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The Impact of Cadmium Stress on Rice Growth, Development and Protective Mechanisms

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Abstract. Cadmium (Cd) is a significant pollutant in paddy fields, and its accumulation in rice (Oryza sativa L.), along with its eventual entry into the food chain, has led to a serious global environmental and people's health issue related to diet. This research proposal aims to investigate the effects of varying concentrations and exposure durations of Cd on rice growth. The researchers will focus on identifying the specific parts of the rice plant most affected by cadmium, including rice ears, leaves and stems. In addition, by examining the morphological, physiological, and biochemical changes in rice due to Cd exposure, this study aims to provide insights into the mechanisms of Cd-induced stress and potential mitigation strategies. These studies are critical for developing effective management practices to reduce Cd contamination in rice cultivation and enhance public food safety.

Keywords: Cadmium (Cd), Rice (Oryza sativa L.), Public food safety, Management mitigation strategy

1. Introduction

In recent decades, natural resources have become significantly polluted by human activities, such as industrial processes, deforestation, urbanization, and mining. Gradually, these activities are increasing the toxic pollutants, particularly cadmium (Cd) [1]. However, cadmium (Cd) is a major pollutant with significant biological toxicity, unnecessary for living organisms, and toxic to all forms of life, particularly affecting the quality of farmland soil. Because of its high solubility and fluidity, the toxic effects of Cd on plants are manifested in various metabolic activities [2]. Contamination of agricultural soils with Cd has become one of the most toxic and widespread environmental problems [3]. Notably, rice is one of the world's most important staple and economic foods, feeding more than half of the global population and providing essential carbohydrates [4]. As a primary source of energy, the health and growth of rice are crucial for food security.

In addition, the major harmful effects of cadmium on plants primarily include damage to plant organs, such as roots and stems, physiological and biochemical characteristics, including an increase in reactive oxygen species in rice ears and a reduction in photosynthesis in the leaves [5]. These detrimental effects on rice, a primary source of energy for over half the world's population, highlight the urgent need to address cadmium contamination for global food security.

Furthermore, statistical surveys indicate that cadmium (Cd) primarily affects major economic crops such as rice, wheat, and maize, all of which are highly susceptible to Cd contamination. This

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contamination severely impairs their growth, yield, and safety for consumption. As a stable environmental contaminant, cadmium enters ecosystems through industrial discharges, sewage sludge, mining activities, and combustion processes, accumulating in soil, water, and the atmosphere and significantly affecting agricultural production and ecosystems. Among these agricultural crops, rice particularly vulnerable due to its cultivation in waterlogged paddies, where Cd contamination is more prevalent. Human exposure to cadmium occurs through food, water, and cigarette smoke. Once absorbed, Cd is efficiently retained in the body, accumulating over a lifetime with a half-life of 25–30 years. Prolonged high Cd exposure can lead to bone and kidney damage, oxidative stress, and, in severe cases, carcinogenic effects.

In this paper, researchers hypothesize that increasing concentrations and treatment durations of cadmium (Cd) will significantly decrease the development of rice roots and stems. Furthermore, Cd toxicity is expected to increase reactive oxygen species (ROS) levels in rice ears and decrease photosynthesis in rice leaves. The study will explore recent advancements in understanding the impact of cadmium on rice cultivation.

2. Material and methods

The growth conditions of rice roots and stems will be assessed using morphological analysis by optical microscope. ROS levels in rice stems will be measured using fluorescent probes, such as DCFH-DA. The decrease in leaf photosynthesis will be measured using a spectrophotometer.

2.1. Cd decreases growth of rice stems and roots by microscope

2.1.1. Design of the experiment

This experiment, conducted in the greenhouse of Shenyang Agricultural University, used Oryza sativa L. as the rice material. The reagents included $CdCl_2$, paraffin wax, ethanol, xylene, safranin, fast green, and distilled water. The equipment consisted of a light microscope, microtome, embedding station, staining racks, glass slides, and cover slips [6].

2.1.2. Procedures

First, for rice cultivation, rice seeds were germinated and grown in Hoagland nutrient solution until they reached the three-leaf stage. Cadmium stress treatment plants were exposed $CdCl_2$ solution of different concentrations (0.01, 0.1, 10, 100, 1000μm) for 30 days (1, 5, 10, 15, 20, 25, 30days). After treatment, stem and root samples were collected from each plant. The samples were fixed in FAA solution (formalin-acetic acid-alcohol) for 24 hours. The samples were dehydrated through a graded ethanol series (50%, 70%, 90%, 100%), cleared in xylene, and embedded in paraffin wax. The paraffin-embedded samples were sectioned into thin slices (5-10μm) using a microtome [7]. Furthermore, for staining, the sections were mounted on glass slides, deparaffinized, and rehydrated through a series of ethanol solutions. The sections were stained with safranin (for lignin and cell walls) and fast green (for cytoplasm and cellulose). After Dehydration, the sections were cleared in xylene, and mounted with coverslips. Finally, stained sections were observed under a light microscope at 100x magnification. Images were captured to observe the structural changes in the stem and root tissues, focusing on cell wall integrity, cell size, and the presence of abnormalities [8].

Prepare the organic soil with a P^H value of approximately 6.0-6.5, incorporating various beneficial elements to cultivate healthy rice. Repeat the above procedures (1,3,4,5) on the control

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group. This will facilitate the observation of differences under various cadmium concentration treatments compared to cultivation in normal organic soil, which is significant for the management of environmental toxins.

2.2. Cd increases ROS of rice ears by DCFH-DA

2.2.1. Design of the experiment

The rice material used in this experiment was Oryza sativa L., with reagents including $CdCl_2$, DCFH-DA, PBS, DMSO, and culture medium, and equipment comprising a fluorescence microscope, fluorescence microplate reader, centrifuge, and pipettes with tips.

2.2.2. Procedures

First, for rice cultivation, sterilize rice seeds and place them in culture dishes with suitable medium. Incubate in an incubator until the heading stage. Collect healthy rice ears and divide them into control and experimental groups. Treat the experimental group with a certain concentration of $CdCl_{2.}$ For sample preparation, wash the treated rice ears with PBS to remove surface impurities. Cut samples into small pieces and place them in centrifuge tubes.

Furthermore, for DCFH-DA staining, dissolve DCFH-DA in DMSO to a final concentration of 10 mM, then dilute with PBS to 10μm. Add DCFH-DA working solution to each sample, ensuring full immersion. Incubate in the dark for 30 minutes. Finally, for Fluorescence Detection. Observe the stained samples under a fluorescence microscope and record fluorescence intensity [9].

2.3. Cd decreases photosynthesis in rice leaves by spectrophotometer

2.3.1. Design of the experiment

The materials used in this experiment include (Oryza sativa L.), with reagents such as $CdCl_2$, 95% ethanol, and distilled water, while the equipment comprises a spectrophotometer, centrifuge, analytical balance, test tubes, mortar and pestle, pipettes, and an analytical balance.

2.3.2. Procedures

First, for rice cultivation, rice seeds were germinated and grown in Hoagland nutrient solution until the three-leaf stage. For cadmium stress, treatment plants were exposed with $CdCl_2$ solution of different concentrations (0.01, 1, 10, 100, 1000μM) for 30 days (1, 5, 10, 15, 20, 25, 30 days). Furthermore, for chlorophyII extraction, after treatment, the third fully expanded leaf from each plant was collected and cut it into small pieces (approximately 0.1 grams). The leaf pieces were placed in a mortar, add 10 ml of 95% ethanol or 80% acetone, and the mixture was ground into a homogenate. The homogenate was transferred to test tubes and kept it in the dark for 1 hour, shaking every 10 minutes. The homogenate was then Centrifuged at 4000 rpm for 10 minutes and collected the supernatant for measurement. Finally, the absorbance of the supernatant was measured at 663nm and 645nm using a spectrophotometer. ChorophyII a and b contents were calculated using the formulas [10].

According to the above methods and procedures, researchers could learn the effects of various concentrations of cadmium on the photosynthesis and ChlorophyII reduction in rice leaves. Testing

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ChlorophyII content can effectively demonstrate whether photosynthesis is increased or decreased in rice leaves [11].

2.3.3. Positive and negative controls for the experiments

Control groups will be utilized in this experiment to account for any unknown variables that may affect the experimental outcome and to provide a baseline for comparison with the test group. There are three measurements that test if Cd decreases the growth rice stems and roots (The positive control is H_2O_2, the negative control is H_2O). For testing if Cd increases the ROS level of Rice ears experiment (The positive control is ROS up, the negative control is PBS). For testing if Cd decreases photosynthesis in rice leaves experiment (The positive control is high or low temperature, the negative control is H_2O).

3. Statistical analysis

The experiments were conducted at least three times, and data are presented as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 6.0 software. An unpaired Student's t-test was applied to analyze the growth of rice stems and roots observed under a microscope, and the photosynthesis levels in rice leaves were measured by a spectrophotometer. A Chi-squared test of independence was used for the data from the ROS level detection in rice ears using the DCFH-DA probe. A significance level of $p<0.05$ was considered statistically significant.

3.1. Experiment 1: Cd decreases the growth of rice stems and roots

Treatment with varying concentrations of $CdCl_2$ (0.01, 0.1, 1, 10, 100, 1000 μM) over durations of 1, 5, 10, 15, 20, 25, and 30 days may show a dose-dependent reduction in growth. Higher concentrations and longer exposure times are likely to result in more significant growth inhibition of stems and roots, evidenced by decreased cell size and integrity under the microscope. Lower concentrations and shorter durations may show minor or no noticeable changes [1].

3.2. Experiment 2: Cd increases ROS in rice ears

Treatment with $CdCl_2$ may lead to increased ROS levels in rice ears, detectable by fluorescence intensity using DCFH-DA staining. Higher concentrations and longer treatment durations are expected to produce greater fluorescence, indicating elevated ROS. Low concentrations or shorter treatments may show slight or no changes in ROS levels compared to the control [12].

3.3. Experiment 3: Cd decreases photosynthesis in rice leaves

Chlorophyll content in rice leaves, assessed via spectrophotometry, will likely decrease with increasing $CdCl_2$ concentrations and extended treatment durations. Absorbance readings at 663 nm and 645 nm are expected to drop, reflecting reduced chlorophyll a and b levels. Minimal changes might be observed at low $CdCl_2$ concentrations and shorter exposure periods [2].

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4. Results

Table 1. The combination of all possible results for test groups

Combination Results # (CR#)	Cd decreases the growth of rice stems and roots, as observed under a microscope?	Cd increases the ROS level in rice ears, as detected by DCFH-DA?	Cd decreases photosynthesis in rice leaves, as measured by a spectrophotometer?	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	+	-	-	Partial
6	-	-	+	Partial
7	-	+	-	Partial
8	-	-	-	Fully Contradicts

The “+” sign means that the phenomenon in each column heading is observed in the experiments and is statistically significant compared to the control groups. The “-” sign means that the phenomenon is not observed in the experiments, which may be due to the observation contradicting the hypothesis or is not statistically significant compared to the control groups.

CR1: As shown in Table 1, CR#1 demonstrates that Cd reduces the growth of rice stems and roots by light microscope, as observed a light microscope, increases ROS levels in rice ears by DCFH-DA, and decreases photosynthesis and chlorophyll in rice leaves, as measured by spectrophotometer. These findings support the hypothesis that cadmium (Cd) exerts toxic effects on rice.

CR2: As shown in Table 1, CR#2 demonstrates that Cd decreases the growth of rice stems and roots, as observed under a light microscope, and increases ROS of rice ears, as detected by DCFH-DA. However, photosynthesis in the rice leaves has not decreased.

CR3: As shown in Table 1, CR#3 demonstrates that Cd decreases growth of the rice stems and roots, as observed under a light microscope, and decreases photosynthesis in the rice leaves, as measured by a spectrophotometer. However, Cd has not increased ROS level in Rice ears, as detected by DCFH-DA. This could indicate that rice ears might possess robust antioxidant enzyme systems such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which effectively neutralize the ROS induced by Cd, resulting in no significant increase in ROS levels.

CR4: As shown in Table 1, CR#4 demonstrates that Cd increases ROS in rice ears and decreases photosynthesis in rice leaves. However, Cd has not inhibited the growth of rice stems and roots. This may suggest that Cd might accumulates more in leaves and ears than in stems and roots, leading to more pronounced effects in tissues with higher Cd concentrations, thereby impacting photosynthesis and ROS levels more severely. In addition, stems and roots may possess more efficient detoxification systems that can manage and neutralize Cd stress, preventing growth inhibition. These mechanisms could include higher levels of phytochelatins, which bind to Cd and reduce its toxicity.

CR5: As shown in Table 1, CR#5 demonstrates that Cd reduces the growth of rice stems and roots. However, Cd has neither increased ROS levels in Rice ears nor decreased photosynthesis in rice leaves. This suggests that cadmium may concentrate more in the stems and roots, with less

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accumulation in the leaves and ears. As a result, there are significant impacts on stem and root growth but minimal effects on photosynthesis and ROS levels in other tissues.

CR6: As shown in Table 1, CR#6 demonstrates that Cd decreases photosynthesis in rice leaves. However, Cd has not inhibited the growth of rice stems and roots nor increased ROS of Rice ears. This indicates that cadmium might accumulate more in rice ears, leading to increased ROS, while lower accumulation in stems and roots minimizes its impact on their growth. Similarly, Cd accumulation in leaves may not be sufficient to significantly affect photosynthesis.

CR7: As shown in Table 1, CR#7 demonstrates that Cd increases the ROS level of Rice ears, as detected by DCFH-DA. However, Cd has not inhibited the growth of rice stems and roots, nor has it reduced photosynthesis in rice leaves. This may indicate that rice ears might be more sensitive to cadmium, leading to a significant increase in ROS levels. In contrast, stems and roots may possess stronger antioxidant defense mechanisms that mitigate Cd toxicity, allowing them to maintain normal growth. Nevertheless, Cd accumulation in leaves may not be substantial enough to affect photosynthesis.

CR8: As shown in Table 1, CR#8 demonstrates that Cd has not inhibited the growth of rice stems and roots, increased ROS levels in rice ears, nor decreased photosynthesis and chlorophyll content in rice leaves. These results do not support the hypothesis that cadmium (Cd) has toxic effects on rice.

5. Discussion

CR1: Based on these experiments, researchers observed that Cd reduces the growth of rice stems and roots, increases ROS levels in rice ears, and decreases photosynthesis and chlorophyll content in rice leaves. These findings demonstrate that cadmium (Cd) has toxic effects on rice (Oryza sativa), as evidenced by multiple physiological and biochemical disruptions.

Cd toxicity impairs cellular processes, leading to reduced cell elongation and division, which in turn stunts rice roots and stem growth [13]. The rise in reactive oxygen species (ROS) levels in rice ears, detected using DCFH-DA staining, is a hallmark of Cd-induced oxidative stress [14]. ROS, such as hydrogen peroxide and superoxide anions, accumulate under heavy metal stress, causing oxidative damage to lipids, proteins, and nucleic acids.Additionally, Cd interferes with chlorophyll biosynthesis by replacing magnesium ions in the chlorophyll molecules, resulting in chlorosis and diminished photosynthetic efficiency [15]. The hypothesis is fully consistent with the experimental results.

To further advance this research, scientists should focus on elucidating the molecular mechanisms underlying Cd toxicity in rice. Investigating the expression levels of genes involved in the antioxidant defense system, photosynthetic pathways, and growth regulation will provide deeper insights into how Cd disrupts these processes in the future.

CR2: Based on these experiments, researchers found that Cd reduces the growth of rice stems and roots, increases ROS levels in rice ears and does not significantly impact photosynthesis in rice leaves. These findings demonstrate that cadmium (Cd) has a toxic effect on rice (Oryza sativa L.), as evidenced by multiple physiological and biochemical alterations.

There may be three possible causes for these results. First, regarding the inhibition of rice growth, Cd disrupts nutrient uptake and interferes with cellular processes, leading to reduced growth. Second, Cd induces oxidative stress by generating excessive reactive oxygen species (ROS), overwhelming the plant’s antioxidant defense system and resulting in increased ROS levels. However, the lack of change in photosynthesis might be due to insufficient Cd concentrations or exposure time, which were not enough to affect this process.

According to the experiment results, researchers should focus on examining the effects of prolonged Cd exposure on photosynthesis and overall plant health in the future.

CR3 and CR5: Based on these experiments, the results consistently show that Cd decreases the growth of rice stems and roots but does not increase ROS levels in Rice ears. However, the findings are inconclusive regarding whether Cd affects photosynthesis in rice leaves. Therefore, this study demonstrates that cadmium (Cd) exerts toxic effects on rice (Oryza sativa L.), as evidenced by various physiological and biochemical alterations. The hypothesis partially aligns with the experimental results.

For future research, researchers should focus on standardizing conditions and determining the precise relationship between Cd concentration, exposure time, and photosynthesis efficiency.

CR4 and CR6: Based on these experiments, the results consistently show that cadmium (Cd) decreases photosynthesis in rice leaves but does not significantly affect the growth of rice stems and roots. However, the results regarding whether Cd increases or decreases reactive oxygen species (ROS) levels in rice ears remain inconclusive. Overall, the study demonstrates that Cd has a toxic effect on rice (Oryza sativa L.), causing multiple physiological and biochemical alterations. One possible explanation for varying ROS levels is that Cd-induced ROS production may, in some cases, be countered by the plant's antioxidant defense systems, leading to decreased ROS levels. However, when these systems are overwhelmed, however, ROS levels may increase.

For future research, scientists should focus on exploring the antioxidant defense mechanisms in more detail to understand the variability in ROS levels and identify key regulators involved in the oxidative stress response.

CR7: Based on these experiments, researchers could learn these results that Cd decreases photosynthesis in rice leaves, but Cd does not decrease the growth of rice stems and roots, nor increase the ROS level of rice ears. some possible explanations for these results include that the lack of significant impact on the growth of rice stems and roots suggests that these parts of the plant may have higher metal ion exclusion or detoxification capabilities. The roots might protect themselves by expelling or sequestering Cd. Rice plants could also mitigate Cd toxicity by increasing the activity of antioxidant enzymes and synthesizing metal chelators like phytochelatins. The unchanged reactive oxygen species (ROS) levels imply that rice plants possess effective antioxidant defense mechanisms to cope with Cd stress.

For future research, researchers should focus on studying the growth and physiological responses of different rice varieties to cadmium identifying cadmium-tolerant varieties.

CR8: Based on these experiments, the results do not support the hypothesis at all. Specifically, Cd has not reduced the growth of rice stems and roots, increased reactive oxygen species (ROS) in rice ears, nor decreased photosynthesis and chlorophyll content in rice leaves. Thus, these findings demonstrate that cadmium (Cd) does not exhibit a toxic effect on rice (Oryza sativa L.), as seen through various physiological and biochemical alterations.

One potential explanation for these results is that rice plants may possess specific tolerance mechanisms to counteract Cd stress. Certain rice varieties might have genetic or biochemical pathways that help mitigate Cd's harmful effects. For future research, it would be beneficial to explore these specific tolerance mechanisms. Investigating the expression levels of genes involved in metal detoxification and antioxidant pathways could offer insights into how rice ears cope with Cd exposure.

Discussion of the possible results for the variables of concentration and treatment duration. Cadmium (Cd) exposure in rice affects the plants based on the concentration and duration of exposure. At low levels (0.01–1 μM), rice may show minimal changes in growth, ROS levels, or

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photosynthesis, indicating some tolerance. However, at higher concentrations (10–1000 μM), significant growth inhibition, increased ROS, and decreased photosynthesis are expected, reflecting oxidative stress and metabolic disruption. Short-term exposure (1–10 Days) may cause early stress signs like a slight ROS increase and reduced photosynthesis, while long-term exposure (15–30 Days) can lead to more severe damage and physiological dysfunction, demonstrating the cumulative toxicity of Cd.

Future research should focus on genetic modifications to improve Cd tolerance, examine Cd's interaction with other stressors, and explore middle-range concentrations and varied exposure times to better understand Cd's overall impact on rice. This will help develop strategies to mitigate its harmful effects.

6. Conclusions

This study examined the impact of cadmium (Cd) on growth, reactive oxygen species (ROS) levels, and photosynthesis in rice (Oryza sativa L.). The findings revealed that higher Cd concentrations and prolonged exposure significantly inhibited the development of rice roots and stems. Furthermore, Cd toxicity resulted in elevated ROS levels in rice ears and reduced photosynthesis in the leaves. These results deepen our understanding of Cd's toxic effects on rice, emphasizing its negative influence on both plant growth and physiological processes. The study underscores the critical need to manage Cd contamination in agricultural systems to safeguard crop health and productivity. Future research should prioritize developing and implementing strategies to mitigate Cd toxicity, contributing to sustainable agriculture and global food security.

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