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ENHANCING CADMIUM TOLERANCE IN COMMON BEAN PLANTS BY SEED PRIMING WITH PUTRESCINE

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Abstract: This study evaluates the efficacy of putrescine-based seed biopriming at concentrations of 0, 0.25, 0.5, and 1 mmol in mitigating oxidative stress induced by 50 mg kg^{-1} cadmium (Cd) in common bean plants. Cadmium exposure significantly elevated oxidative stress markers, such as hydrogen peroxide (H_2O_2), while suppressing antioxidative enzyme activities, including ascorbate peroxidase (APX). Putrescine treatments, particularly at 0.5 and 1 mmol, enhanced antioxidative defenses by increasing superoxide dismutase (SOD) and APX activities and reducing H_2O_2 levels, thereby alleviating oxidative damage.

Photosynthetic performance improved markedly with putrescine application, as evidenced by higher chlorophyll a content, an optimized chlorophyll a/b ratio, and increased total carotenoid levels, indicating enhanced photosynthetic efficiency under cadmium stress. Among the treatments, Cd-P3 (1 mmol putrescine) demonstrated the most significant improvements, reversing the detrimental effects of cadmium on photosynthetic pigments and plant health. Additionally, putrescine enhanced the accumulation of total phenolic and flavonoid compounds, contributing to improved antioxidant capacity. This was supported by higher DPPH radical scavenging activity and FRAP values, highlighting its strong antioxidative potential.

In summary, putrescine seed priming offers a promising strategy for mitigating cadmium toxicity in plants. By modulating antioxidant systems, stabilizing photosynthetic pigments, and promoting bioactive compound synthesis, putrescine enhances plant resilience to heavy metal stress. These findings underscore its potential application in agricultural practices to improve crop tolerance to abiotic stresses.

Keywords: Putrescine seed priming, Cadmium stress mitigation, Antioxidant enzyme activity, Photosynthetic pigments, Heavy metal stress tolerance

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1. Introduction

Modern agriculture is facing significant transformations driven by escalating environmental challenges, further intensified by the rapid growth of the global population (Yilmaz et al., 2024). The Green Revolution, while significantly enhancing agricultural productivity, has also led to unintended environmental consequences, including soil degradation and pollution of soil and water resources due to the excessive use of chemical inputs (Yilmaz and Yilmaz, 2025). A critical outcome of this intensive chemical use is the accumulation of heavy metals in agricultural soils. Soil contamination with heavy metals significantly disrupts ecosystems and poses severe risks to human health by compromising food quality, reducing arable land, and enabling the transfer of toxic elements through direct soil contact, polluted water, and the food chain (Haider et al., 2021). Among these contaminants, cadmium (Cd) is particularly concerning due to its high toxicity and environmental persistence. The primary sources of cadmium contamination in agricultural soils include industrial emissions, the application of cadmium-containing fertilizers, and the use of wastewater for irrigation (Lemessa et al., 2022). Although cadmium serves no biological purpose, its presence is highly detrimental to both plant and animal systems (Genchi et al., 2020). This metal is predominantly associated with industrial processes and phosphate-based fertilizers (Gupta et al., 2014). While cadmium is relatively rare in the Earth's crust, with an average concentration of approximately 0.2 mg kg^{-1}, it often co-occurs with zinc, lead, and copper sulfide ores (Kubier et al., 2019; Genchi et al., 2020). Its behavior and distribution in soils are influenced by several factors, including pH, clay content, soluble organic matter, and the presence of organic and inorganic ligands (Hamid et al., 2020).

Cadmium toxicity exerts multi-dimensional impacts on plants, affecting morphological, physiological, biochemical, and molecular processes (Farid et al., 2013). It disrupts essential metabolic activities such as photosynthesis and respiration, resulting in reduced root activity, stunted seedling growth, chlorosis, and ultimately plant death (Li et al., 2024; Ningombam et al., 2024). The physiological disorders caused by cadmium

toxicity are closely linked to oxidative stress, driven by excessive reactive oxygen species (ROS) production (El Rasafi et al., 2022). To counter these effects, plants have evolved various defense mechanisms, enabling survival in cadmium-contaminated soils (Raza et al., 2020). These mechanisms can be broadly classified into two strategies: reducing metal uptake to minimize toxicity and enhancing tolerance through the accumulation, storage, or immobilization of harmful elements within specific tissues (Kushwaha et al., 2015; Sarwar et al., 2017).

Recent advancements in stress management emphasize the efficacy of seed biopriming, wherein seeds are treated with beneficial agents to enhance resilience to abiotic stresses. Among these agents, putrescine, a polyamine, has shown promising potential in mitigating cadmium toxicity in various plants, including *Coriandrum sativum*, *Triticum aestivum*, *Camelina sativa*, and *Brassica napus*. Studies indicate that putrescine enhances antioxidant enzyme activity, promotes nutrient uptake, and supports plant health under heavy metal stress conditions (Tajti et al., 2018; Jahan et al., 2021; Sardar et al., 2022). As a critical polyamine, putrescine regulates plant growth and development by influencing key processes such as root elongation, flowering, fruit maturation, and stress responses (González-Hernández et al., 2022). Additionally, it functions as a signaling molecule and modulator of phytohormonal pathways, improving stress tolerance by regulating ROS levels, maintaining ion homeostasis, and interacting with hormones like abscisic acid (ABA), salicylic acid (SA), and indole-3-acetic acid (IAA) (Hussain et al., 2011; Liu et al., 2015; Mustafavi et al., 2018).

The common bean (*Phaseolus vulgaris* L.) is a nutritionally rich legume crop widely cultivated across diverse agro-climatic regions (Yılmaz et al., 2023). Renowned for its high protein, vitamin, and mineral content, it serves as a staple food in many parts of the world (Yılmaz, 2024). Despite its agricultural and economic importance, the common bean is highly sensitive to abiotic stresses, including heavy metal toxicity, which significantly impacts its growth, yield, and quality (Lone et al., 2021).

This study investigates the potential of putrescine-based seed biopriming to mitigate cadmium-induced stress in common bean plants subjected to 50 mg kg^{-1} of Cd. Seed treatments with putrescine at concentrations of 0, 0.25, 0.5, and 1 mmol were evaluated for their effects on oxidative stress markers, including malondialdehyde (MDA), antioxidant enzymes such as ascorbate peroxidase (APX) and superoxide dismutase (SOD), and photosynthetic pigments. The analyzed pigments included chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, and the chlorophyll a/b ratio. This is the first research to thoroughly investigate its effects on oxidative stress, antioxidant enzyme activity, and photosynthetic performance in common bean plants under cadmium stress.

2. Materials and Methods

2.1. Plant Material

The study was conducted using the dwarf common bean variety "Alberto," obtained from Harmas Global Agriculture and Industry Inc. The experiment took place under controlled environmental conditions in a climate chamber at the Faculty of Agriculture, Bolu Abant İzzet Baysal University, during November and December 2024. The climate chamber was maintained at a constant temperature of 24 °C with 70% relative humidity, and the plants were grown under a 16-hour light/8-hour dark photoperiod, ensuring optimal conditions for the study.

2.2. Experimental Design

Each pot, with a capacity of 1.5 kg, was filled with a substrate composed of two-thirds soil and one-third peat. Before sowing, cadmium was introduced into the soil at a concentration of 50 mg kg^{-1}. The seeds underwent surface sterilization by immersion in a 4% sodium hypochlorite solution for 5 minutes, followed by thorough rinsing with distilled water (4–5 times). After sterilization, the seeds were subjected to putrescine treatments at four different concentrations (0, 0.25, 0.5, and 1 mmol) by soaking them in the respective solutions at room temperature for 12 hours (Hussein et al., 2023). Following the biopriming procedure, three seeds were sown per pot. The plants were cultivated for three weeks under a randomized plot design with three replications. At the end of the growth period, leaf samples were collected and promptly stored at -80°C for subsequent analyses

2.3. Chlorophyll, and Carotenoid Contents

Total chlorophyll, carotenoid content, and chlorophyll a and b levels were assessed using the method described by Arnon (1949). For the chlorophyll analysis, 0.1 g of fresh leaf tissue was ground in 80% acetone, and the absorbance was measured at wavelengths of 663 nm, 645 nm, and 470 nm with a UV-visible spectrophotometer. Carotenoid analysis involved homogenizing 100 mg of leaf tissue in 80% (v/v) acetone, followed by filtration through filter paper, and measuring the absorbance of the filtrate at 470 nm. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were determined in milligrams per gram of fresh weight (mg g^{-1} FW) using the following equations:

Carotenoid (mg g^{-1}) = [((1000 × A470) − (2.27 × Chla) − (81.4 × Chlb)) / 227] × V / g

Chlorophyll a (mg g^{-1} F.W.) = (12.7 × A663 − 2.69 × A645) × V / 1000 × g

Chlorophyll b (mg g^{-1} F.W.) = (22.9 × A645 − 4.68 × A663) × V / 1000 × g

Total chlorophyll (mg g^{-1} F.W.) = (20.2 × A645 + 8.02 × A663) × V / 1000 × g

In these formulas, V; represents the volume of the extract, g is the weight of the sample, Chla; denotes chlorophyll a, Chlb; indicates chlorophyll b, and A refers to absorbance at the specified wavelengths.

2.4. Hydrogen Peroxide (H_2O_2) Analysis

Hydrogen peroxide (H_2O_2) levels were quantified spectrophotometrically based on its reaction with potassium iodide (KI) as described by Alexieva et al. (2001). For this analysis, 500 mg of leaf tissue was homogenized in 2.5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 12.000×g for 15 minutes. The reaction mixture was prepared by combining 0.5 mL of the resulting supernatant with 0.5 mL of 100 mM potassium phosphate buffer (pH 7.0) and 2 mL of 1 M KI reagent. After incubation in the dark for 1 hour, absorbance was recorded at 390 nm. The hydrogen peroxide concentration was determined using a standard curve generated from serial dilutions of H_2O_2 solutions (100 µM).

2.5. Ascorbate Peroxidase (APX) and Superoxide Dismutase (SOD) Analysis

Ascorbate peroxidase (APX) activity was analyzed by monitoring the reduction in absorbance at 290 nm. Samples (200 mg) were homogenized in 2 mL of an extraction buffer containing sodium phosphate, sodium EDTA, and ascorbic acid, followed by centrifugation at 15.000 rpm. The reaction mixture included sodium phosphate buffer (pH 7.0), ascorbic acid, and EDTA, to which 0.1 mL of the sample extract and 0.1 mL of H_2O_2 were added. Activity was determined using an ascorbic acid standard curve (Yilmaz and Kulaz, 2019). Superoxide dismutase (SOD) activity was assessed by evaluating its capacity to inhibit the reduction of nitro blue tetrazolium (NBT). Homogenized samples (200 mg) in a buffer containing sodium phosphate and EDTA were centrifuged at 15.000 rpm. The reaction mixture included methionine, NBT, EDTA, sodium phosphate, sodium carbonate, and riboflavin. Following light exposure (75 µmol m^{-2} s^{-1}) for 15 minutes, absorbance at 560 nm was used to calculate SOD activity based on the degree of NBT photochemical reduction inhibition (Beauchamp and Fridovich, 1971).

2.6 Antioxidant Assays for Phenolic, Flavonoid Content, and Antioxidant Activity

The total phenolic content was determined using a modified microscale approach as described by Waterhouse (2002). The assay involved mixing the sample with Folin-Ciocalteu reagent and sodium carbonate, followed by incubation in the dark for two hours. Absorbance was then measured at 760 nm using a spectrophotometer. Gallic acid served as the standard, and the results were expressed in mg/g gallic acid equivalents (GAE). The total flavonoid content was determined using a modified method adapted from Feduraev et al. (2022). Plant extracts or standard solutions were reacted sequentially with sodium nitrite, aluminum chloride, and sodium hydroxide, followed by dilution to the final volume. Absorbance was recorded at 430 nm, with quercetin as the standard, and the results were expressed in mM. The antioxidant capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay based on the method by Ünal et al. (2023). Samples were mixed with DPPH in ethanol, and absorbance was measured at 517 nm after 15 minutes of reaction time. Ascorbic acid was employed as the standard to calculate scavenging activity, and the EC_{50} value, indicating the concentration required to achieve a 50% reduction in DPPH radicals, was expressed in mg mL^{-1}.

The ferric reducing antioxidant power (FRAP) assay was performed following the protocol of Benzie and Strain (1996). The samples and standards were reacted with a FRAP reagent diluted 1:1, and absorbance was recorded at the specified wavelength. L-ascorbic acid was used as the standard, and the results were reported in mM.

2.7. Statistical Analysis

The experiment was designed as a randomized plot layout, incorporating three biological replicates and three technical replicates for each treatment. To assess the impact of cadmium stress and the applied treatments, a one-way analysis of variance (ANOVA) was performed. Pairwise comparisons between the control group and the treatments were conducted using Duncan's Multiple Range Test. Correlation analyses were conducted to explore the relationships between yield parameters and antioxidant enzyme activities under water stress, using Pearson's coefficient. Data were visualized with the 'corrplot' R package (Wei et al., 2017).

3. Results and Discussion

3.1. Plant Defense Mechanisms in Response to Cadmium Stress

The statistical analysis results revealed that the treatments, including different doses of putrescine (0, 0.25, 0.5, and 1 mmol) and cadmium (50 ppm), had a highly significant effect on the measured parameters, including SOD (superoxide dismutase) activity, hydrogen peroxide (H_2O_2) content, APX (ascorbate peroxidase) activity, chlorophyll a, chlorophyll b, chlorophyll a/b, total chlorophyll, total carotenoid content, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, FRAP antioxidant capacity, and CUPRAC antioxidant capacity, with an F-value indicating statistical significance at $p < 0.001$ (Table 1, Figure 1).

Table 1. ANOVA table showing the minimum, maximum, mean, standard error, F-value, and %CV for rhizobacterial applications in common bean

Variables	Min.	Max.	Mean	Std. Error	F value
SOD	70.42	220.97	138.09	6.97	33.64***
H_2O_2	1094	2464.2	1704.9	83.91	249.26***
APX	0.27	1.27	0.71	0.06	187.53***
Chlo a	0.01	0.03	0.016	0.001	121.22***
Chlo b	0.004	0.02	0.010	0.001	164.97***
Chlo a/b	0.31	5.04	2.24	0.29	369.27***
TChlo	0.019	0.03	0.026	0.001	30.01***
TCar	125.0	244.94	197.84	6.89	203.72***
TPC	6.44	15.14	11.17	0.47	199.04***
TFC	2.45	6.92	4.84	0.24	46.02***
DPPH	21.3	66.85	43.82	2.63	198.76***
FRAP	15.11	45.54	31.93	1.81	89.51***

Significant differences according to Duncan test; *** (P≤0.001)

The Cd treatment (121.07 U g^{-1} FW) increased SOD activity by 46.6% compared to the control (82.60 U g^{-1} FW), highlighting oxidative stress induced by cadmium. Among the putrescine treatments, P3 (166.75 U g^{-1} FW) was the most effective, showing a 102% increase compared to the control. Against cadmium alone, Cd-P3 (197.39 U g^{-1} FW) further enhanced SOD activity by 63%, demonstrating its superior ability to mitigate oxidative stress.

Cadmium application significantly increased H_2O_2 levels (2444.60 µmol g^{-1} FW), a 24% rise compared to the control (1965.50 µmol g^{-1} FW). In contrast, putrescine treatments reduced H_2O_2 levels, with P3 (1133.70 µmol g^{-1} FW) achieving the greatest reduction (42%). Among cadmium-treated groups, Cd-P3 (1615.00 µmol g^{-1} FW) reduced H_2O_2 by 34%, confirming the effectiveness of putrescine in mitigating oxidative damage.

APX activity was highest in the control group (1.26 mM g FW min^{-1}). Cadmium alone caused a 75% reduction, indicating severe oxidative stress. Putrescine treatments significantly restored APX activity, with P1 (1.01 mM g FW min^{-1}) showing the strongest protection (only 20% reduction). Cd-P3 (0.99 mM g FW min^{-1}) exhibited a 219% improvement over cadmium alone, highlighting its remarkable protective effect. Overall, Cd-P3 consistently demonstrated superior performance in mitigating cadmium-induced oxidative stress.

The highest chlorophyll a content was recorded in the P3 treatment (0.024 mg g^{-1} FW), followed by Cd-P3 (0.021 mg g^{-1} FW). Cadmium alone (Cd, 0.007 mg g^{-1} FW) resulted in the lowest chlorophyll a level, showing a significant decline compared to the control (0.013 mg g^{-1} FW). Among cadmium treatments, Cd-P3 demonstrated the most effective mitigation, with a 200% increase in chlorophyll a content compared to cadmium alone.

Interestingly, chlorophyll b content was highest in the Cd treatment (0.021 mg g^{-1} FW), reflecting stress-induced chlorophyll accumulation. Among cadmium + putrescine treatments, Cd-P1 (0.014 mg g^{-1} FW) showed the best mitigation effect, followed by Cd-P2 (0.013 mg g^{-1} FW) and Cd-P3 (0.010 mg g^{-1} FW). In contrast, the lowest chlorophyll b content was recorded in the P3 treatment (0.004 mg g^{-1} FW), indicating that putrescine alone without cadmium has limited impact on chlorophyll b levels.

The chlorophyll a/b ratio, an indicator of photosynthetic efficiency, was highest in the P3 treatment (4.92), followed by P2 (3.67) and P1 (2.97), demonstrating the enhancing effect of putrescine alone. Among cadmium treatments, Cd-P3 (2.09) showed the greatest improvement compared to cadmium alone (0.34), highlighting putrescine's role in alleviating the negative effects of cadmium stress on the chlorophyll a/b balance.

The highest total chlorophyll content was observed in the Cd-P3 treatment (0.031 mg g^{-1} FW), followed by Cd-P2 (0.030 mg g^{-1} FW) and P3 (0.029 mg g^{-1} FW). Cadmium alone (Cd, 0.028 mg g^{-1} FW) exhibited slightly higher levels than the control (C, 0.022 mg g^{-1} FW), likely due to stress-induced chlorophyll changes. Among all treatments, Cd-P3 was the most effective in enhancing total chlorophyll content, suggesting that putrescine applications, particularly at higher doses, mitigate cadmium stress and support chlorophyll synthesis.

For total carotenoids, the highest content was recorded in the Cd-P3 treatment (239.36 mg g^{-1} FW), followed by Cd-P2 (222.85 mg g^{-1} FW) and Cd-P1 (213.57 mg g^{-1} FW), demonstrating the effectiveness of putrescine in mitigating cadmium stress. Cadmium alone (Cd, 168.08 mg g^{-1} FW) showed a significant reduction compared to the control (C, 127.68 mg g^{-1} FW), reflecting the oxidative damage caused by cadmium. Among treatments without cadmium, P3 (211.52 mg g^{-1} FW) exhibited the highest carotenoid content, highlighting its protective role. Overall, Cd-P3 provided the most substantial enhancement of carotenoid content, effectively reducing cadmium-induced oxidative stress and supporting antioxidant capacity.

The highest total phenolic content (TPC) was observed in the Cd-P3 treatment (14.67 mg mL^{-1} GAE eq), followed by Cd-P2 (12.77 mg mL^{-1} GAE eq) and Cd-P1 (12.36 mg mL^{-1} GAE eq), underscoring the ameliorative effect of putrescine under cadmium stress. Cadmium alone (Cd, 10.25 mg mL^{-1} GAE eq) caused an increase compared to the control (C, 6.62 mg mL^{-1} GAE eq), indicating stress-

induced phenolic accumulation. Among treatments without cadmium, P3 (11.70 mg mL^{-1} GAE eq) showed the highest TPC. Overall, Cd-P3 demonstrated the greatest improvement, highlighting its role in enhancing phenolic content and alleviating oxidative stress caused by cadmium.

For total flavonoid content (TFC), the highest level was recorded in the Cd-P3 treatment (6.69 mg mL^{-1} QE eq), followed by Cd-P2 (5.74 mg mL^{-1} QE eq), demonstrating putrescine's effectiveness in mitigating cadmium stress. Among treatments without cadmium, P3 (5.23 mg mL^{-1} QE eq) showed the highest TFC. Cadmium alone (Cd, 3.91 mg mL^{-1} QE eq) resulted in a moderate increase compared to the control (C, 2.75 mg mL^{-1} QE eq), reflecting stress-induced flavonoid synthesis. Cd-P3 provided the most significant enhancement, emphasizing its strong role in reducing oxidative stress and promoting flavonoid accumulation.

The highest DPPH radical scavenging activity was observed in the P3 treatment (65.01 mg mL^{-1} ASA eq), followed by Cd-P3 (57.14 mg mL^{-1} ASA eq), reflecting the potent antioxidant effect of putrescine, particularly in the absence of cadmium. Among cadmium treatments, Cd-P3 showed the greatest improvement, with a 64% increase in antioxidant activity compared to cadmium alone (34.81 mg mL^{-1} ASA eq). The control group (C, 22.02 mg mL^{-1} ASA eq) exhibited the lowest activity, underscoring the stress-induced increase in antioxidant capacity due to cadmium and putrescine treatments. Overall, P3 and Cd-P3 significantly enhanced the antioxidant defense system, highlighting their effectiveness in alleviating cadmium-induced oxidative stress.

The highest FRAP value was recorded in the P3 treatment (45.04 mg mL^{-1} ASA eq), followed by Cd-P3 (40.19 mg mL^{-1} ASA eq), highlighting the strong antioxidant potential of putrescine, both in the presence and absence of cadmium. Among cadmium treatments, Cd-P3 demonstrated a significant improvement, with a 59% increase in FRAP activity compared to cadmium alone (25.36 mg mL^{-1} ASA eq). The control group (C, 15.52 mg mL^{-1} ASA eq) showed the lowest antioxidant capacity, reflecting the stress-induced enhancement achieved through cadmium and putrescine treatments. Overall, P3 and Cd-P3 were the most effective in enhancing antioxidant activity.

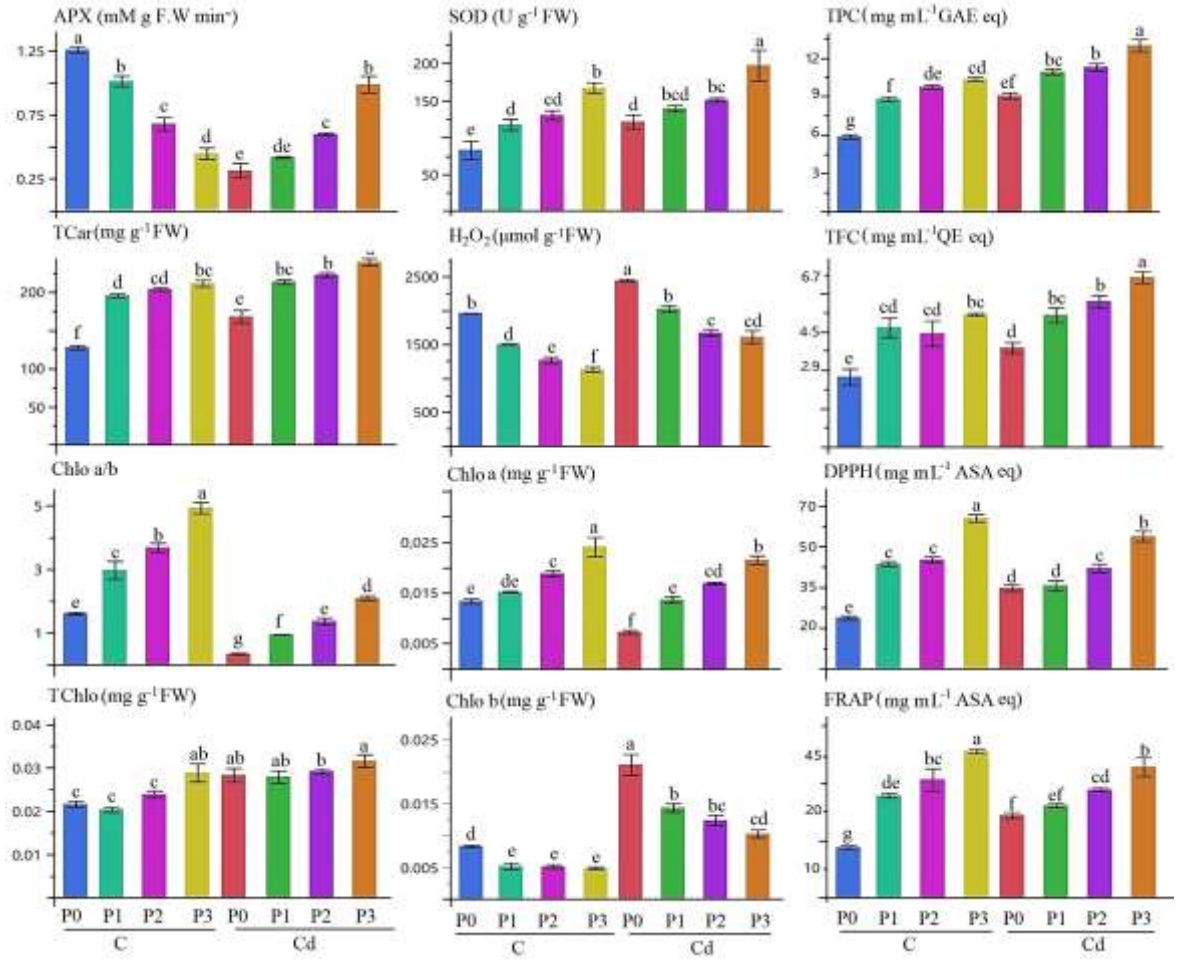

Figure 1. Effects of putrescine (0, 0.25, 0.5, and 1.0 mmol) on chlorophyll a (Chlo a), chlorophyll b (Chlo b), chlorophyll a/b ratio (Chlo a/b), total chlorophyll (TChlo), total carotenoid content (TCar), ascorbate peroxidase activity (APX), superoxide dismutase activity (SOD), hydrogen peroxide content (H_2O_2), DPPH radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC) in common bean plants under cadmium stress (50 mg kg^{-1}) (C= control; Cd= cadmium).

3.2 Correlations Among Studied Characteristics

The correlation analysis revealed many significant relationships between physiological, biochemical, and antioxidative parameters (Figure 2). A strong negative correlation between hydrogen peroxide (H_2O_2) and antioxidant enzymes such as SOD (r= -0.40) and APX (r= -0.26) indicates that increased oxidative stress, marked by higher H_2O_2 levels, suppresses the enzymatic defense system. Conversely, positive correlations among chlorophyll pigments (CHL A, CHL B, and Total Chlorophyll) demonstrate the synchronized regulation of the photosynthetic apparatus under cadmium stress. The strong negative correlation of CHL A/B with H_2O_2 (r= -0.92) further emphasizes the detrimental effect of oxidative stress on the photosynthetic efficiency.

CUPRAC, a key measure of antioxidant capacity, exhibited robust positive correlations with TPC (r= 0.70), TFC (r= 0.72) and DPPH (r= 0.93) highlighting the critical role of phenolic and flavonoid compounds in scavenging reactive oxygen species (ROS) and mitigating oxidative damage. Similarly, FRAP (r= 0.92) showed strong correlations with CUPRAC, underscoring the complementary nature of these antioxidant capacity assays. These findings collectively emphasize the pivotal role of antioxidant systems in protecting chlorophyll content, enhancing carotenoid levels, and reducing oxidative stress caused by cadmium toxicity. Overall, the results underscore the intricate interplay between antioxidative defenses, photosynthetic pigments, and bioactive compounds in alleviating cadmium-induced oxidative damage, providing insights into potential mechanisms underlying plant resilience under heavy metal stress.

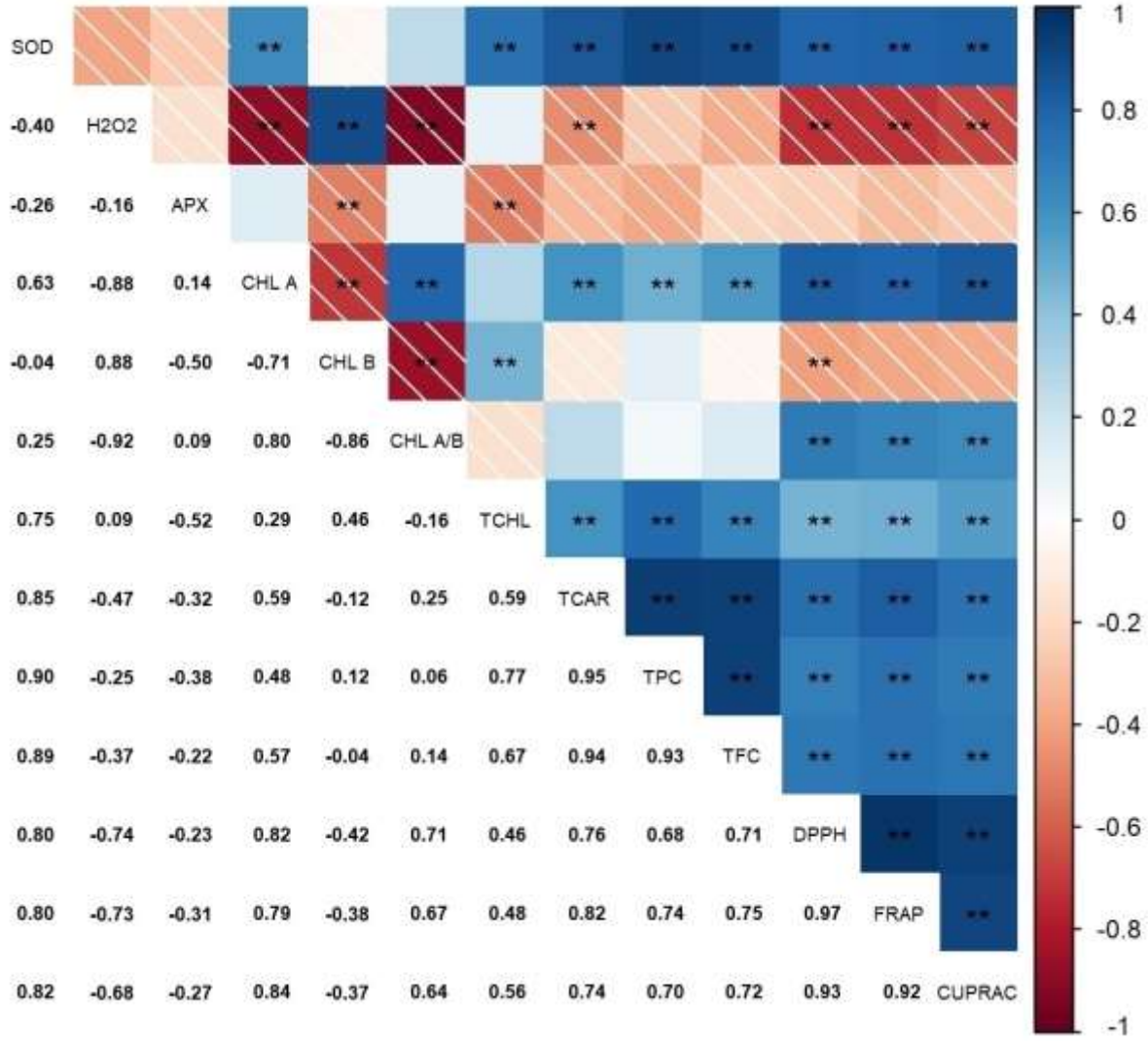

Figure 2. Correlations between the studied characteristics in common bean. *, and ** indicates significance at p≤0.05, p≤0.01, respectively (SOD= superoxide dismutase activity, H_2O_2= hydrogen peroxide, APX= ascorbate peroxidase activity, CHL A= chlorophyll a, CHL B= chlorophyll b, CHL A/B= chlorophyll a/b ratio, TCHL= total chlorophyll, TCar= total carotenoid content, TPC= total phenolic content, TFC= total flavonoid content, DPPH= DPPH radical scavenging activity, FRAP= ferric reducing antioxidant power, CUPRAC= cupric reducing antioxidant capacity)

4. Discussion

This study demonstrates the protective role of putrescine in mitigating cadmium (Cd)-induced oxidative stress in common bean plants. The findings reveal significant improvements in antioxidative enzyme activities, reductions in oxidative damage markers, and enhancements in photosynthetic pigments and antioxidant compounds under putrescine treatments, particularly at higher doses. These results align with previous studies highlight the efficacy of polyamines, such as putrescine, in alleviating heavy metal stress. Putrescine, a type of polyamine, has been widely recognized for its essential role in enhancing plant resilience against various abiotic stresses, including cadmium toxicity (González-Hernández et al., 2022). Cadmium is a heavy metal that induces oxidative stress in plants, leading to the excessive generation of reactive oxygen species (ROS) and subsequent cellular damage (Mansoor et al., 2023). The adverse effects of cadmium exposure are primarily attributed to increased hydrogen peroxide levels and lipid peroxidation, which significantly impair plant growth and physiological functions. To counteract these effects, plants activate their antioxidant defense systems, which are crucial for detoxifying ROS and preserving cellular integrity (Kumar and Pathak, 2018; Muneer et al., 2012). The application of putrescine has been shown to enhance the antioxidative metabolism of plants, thereby mitigating cadmium-induced damage. For instance, exogenous putrescine alleviated cadmium stress in coriander by enhancing antioxidant enzyme activity, improving photosynthesis and growth, and reducing oxidative damage markers such as malondialdehyde (MDA) (Sardar et al., 2022). In rice, putrescine pre-treatment enhanced cadmium resistance by increasing root cell wall hemicellulose levels to bind more Cd, regulating key Cd transport genes to limit Cd absorption, promoting vacuolar sequestration, and stimulating nitric oxide (NO) generation, a signaling molecule crucial for stress tolerance (Wang et al., 2023).

Additionally, putrescine is known to induce the synthesis of phytochelatins—peptides that chelate heavy metals—thereby facilitating their detoxification (Pál et al., 2017). It also significantly increases the activity of key antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase (POD), which play pivotal roles in scavenging ROS and protecting plant cells from oxidative damage (Tajti et al., 2018). The enhancement of these enzymatic activities is often linked to the upregulation of genes involved in antioxidant defense pathways, suggesting that putrescine not only acts as a direct protective agent but also modulates gene expression related to stress responses (Mohammadi-Cheraghabadi et al., 2021). Moreover, putrescine has demonstrated efficacy in improving plant growth and physiological responses under cadmium stress. Research indicates that cadmium exposure results in a significant decline in chlorophyll content, often accompanied by leaf chlorosis (Zhao et al., 2021). This reduction in chlorophyll levels is attributed to cadmium's inhibitory effects on pigment biosynthesis enzymes and its interference with the electron transport chain within chloroplasts (Muradoglu et al., 2015). Pre-treatment with putrescine has been shown to alleviate these adverse effects, restoring chlorophyll content and promoting plant biomass, thereby supporting overall plant health (Tajti et al., 2018; Badihi et al., 2021; Hussein et al., 2023). This protective effect is primarily attributed to putrescine's ability to stabilize cell membranes and enhance photosynthetic efficiency, which is critical for maintaining energy balance under stress conditions (Gupta et al., 2012). Furthermore, putrescine application has been associated with improved water retention and nutrient uptake, further contributing to enhanced plant resilience against heavy metal toxicity (Mohammadi-Cheraghabadi et al., 2021). The antioxidant capacity of putrescine can be effectively assessed using various assays, such as TPC, TFC, and antioxidant assays like CUPRAC, FRAP, and DPPH. These assays evaluate the ability of putrescine-treated plants to neutralize free radicals, providing insights into their potential protective effects against oxidative stress (Tumilaar et al., 2024). Studies have consistently shown that putrescine treatment increases TPC and TFC levels, which correlate with enhanced antioxidant activity measured through assays such as CUPRAC and DPPH (Gul et al., 2018; Zeynali et al., 2023; Hussein et al., 2023). In summary, putrescine acts as a multifaceted stress mitigator by modulating antioxidant systems, stabilizing cellular structures, and improving physiological responses. These findings underscore its potential as a practical and sustainable strategy for enhancing plant tolerance to cadmium stress and other abiotic challenges.

5. Conclusion

This study highlights the efficacy of putrescine, applied as seed priming, in mitigating cadmium-induced stress in plants. The findings demonstrate that putrescine significantly enhances antioxidative enzyme activities, stabilizes photosynthetic pigments, reduces hydrogen peroxide (H_2O_2) accumulation, and promotes the synthesis of antioxidant compounds under cadmium stress conditions. These results underscore its potential as a practical and sustainable strategy for improving plant resilience to heavy metal toxicity. Future research should focus on elucidating the molecular mechanisms underlying these effects and assessing the long-term applicability of putrescine seed priming across diverse crop species under field conditions.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	H.Y.	V.Ç.
C	50	50
D	100	
S		100
DCP	100	
DAI	100	
L	100	
W	100	
CR		100
SR	50	50
PM		100
FA		100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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