

Melatonin and Gamma-aminobutyric Acid Mitigates Cadmium Toxicity in Rice by Regulating Antioxidant Activities, Osmolytes Synthesis, and Reducing Oxidative Damages and Cadmium Accumulation

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Abstract

Soil cadmium (Cd) contamination poses a serious challenge to crops. The exogenic application of growth hormones is an important strategy in addressing the challenge of Cd pollution. The study explores the impact of combined melatonin (MT) and gamma-aminobutyric acid (GABA) in mitigating Cd toxicity with these treatments; control, Cd stress (250 µg kg⁻¹), Cd stress + melatonin (MT: 100 µM), Cd stress + GABA (1 mM) and Cd+ MT + GABA. Cadmium toxicity significantly decreases rice growth and yield productivity by increasing oxidative markers, Cd accumulation, and decreasing photosynthetic pigments, osmolyte productions and nutrients availability. Melatonin and GABA significantly decreased the adversities of Cd and increased rice productivity. Co-applying MT and GABA decreased hydrogen peroxide (H₂O₂), malondialdehyde (MDA), electrolyte leakage (EL) by 34.59%, 44.74% and 105.40%, soil Cd availability, and increased chlorophyll synthesis (36.61- 54.67%) and antioxidant activities (48.38-121.34%), therefore lead to an increase in growth and yield. Further, MT and GABA also reduced Cd accretion in rice roots and shoots and increased the nutrients availability favoring the growth of rice plants in Cd stress conditions. These results underpin the potential combined MT + GABA application in improving crop productivity and remediating Cd polluted soils.

Keywords: antioxidants, cadmium, GABA, hydrogen peroxide, yield.

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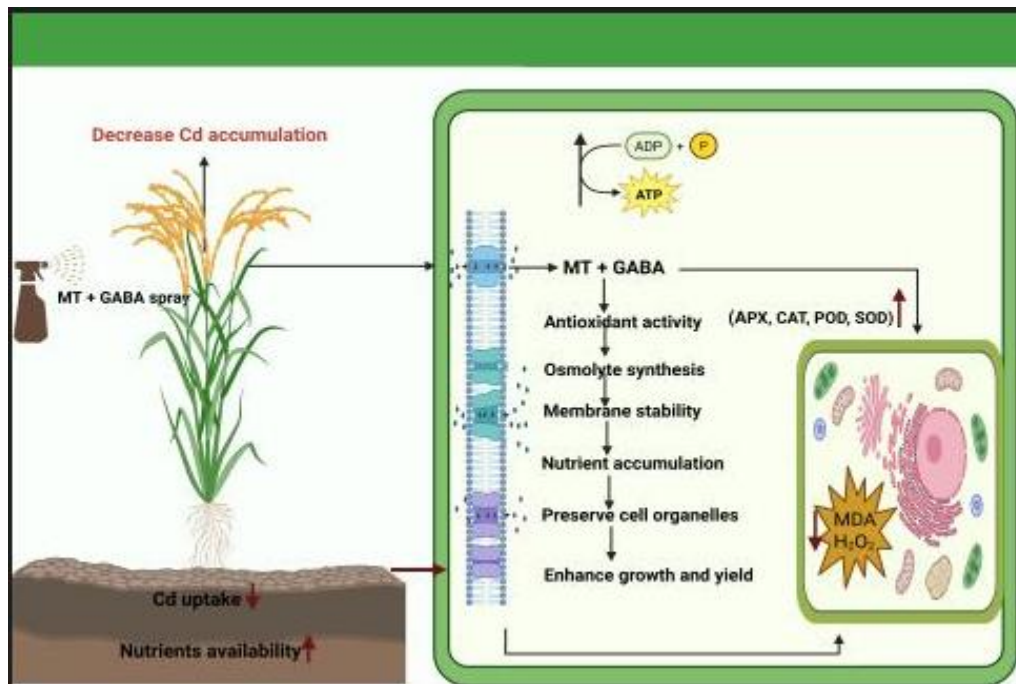
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Graphical abstract



1. Introduction

Soil heavy metal (HM) pollution and air pollution are serious challenges to environmental quality and ecosystem sustainability (Mitra *et al.* 2022; Mohandas *et al.*, 2025a,b,c). Heavy metals are continuously soaring up in soils and water, thus it is essential to address this problem for safer food production (Rahimzadeh *et al.* 2017; Sivasubramanian *et al.*, 2025). Among different HM, cadmium (Cd) is a seriously toxic metal posing serious threat to crop production, human health, and ecosystem sustainability (Peng and Shahidi 2021). It is a non-essential metal; however, its concentration is rapidly soaring due to mining, smelting, synthetic fertilizers, traffic, waste incinerators, and industrial effluents (Genchi *et al.*, 2020; Wang and Yang 2021). Apart from these anthropogenic sources, Cd also enters the environment through weathering, forest fires, wind dust, and eruptions from volcanoes (Liu *et al.* 2013). The foods grown on Cd-contaminated soils lead to its entry into humans, which in turn causes kidney and liver damage, emphysema, and heart diseases (Li *et al.* 2022).

Cadmium is highly toxic to plants; however, plants quickly absorb Cd through roots, which negatively affects growth, cell division, chlorophyll synthesis, photosynthetic efficiency, and assimilate production (Moravčíková and Žiarovská 2023). The excessive concentration of Cd also causes oxidative damage and disturbs membranes, DNA, electron transport, and photosynthetic apparatus (Marron 2015). Cadmium toxicity induce stomata closing, inhibits water uptake and carbon fixation (Huybrechts *et al.* 2020) thereby causes significant growth losses (Huybrechts *et al.* 2020). Additionally, Cd toxicity also disturbs nutrient uptake, decreases water uptake, damages the

photosynthetic apparatus, and reduces antioxidant activities (Hassan *et al.* 2024). Cadmium pollution is a serious challenge to achieve agricultural goals, (Chen *et al.* 2018), therefore, it is mandatory to manage the Cd-polluted soils for sustainable and safer crop productivity.

Different practices such as micro-nutrients, hormones, biochar, organic amendments, and controlled irrigation are using in mitigating Cd toxicity (Wang *et al.* 2022). Recently, role plant hormones is well acknowledged in mitigating toxic impacts of abiotic stress. Melatonin (MT) is an imperative hormone with tremendous potential in mitigating toxicity of heavy metals (El-Yazied *et al.* 2022) and improving plant performance (Moustaka *et al.* 2024). Melatonin-mediated improvement in growth under HM pollution is linked with improved osmolyte synthesis, leaf photosynthesis, and antioxidants activities (Malik *et al.* 2022). In the tomato crop, exogenously applied MT increased Cd toxicity via boosting the antioxidant activity and synthesis of phytochelatin (PC) (Hasan *et al.* 2015). Melatonin increases chlorophyll synthesis, water uptake, osmolyte synthesis, and decreases the oxidative damage and Cd accumulation (Cai *et al.* 2017).

Gamma-aminobutyric acid (GABA) also showed appreciable results to counteract abiotic stresses. It improves stomata opening, regulates the osmotic pressure (Xu *et al.* 2021), improves carbon assimilation, and antioxidant activity, thus regulating plant growth in stress conditions. Under Cd stress, GABA uptake reduces Cd uptake and improves plant performance by increasing endogenous MT synthesis (Li *et al.* 2022). The role of single MT and GABA application in mitigating the toxic impacts of HMs. However, the interactive effect of MT and GABA in mitigating the adverse impacts of Cd is poorly understood.

Therefore, we hypothesized that the interactive effects of MT and GABA can substantially reduce the toxic impacts of Cd on rice as compared to their application alone. Therefore, this study aimed to explore the interactive impacts of GABA on growth, yield, plant functioning, Cd uptake, accumulation, and soil properties.

2. Materials and methods

2.1. Growth conditions and plant materials

The study was performed at Baicheng Normal University and soil was taken from experimental soil (0-20 cm) and sieved to fill the pots with 10 kg capacity after removing debris. The soil was silt-loam with acid pH (5.97), total nitrogen (TN: 1.66 g kg⁻¹), and available phosphorus (AP) and available potassium (AK) concentrations of 38.42 and 117.19 mg kg⁻¹. The pots were filled, and CdCl₂ was to impose Cd toxicity. The soil was equilibrated for two months, and during this period, a field capacity level of 70% was maintained.

2.2. Experimental treatments

The current study was designed in a complete randomized design comprising of three replications along with five treatments: control, Cd stress (250 µg kg⁻¹), Cd stress + melatonin (MT: 100 µM), Cd stress + GABA (1 mM), and Cd+ MT + GABA. The pots were carefully handled, and recommended practices were followed to ensure good stand establishment. Melatonin and GABA were applied as a foliar spray at the tillering stage. The application of these rates of MT and GABA proved beneficial in mitigating cadmium stress in rice (Nayyar *et al.* 2014; Ashraf *et al.* 2022; Jiang *et al.* 2022). The certified reference materials, along with sterilized instruments and analytical grade chemicals, were used to get reliable results. All biochemical assay was performed in three replicates with established protocols to get reliable results.

2.3. Measurement of growth and photosynthetic traits

The fresh leaves of rice were taken and chlorophyll (Chl) and carotenoid (Cart.) concentrations by the methods of Arnon (1949). The freshly collected leaves (0.5 g) were homogenized in 80% methanol and extract was collected, and absorbance was recorded at 645, 480, and 663 for determining Chl-a and Chl-b and Cart concentrations. The fresh leaves were randomly collected and weighed (FW), and then they were soaked in water and after 24 hours they were weighed (TW). Later, these leaves were removed from water and oven dried (65°C) until constant weight (DW). Finally, leaf relative water contents (RWC) were calculated with following formula: $RWC (\%) = \frac{FW - DR}{TW - DR} \times 100$.

2.4. Measurement of biochemical attributes

Fresh rice leaves (0.5 g) were collected and dipped in water for 30 minutes, and the electrical conductivity (EC₁) was measured. Thereafter, the same leaves were taken and boiled in water (90 °C) for 1 hour. After that, the leaves were removed from the water, and the second EC (EC₂) was measured. Finally, EL was calculated using the following formula: $EL = \frac{EC_1}{EC_2} \times 100$. The freshly collected leaves (0.5 g) were homogenized using the potassium phosphate

buffer (PPB) and centrifuged (14000 rpm) for 15 minutes. The obtained extract was treated with Bradford reagent (2 mL), and absorbance was measured at 595 nm (Bradford, 1976). For free amino acids (FAA), leaves were ground using PPB, and then the extract was mixed with ninhydrin (1 mL) and pyridine (1 mL). Later, the mixture was boiled for 30 min, and the absorbance was read at 570 nm (Hamilton and Van-Slyke 1943). To quantify hydrogen peroxide (H₂O₂), we homogenized 0.5 g of fresh leaf tissue in 5 mL of trichloroacetic acid (TCA). The resulting homogenate was centrifuged, and we then mixed 1 mL of the supernatant with a reaction solution containing potassium phosphate buffer (PBB) and 1 M potassium iodide (KI). The absorbance of this mixture was measured at 390 nm. For malondialdehyde (MDA), leaves were ground in trichloroacetic acid (TCA) solution and centrifuged to obtain the extract, and then 5 mL of thiobarbituric acid (TBA) was added and boiled (100 °C) for 30 minutes, and later the absorbance (532 nm) was taken. For ascorbate peroxidase (APX) activity leaves (0.5 g) were homogenized in PPB buffer and centrifuged (10000 rpm) for 15 min, and absorbance noted at 290 nm (Nakano and Asada, 1987). For the catalase (CAT) activity, 0.5 g freshly collected leaves were blended by using PPB and centrifuged (10000 rpm), and absorbance was noted at 240 nm (Aebi 1984). For determining superoxide dismutase (SOD) activity, we prepared the mixture containing 400 µL H₂O₂, 25 mL buffer, 100 µL Triton, 50 µL extract was made and reading done at 560 nm (Mukherjee and Choudhuri 1983). In case of peroxidase (POD), freshly collected leaves were homogenized in PPB, and later, the absorbance noted at 470 nm, and POD activity was measured by the methods of Zhang (1992). For ascorbic acid (AsA), 0.5 g of leaves was homogenized in TCA solution (5 mL) and centrifuged (10000 rpm) for 15 minutes, and later AsA contents were determined with the methods of Zhang (1992).

2.5. Measurement of growth traits, Cd concentration in plant tissues and soil properties

The height of each plant was measured, and tillers were counted and average was done. Five panicles from each replication were selected to determine their length and grains per panicle. The plants were hand harvested for assessing the grain and biomass yield. The rice samples were collected, dried (65°C), and powdered to determine Cd concentration. The samples were digested (180°C) by using two acids (HCl and HClO₄, 4:1), then this mixture was filtered and diluted to 50 mL, and Cd in the samples was measured by atomic absorption spectrophotometry. The soil samples were collected, and pH meter was used for measuring pH in a soil and water solution (3:1). Nitrogen in soil samples was assessed with Kjeldahl apparatus, while AP and AK were assessed with the Olsen and ammonium acetate extraction flame photometry method. The translocation factors and biological accumulation coefficient were calculated with the procedures of Malik *et al.* (2010). The soil samples were digested in HNO₃:HClO₄ (4:1), thereafter they were filtered and diluted by adding water and Cd concentration was estimated with atomic absorption spectrometry.

2.6. Data Analysis

A one-way ANOVA was conducted to assess significance among treatments, and Tukey's honestly significant difference (HSD) was employed for separating means. Moreover, Sigma-plot 10 was used for making figures, and PCA and correlation matrix were prepared by R-studio.

3. Results

3.1. Effect of MT and GABA on photosynthetic pigments

The chl-a, chl-b, and carotenoid were decreased by 56.69%, 70% and 42.14% respectively, under Cd stress (**Table 1**). Melatonin and GABA mitigated this reduction and resulted in a remarkable increase in photosynthetic traits. The combined MT and GABA remained the top-performing and resulted in an increase of 36.61%, 54.67, and 32.20% in chl-a, chl-b, and Car synthesis in Cd-polluted soil (**Table 1**).

3.2. Effect of MT and GABA on oxidative markers, osmolytes and antioxidants activities

Cadmium toxicity caused a marked decrease in RWC of rice plants. Cd toxicity reduced leaf RWC by 43.25% respectively (**Table 1**) while MT and GABA considerably increased leaf RWC (**Table 1**). The production of all the oxidative markers substantially increased in Cd stress. The results depicted that Cd toxicity increases EL, MDA, and H₂O₂ synthesis by 299.29%, 298.45% and 323.19% as the control (**Table 1**). Co-applying MT and GABA causes a marked decrease in EL, MDA, and H₂O₂ production (**Table 1**). Cadmium toxicity increased the proline synthesis, while it decreased the synthesis of total soluble protein (TSP) and FAA (**Figure 1**). Both MT and GABA increased the synthesis of all the osmolytes under Cd stress conditions. For instance, co-applying MT and GABA enhanced the proline, TSP, and FAA by 45.67%, 75.68% and 102.73% respectively, under Cd stress conditions (**Figure 1**). Melatonin and GABA boosted the antioxidant activity. Co-applying MT and GABA enhanced APX, CAT, POD, SOD, and AsA activities by 62.46%, 89.40%, 48.38%, 66.19% and 121.34% respectively than control (**Figure 1**).

3.3. Effect of MT and GABA on growth and yield traits

Cadmium toxicity decreased the RL, their fresh and dry matter production by 49.49%, 79.84% and 84.65% respectively (**Table 2**). Interestingly, exogenous treatments with MT and GABA significantly increased root length (RL) and their fresh and dry matter production by 38.93%, 53.91% and 68.58% respectively in Cd-polluted soil (**Table 2**). Cadmium stress considerably decreased yield and yield traits.

The exogenous applied MT and GABA significantly increased the plant height (PH), tillers per plant (TPP), hundred kernel weight (HKW), grain yield (GY), biological yield (BY), and harvest index (HI) of rice plants 22.47%, 16.08%, 58.30%, 62.28%, 30.28% and 24.31% respectively (**Table 2**).

3.4. Effect of MT and GABA on root and shoot Cd concentration on TF, BAC and BAF

The results showed that Cd stress results in a significant increase in root and shoot Cd concentration (**Figure 2**). MT

and GABA application significantly diminished Cd accretion in the plant, and a high reduction was noted with co-applying MT and GABA (**Figure 2**). Furthermore, these findings indicated that translocation factor (TF), bio-accumulation concentration (BAC), and bio-concentration factor (BCF) of Cd were significantly diminished with the supplementation of MT and GABA. The highest reduction in TF, BAC, and BCF was obtained after co-applying MT and GABA than their single application (**Figure 2**).

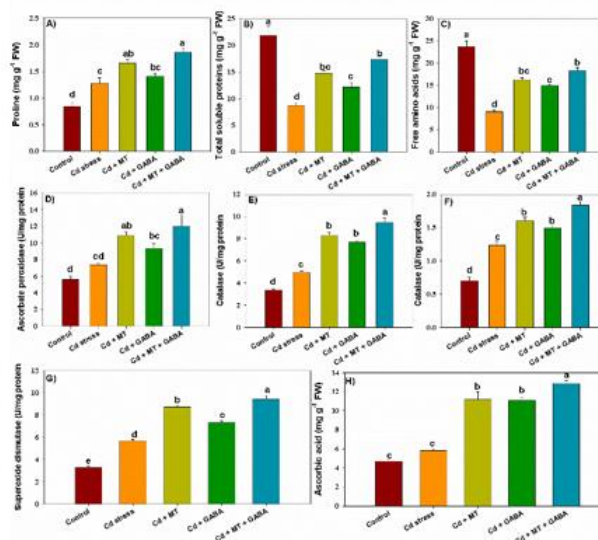


Figure 1. Impact of exogenous applied MT and GABA on the osmolyte synthesis and antioxidant activities of rice planted in Cd polluted soil. The presented data is mean of three replications with \pm SD and different letters depicts the significance at $P \leq 0.05$.

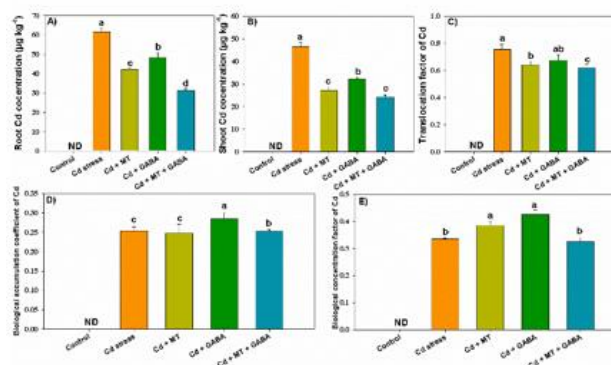


Figure 2. Impact of exogenous applied MT and GABA on root and shoot Cd concentration, TF, BAC and BCF of rice planted in Cd polluted soil. The presented data is mean of three replication with \pm SD and different letters depicts the significance at $P \leq 0.05$.

3.5. Effects of MT and GABA on soil properties

The results depicted that MT and GABA supplementation significantly decreased the soil Cd concentration after harvesting rice plants (**Figure 3**). Co-applying MT+GABA decreased soil Cd by 90.61%, while MT and GABA decreased soil Cd concentrations by 66.65% and 61.83% respectively (**Figure 3**). Further, the results also indicated that Cd stress and exogenously applied GABA and MT had non-significant impacts on soil pH (**Figure 3**). Cd reduced AP, AK, and TN concentration; conversely, MT and GABA caused a marked increase in the availability of AP, AK, and TN (**Figure 3**).

Table 1: Impact of exogenous applied MT and GABA on the photosynthetic pigments, oxidative markers and osmolyte synthesis rice planted in Cd polluted soil

Treatments	Chlorophyll-a (mg g-1 FW)	Chlorophyll-b (mg g-1 FW)	Carotenoid (mg g-1 FW)	Relative water contents (%)	Electrolyte leakage (%)	Malondialdehyde (μ mol g-1 FW)	Hydrogen peroxide (μ mol g-1 FW)
Control	4.45a±0.135	2.55a±0.091	7.15a±0.026	92.93a±1.79	17.13d±1.11	2.59d±0.13	1.94e±0.11
Cd stress	2.84d±0.059	1.50c±0.041	5.03c±0.102	64.87d±1.93	68.40a±1.47	10.32a±0.33	8.21a±0.18
Cd + MT	3.52c±0.029	2.06b±0.048	6.05b±0.057	80.20b±1.63	35.79c±2.49	8.10b±0.09	7.06c±0.04
Cd + GABA	3.28c±0.042	1.87b±0.075	5.83b±0.061	73.00c±1.88	42.10b±1.74	8.37b±0.181	7.58b±0.24
Cd + MT + GABA	3.88b±0.073	2.32a±0.082	6.65a±0.0239	85.33b±2.41	33.30c±0.82	7.13c±0.074	6.10d±0.09

The presented data is mean of three replication with ± SD and different letters depicts the significance among means at P ≤ 0.05. Cd: cadmium, MT: melatonin, GABA: γ-aminobutyric acid.

Table 2: Impact of exogenous applied MT and GABA on the agronomic traits of rice planted in Cd polluted soil

Treatments	RL (cm)	RFW (g)	RDW (g)	PH (cm)	TPP	HKW	GY/pot (g)	BY/yield (g)	HI (%)
Control	53.37a±2.33	14.01a±0.42	7.70a±0.99	121a±2.94	11.00a±0.82	5.11a±0.10	62.97a±1.84	212.48a±7.20	30.85a±1.13
Cd stress	35.70c±2.07	7.79d±0.56	4.17c±0.06	89c±3.22	8.33b±0.47	3.07c±0.13	36.80c±3.21	148.39d±3.33	24.84b±2.39
Cd + MT	46.08b±1.55	10.29bc±0.68	6.64ab±0.10	103b±2.16	10.00ab±1.41	4.40b±0.07	56.35ab±0.72	188.00bc±2.45	29.98a±.066
Cd + GABA	44.12b±2.02	9.22cd±0.54	6.09b±0.09	102b±2.06	9.00ab±0.44	4.16b±0.04	50.33b±0.78	172.71c±4.97	29.19ab±1.2
Cd + MT + GABA	49.60ab±2.57	11.99b±0.64	7.03ab±0.15	109b±2.63	9.67ab±0.47	4.89a±0.06	59.72a±2.51	193.33b±4.19	30.88a±0.76

RL: root length, RFW and RDW indicates fresh and dry weights of roots, PH and TPP are plant height and tillers/plant, HKW: 100 kernel weight, GY and BY are grain and biomass yield and HI is harvest index. Cd: cadmium, MT: melatonin, GABA: γ-aminobutyric acid. The presented data is mean of three replication with ± SD and different letters depicts the significance among means at P ≤ 0.05.

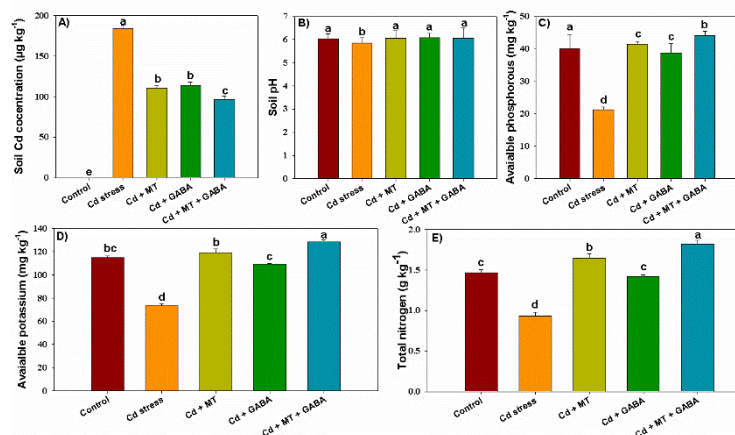


Figure 3: Impact of exogenous applied MT and GABA on soil Cd concentration, soil pH and soil N, P, and K concentration in Cd polluted soil. The presented data is mean of three replications with ± SD and different letters depicts the significance at P ≤ 0.05.

3.6. Principal component and correlation analysis

The first two principal components, Dim1 (95.3%) and Dim2 (3.3%), explain a cumulative 98.6% of the variance, indicating that most of the variation in growth traits is captured by these two dimensions (**Figure 4a**). The results displayed that Cr stress significantly alters plant growth traits. The Cd stress group is clustered far from other groups, suggesting a strong impact of Cd on growth. Among the treatments, the Cd + MT and Cd + GABA groups are positioned closer to the center, implying a partial recovery of growth traits. The Cd + MT + GABA (light blue crosses) group is located near the control, indicating a strong

mitigation effect on Cd stress. The vector directions suggest that traits such as GY, BY, PH, RDW, RFW and HI are strongly influenced by Cd stress and recovery treatments. The correlation matrix shows strong positive correlations (red circles) between various growth parameters. Traits such as GY, BY, PH, RDW, RFW, TPP, and HI exhibit high correlation coefficients, suggesting that these traits are highly interdependent under Cd stress and recovery treatments. The treatments such as MT, GABA, and their combination effectively restore growth performance, leading to improved biomass and grain yield (**Figure 4b**).

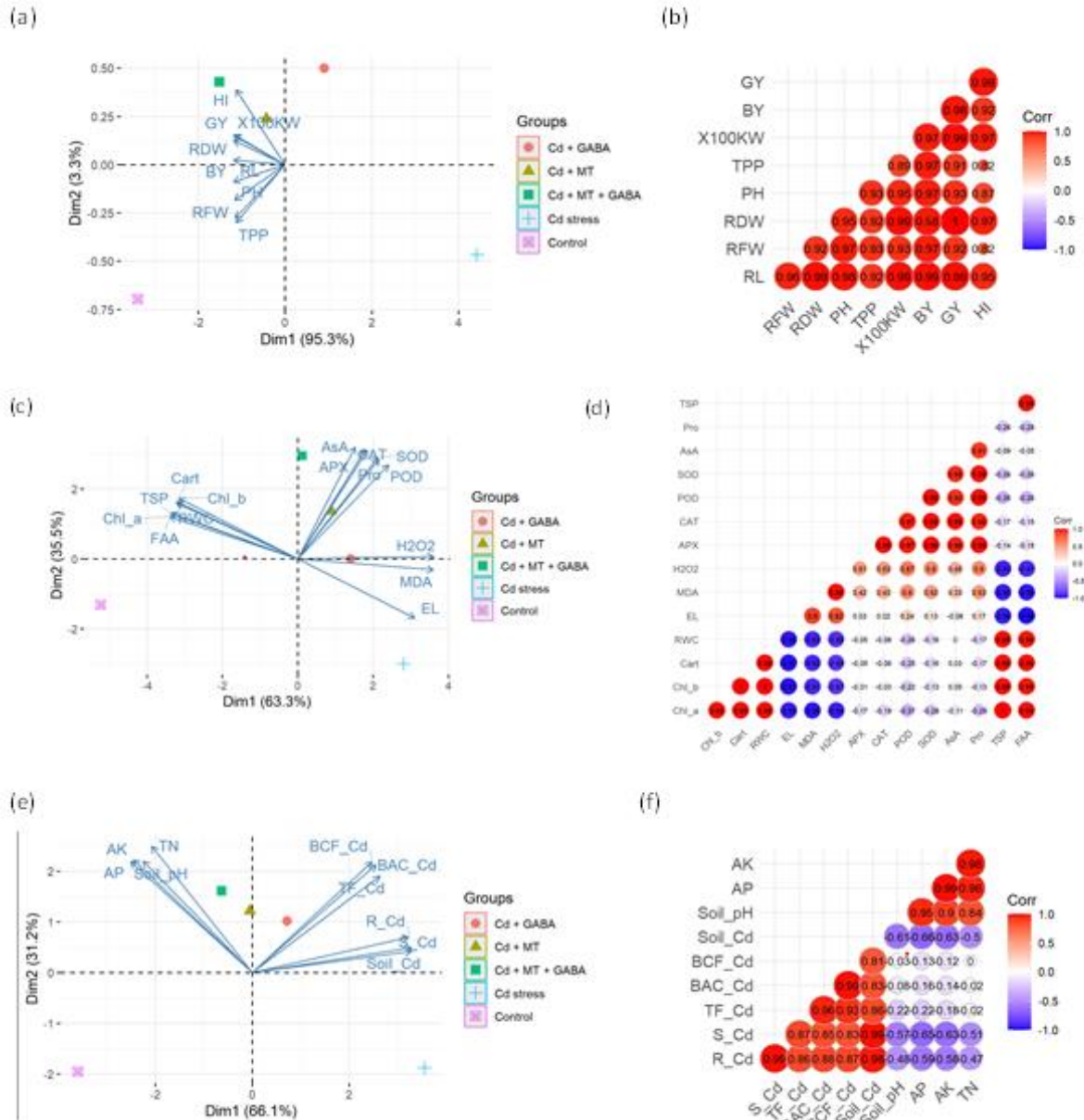


Figure 4: The principal component and correlation analysis for the impact of exogenous applied MT and GABA on growth, morpho-physiological functioning, soil properties and lead accumulation. GY: grain yield, BY: biomass yield, KW: kernel weight, TPP: tillers/plant, PH: plant height, RDW: root dry weight, RFW: root fresh weight, TSP: total soluble protein, Pro: proline, AsA: ascorbic acid, Chl: chlorophyll, RWC: relative water contents, EL: electrolyte leakage, MDA: malondialdehyde, H₂O₂: hydrogen peroxide, TSP: total soluble pro-tein, APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase, SOD: superoxide dismutase. AK: available potassium, AP: available phosphorus, BAC: bio-accumulation factor, BCF: bio-concentration factor.

The PCA components explained 63.3% and 35.5% of the total variance, respectively. Vectors representing antioxidant enzyme activities, oxidative stress markers (H_2O_2 , MDA, EL), and TSP and FAA displayed distinct clustering patterns. Control samples were separated from Cd-treated groups, while combined treatments (Cd + MT and Cd + GABA) showed unique clustering, indicating their potential in mitigating Cd-induced stress (**Figure 4c**). Notably, oxidative stress markers (H_2O_2 , MDA, EL) exhibited a positive correlation with cadmium stress, while antioxidant responses clustered oppositely, suggesting their protective functions. Antioxidant enzymes displayed positive linking with AsA, Pro, TSP, indicating their collaborative role in mitigating oxidative stress. Conversely, markers of oxidative damage negatively correlated with chlorophyll content and carotenoids, suggesting that higher oxidative stress levels lead to reduced photosynthetic efficiency. Additionally, RWC was negatively associated with MDA and EL, highlighting the impact of cadmium stress on cellular water retention. The inclusion of MT and GABA influenced these interactions, underscoring their potential in alleviating cadmium-induced toxicity (**Figure 4d**).

PCA was used to analyze the distribution of different experimental groups and the relationships among soil and plant Cd accumulation parameters under various treatments. The biplot showed distinct clustering patterns for Cd content in plant tissues and soil Cd levels, with control samples positioned differently from Cd-treated groups. Combined treatments (Cd + MT and Cd + GABA) exhibited unique clustering patterns, indicating their role in modulating Cd accumulation and soil parameters (Figure 4e). The correlation matrix highlighted significant relationships between soil properties and Cd accumulation in plant tissues, with strong positive correlations among Cd accumulation factors and negative correlations between soil parameters and plant Cd accumulation. The introduction of MT and GABA modulated these interactions, suggesting their potential in mitigating cadmium accumulation in plants (**Figure 4f**).

4. Discussion

The detrimental impacts of Cd contamination on ecosystem health and organisms presents a significant challenge. Therefore, remediation of Cd-polluted soils is mandatory to safeguard living organisms and the ecosystem (Yang *et al.* 2023). Phyto-hormones got a considerable role for remediating Cd-polluted soils. Melatonin and GABA are important hormones with tremendous potential to improve plant growth under abiotic stresses (Yang *et al.* 2023). Therefore, this stress assessed the impacts of MT and GABA in mitigating Cd toxicity in rice. Cadmium toxicity considerably decreased rice growth and yield (**Table 2**). Further, Cd toxicity also inhibits cell expansion and reduces root growth, causing reduction in growth (Alam *et al.* 2020). Cadmium decreased rice growth by increasing in oxidative damage, reducing nutrient uptake (Figure 3), photosynthetic pigments (**Table 1**), antioxidant activities (Figure 2), and protein degradation (Alam *et al.* 2020). Nevertheless, MT

and GABA significantly increased the rice growth and grain productivity, aligning with previous studies (Guo *et al.* 2022). The exogenous applied GABA improves amylase activities, starch metabolism, osmolyte synthesis, and antioxidant activity; therefore, it reduces the adversities of stress (Cheng *et al.* 2018). Melatonin also maintains better homeostasis, antioxidant, and nutrient uptake, therefore, ensures better growth (Altaf *et al.* 2024). Co-applying MT and GABA enhanced rice growth via increase antioxidant activity, soil properties, and decreasing Cd accumulation (Song *et al.* 2024).

Cadmium negatively effects the photosynthetic efficiency of plants. The higher accumulation of Cd constrains assimilate supply, which in turn impairs the leaf functioning. In the current study, Cd toxicity significantly decreased the chlorophyll synthesis, which was linked with Cd-induced increase in oxidative stress (**Table 1**), and damaged the light-harvesting system and disrupted the structure of the chloroplast (Song *et al.* 2024; Vazquez-Marquez *et al.* 2024). Opposite to this, MT and GABA mitigated the adversities of Cd and ensured better chlorophyll synthesis. This was linked with a reduction in oxidative stress (**Table 1**) and the ability of MT and GABA to maintain the integrity of D1 protein and nutrient uptake (Figure 3), and reduces Cd uptake, which enhances antioxidant activities and supports photosynthesis under stress conditions (Yang *et al.* 2023). Melatonin improves the activity of the photosynthetic apparatus by reducing the stress-induced damage to the thylakoid membrane (Mushtaq *et al.* 2022). MT also increases the photosynthetic-related gene expressions ($CB1_2$ and CAB_7), and an increase in expression of these significantly increases chlorophyll synthesis and leads to better photosynthetic efficiency (Altaf *et al.* 2024).

Cadmium stress significantly decreased RWC of rice plants (**Table 1**), which was linked with Cd-induced damage to membranes, leading to loss of water and resulting in less RWC (Imran *et al.*, 2021). However, MT and GABA maintained the better RWC by improving root growth (**Table 1**). Cadmium toxicity also facilitated the increase in oxidative damage which was evidenced in the form of enhanced H_2O_2 and MDA production (**Table 1**). The foliar applied MT and GABA reduced EL, H_2O_2 , and MDA synthesis by boosting antioxidant activities (Malik *et al.*, 2022). This suggests that co-applying MT and GABA decreased Cd toxicity by decreasing membrane damage and enhancing antioxidant activities (Buttar *et al.* 2020; Lv *et al.* 2023). Likewise, Kumar *et al.* (2019) also witnessed that GABA application reduced the H_2O_2 production under As stress. Notably, co-applying MT and GABA significantly enhanced antioxidant activity and reduced MDA and H_2O_2 production as compared to their sole application. A possible reason for this increase could be that GABA increased the MT synthesis, thus leading to an increase in antioxidant activities (Aghdam and Fard 2017).

Cadmium toxicity increased the antioxidant activities; however, this increase was not enough to decrease the toxic impacts of Cd. This indicates the ability of rice plants to increase antioxidant activities to counteract Cd toxicity

(Chattha *et al.* 2021). Nevertheless, GABA and MT significantly enhanced all the antioxidant activities, which mitigates the toxicity of Cd (Hasanuzzaman *et al.* 2017). Amino acids and proteins are vital for stress tolerance; however, Cd toxicity decreased the synthesis of TSP and FAA. Cadmium toxicity decreased the N uptake (Figure 3), which is a building block of protein and amino acid synthesis. Further, Cd might also disturb the metabolism of protein and amino acids synthesis (Zemanová *et al.* 2014), leading to a reduction in the accumulation of TSP and FAA. The exogenously applied MT and GABA increased the FAA and TSP, which were linked with enhanced N uptake (Figure 3), and antioxidant activities (Figure 1). These results align with previous reports indicating GABA and MT provide tolerance to plants through increasing the synthesis of osmo-regulatory substances (Wang *et al.* 2026).

Maximum Cd contents was noted in roots as compared to shoots plant. This increased accumulation in roots was linked with the fact that the root comes in contact with Cd first, or it is also linked with compartmentalization of Cd in root vacuoles (Aamer *et al.* 2018). The lower Cd concentration was reported in shoots, indicating that less Cd was transported to aerial plant parts. Though MT and GABA significantly decreased Cd accretion in rice. GABA application reduces uptake of Cd by decreasing Cd²⁺ flux and declining gene expression linked with Cd uptake and transportation (Li *et al.* 2022). MT application also decreases Cd accumulation by reducing its uptake. Co-applying GABA and MT significantly decreased Cd uptake, which indicates the interaction between GABA and MT in inhibiting Cd absorption (Lv *et al.* 2023). MT and GABA diminished soil Cd availability and increased the availability of NPK (Figure 3). MT application improves microbial growth, which degrades the Cd and fixes it in the soil, thereby reducing its availability (Liang *et al.* 2017). Applying MT increases root exudate (malate and citrate), which increases bacterial and fungal growth and soil enzyme activities, leading to better nutrient availability (Yang *et al.* 2020).

5. Conclusion

Cadmium toxicity significantly inhibited rice growth, via increasing oxidative damages, causing, Cd accumulation and decreasing chlorophyll synthesis. Nevertheless, co-applying MT and GABA significantly decreased the toxic impacts of Cd. Combined MT and GABA showed a positive impact in remediating Cd-polluted soils and improving rice productivity by increasing soil health, antioxidant activities, osmolyte synthesis, and plant resilience to oxidative damage. Co-applying MT and GABA can serve as a promising approach to mitigate the adversities of Cd. This approach can decrease soil Cd availability, prevents ground-water contamination, and promote safer rice production. Thus, implementing this approach can enhance food safety and sustainable crop productivity. However, the efficiency of MT and GABA can be enhanced by using more sensitive analytical instruments such as ICP-MS for trace metal quantification. Moreover, validating the gene expression data with qRT-PCR alongside enzyme activity assays is also needed to understand its role in

mitigating Cd toxicity. Additionally, conducting field trials across diverse climate and soil conditions can also enhance its efficiency in mitigating Cd toxicity.

Authors' Contributions

Conceptualization, ZG and HY, Writing – original draft: ZG, HY, YS, Data collection: YS, XH and YD, Investigation, YS, XH and YD, Writing, original draft: ZG and HY, Funding acquisition: ZG and HY, Writing – reviewing and editing: JMA, BA, OA, MA and MA. All authors have read and agreed to the published version of the manuscript.”

Data availability

Data will be made available on request.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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