A; BASTIEN, O; LEONHARDT, N; FUJIOKA, S; TICHTINCKY, G; PARCY, F; BOURGUIGNON, J; HUGOUVIEUX, V.: Evidence for functional interaction between brassinosteroids and cadmium response in Arabidopsis thaliana. Journal of Experimental Botany. Vol. 63, No. 3, 1185–1200p, 2012.
159. WANG, P; HENDRON, R.W; KELLY, S.: Transcriptional control of photosynthetic capacity: conservation and divergence from Arabidopsis to rice. New Phytologist. 216: 32–45p, 2017.
160. WANG, B; BIAN, B; WANG, C; LI, C; FANG, H; ZHANG, J; HUANG, D; HUO, J; LIAO, W.: Hydrogen gas promotes the adventitious rooting in cucumber under cadmium stress. PLoS ONE, 1-19p, 2019.
161. WEI, Z; LI, J.: Regulation of Brassinosteroid Homeostasis in Higher Plants. Front. Plant Sci. 1-17p, 2020.
162. WAHAB, A; ABDI, G; SALEEM, M. H; ALI, B; ULLAH, S; SHAH, W; MUMTAZ, S; YASIN, G; MURESAN, C. C; MARC, R. A.: Plants' Physio-Biochemical and Phyto-Hormonal Responses to Alleviate the Adverse Effects of Drought Stress: A Comprehensive Review. Plants, 1-27p, 2022.
163. WISZNIEWSKA, A; KOZMI NSKA, A; HANUS-FAJERSKA, E; DZIURKA, M; DZIURKA, K.: Insight into mechanisms of multiple stresses tolerance in a halophyte As-ter tripolium subjected to salinity and heavy metal stress. Ecotoxicol. Environ. Saf., 12–22p, 2019.
164. XU, D; CHEN, Z; SUN K.: Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (Solanum tuberosum L.). Ecotoxicol. Environ. Safe. 147-153p, 2013.
165. XIAN, J; WANG, Y; NIU, K; MA, H; MA, X.: Transcriptional regulation and expression network responding to cadmium stress in a Cd-tolerant perennial grass Poa Pratensis. Chemosphere, 1-12p, 2020.

166. YAN, Q; FANG, H; WANG, D; XIAO, X; DENG, T; LI, X; WEI, F; LIU, J; LIN, C.: Transfer and transformation characteristics of Zn and Cd in soil rotation plant (Brassica napus L and Oryza sativa L) system and its infuencing factors. Scientifc Reports, 1-21p, 2023.
167. YADAV, P; KAUR, R; KOHL, S. K; SIRHINDI, G; BHARDWAJ, R.: Castasterone assis-ted accumulation of polyphenols and antioxidant to increase tolerance of B. junceaplants towards copper toxicity. Cogent Food & Agriculture, 1-14p, 2016.
168. YUAN, J. S; GALBRAITH, D. W; DAI, S. Y; GRIFFIN, P; STEWART, C. N, J.: Plant systems biology comes of age. Trends Plant Sci. 13(4):165–71p, 2008.
169. YAO, R; FU, W; DU, M; CHEN, Z. X; LEI, A. P; WANG, J. X.: Carotenoids Biosynthesis, Accumulation, and Applications of a Model Microalga Euglena gracilis. Mar. Drugs, 1-15p, 2022.
170. YIN, X; TANG, M; XIA, X; YU, J.: BRASSINAZOLE RESISTANT 1 Mediates Brassinosteroid-Induced Calvin Cycle to Promote Photosynthesis in Tomato. Front. Plant Sci, 1-13p, 2022.
171. YU, N; TEHRIM, S; WANG, L; DOSSA, K; ZHANG, X; KE, T; LIAO, B.: Evolutionary history and functional divergence of the cytochrome P450 gene superfamily between Arabidopsis thaliana and Brassica species un cover effects of whole genome and tandem duplications. BMC Genomics. 1-27p. 2017.
172. YU, J. Q; HUANG, L. F; HU, W. H; ZHOU, Y. H; MAO, W. H; YE, S. F; NOGUÊS, S.: A role for brassinosteroids in the regulation of photosynthesis in Cucumis sativus. Journal of Experimental Botany, Vol. 55, No. 399, 1135-1143p, 2004.
173. ZHANG, J; SHOAIB, N; LIN, K; MUGHAL N, WU, X; SUN, X; ZHANG, L; PAN, K.: Boosting cadmium tolerance in Phoebe zhennan: the synergistic effects of exogenous nitrogen and phosphorus treatments promoting antioxidant defense and root development. Front. Plant Sci, 1-17p, 2024.
174. ZHAO, G; XU, H; ZHANG, P; SU, X; ZHAO, H.: Effects of 2,4-epibrassinolide on photosynthesis and Rubisco activase gene expression in Triticum aestivum L. seedlings under a combination of drought and heat stress. Plant Growth Regul, 81:377–384p, 2017.
175. ZHENG, T; YANG, F; ZHANG, D.: Synergetic Modulation of Plant Cadmium Tolerance via MYB75-mediated ROS Homeostasis and Transcriptional Regulation. Research Square, 1-16p, 2022.
176. ZULLO, M. A. T; ADAM, G.: Brassinosteroid phytohormones - structure, bioactivity and applications. Braz. J. Plant Physiol., 14(3):143-181, 2002.
177. ZHU, J.Y; LI, Y; CAO, D. M; YANG, H; OH, E; BI, Y; ZHU, S; WANG, Z.Y.: The F-box Protein KIB1 mediates brassinosteroidinduced inactivation and degradation of GSK3-like kinases in Arabidopsis. Mol Cell 66: 648–657p. 2016.
178. ZHU, J. K.: Abiotic stress signaling and responses in plants. Cell. 313–324p, 2016.

Figures



(a) (b)

Figure 1

Effect of $CdCl_2$ (0 µM, 50 µM, 100 µM, and 150 µM) and 2-EBL (0 nM, 20 nM, and 40 nM) on seedling height (a) and root length (b) of the species *Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ). Legend: Lowercase letters indicate statistical differences among $CdCl_2$ treatments ($p < 0.05$) based on Tukey's test; uppercase letters indicate statistical differences among 24-EBL treatments ($p < 0.05$) based on Tukey's test.

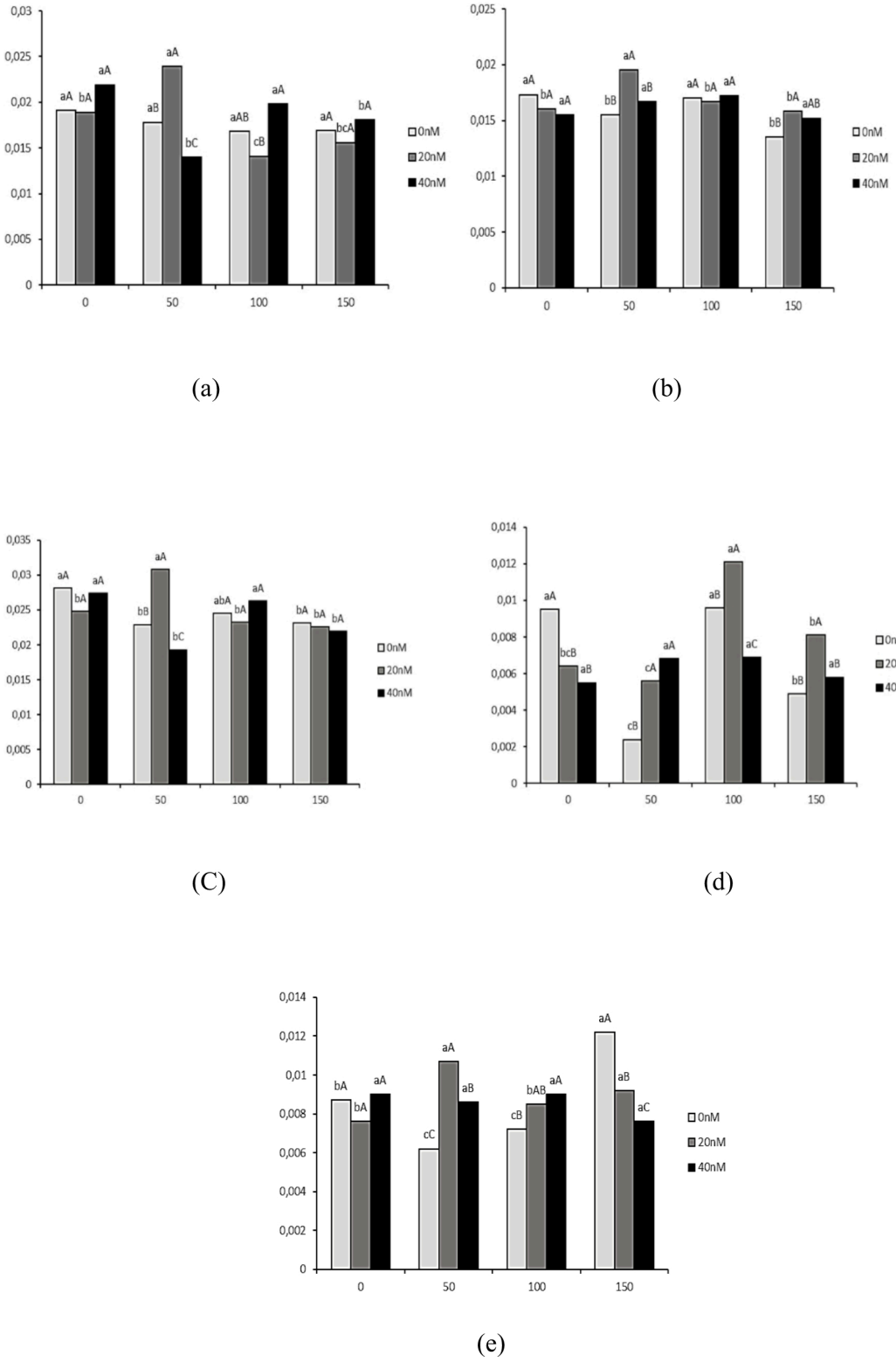


Figure 2

Influence of 24-epibrassinolide on the attenuation of CdCl2 in the distribution of Chlorophyll a (a), Chlorophyll b (b), Total Chlorophyll (a+b) (c), Anthocyanins (d), and Carotenoids (e) in the species Schizolobium parahyba var. amazonicum (PARICÁ). Legend: Lowercase letters indicate statistical differences among CdCl2 treatments ($p < 0.05$) based on Tukey's test; uppercase letters indicate statistical differences among 24-EBL treatments ($p < 0.05$) based on Tukey's test.

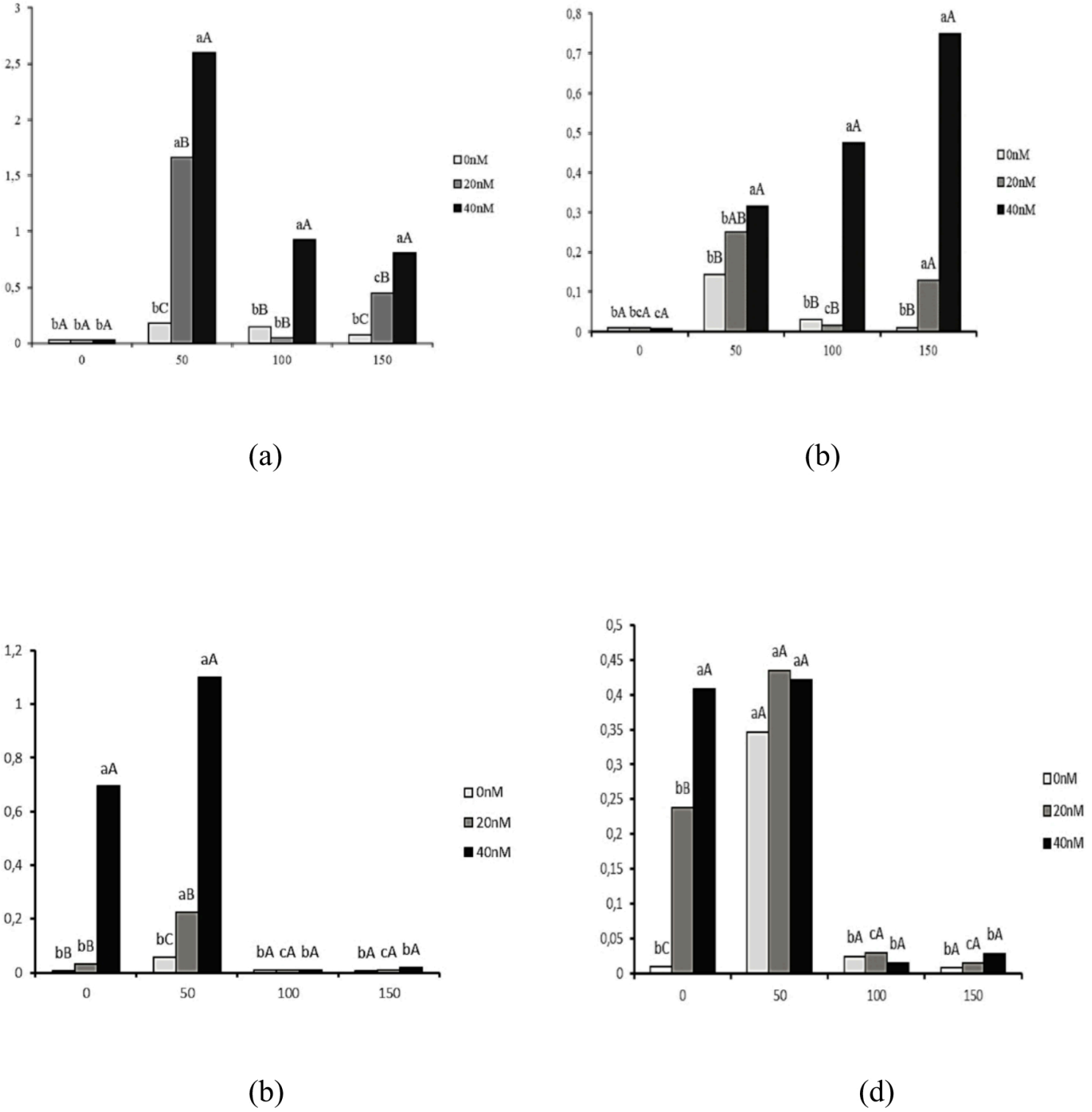


Figure 3

qPCR-RT analysis of the $CPY_{85}A_2$ and BZR1 genes of *Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ) under different dosages of $CdCl_2$ and 24-EBL. (a) qPCR-RT of the *$CPY_{85}A_2$* gene located in the leaflets; (b) qPCR-RT of the *BZR1* gene located in the leaflets; (c) qPCR-RT of the *$CPY_{85}A_2$* gene located in the root system; (d) qPCR-RT of the *BZR1* gene located in the root system. Legend: Lowercase letters indicate statistical differences between $CdCl_2$ treatments ($p < 0.05$) based on

the Tukey test; uppercase letters indicate statistical differences between 24-EBL treatments ($p < 0.05$) based on the Tukey test.

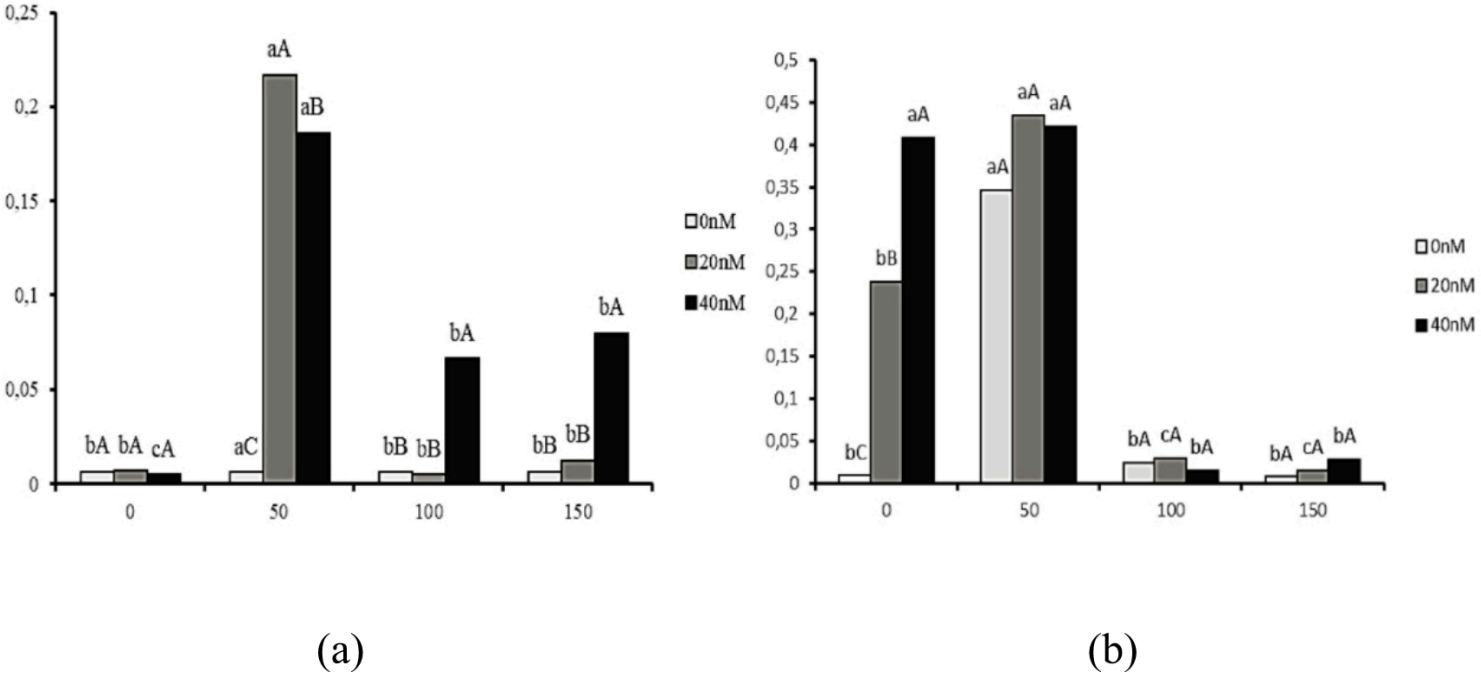


Figure 4

Figure 8: qPCR-RT analysis of the *CAD1* gene in the species *Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby* (PARICÁ) under different dosages of $CdCl_2$ and 24-EBL. (a) qPCR-RT of the *CAD1* gene located in the leaflets; (b) qPCR-RT of the *CAD1* gene located in the root system. Caption: Lowercase letters indicate statistical differences among $CdCl_2$ treatments ($p < 0.05$) based on Tukey's test; uppercase letters indicate statistical differences among 24-EBL treatments ($p < 0.05$) based on Tukey's test.