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Study on the Effect of Cadmium Stress on Rice Seed Germinations

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ABSTRACT

This study investigates the effects of cadmium (Cd) stress at different concentrations on rice seed germination and physiological metabolism. The results show that under medium to high Cd concentrations, both the germination rate and germination potential of rice seeds significantly decreased, with a particularly pronounced inhibitory effect on the root system. The research concludes that Cd stress disrupts rice seed germination through a dual mechanism of "inhibiting starch catabolism" and "impeding substance transport." Firstly, it suppresses the activity of α- and β-amylase, reducing the consumption rate of stored substances and cutting off the energy source for germination. Secondly, it induces oxidative stress (SOD and POD activities exhibit a "low-promotion, high-inhibition" response, while MDA content significantly increases), damaging the cell membrane system and severely hindering the transport efficiency of stored substances to the seedlings. This disrupts the energy supply during rice seed germination, leading to growth inhibition. These findings provide theoretical support for mitigating Cd pollution through root system protection and metabolic regulation.

KEYWORDS

Cd; Rice growth; Crops

1. INTRODUCTION

Paint is a widely used industrial and civil product, which contains toxic heavy metals such as lead (Pb), Cd (Cd), chromium (Cr), mercury (Hg), and arsenic (As), as well as volatile organic compounds (VOCs) such as toluene, xylene, ketones, and esters. When various toxic substances enter the environment, they will have an impact on crops. Fu (2025) investigated the migration patterns of heavy metals in the soil-rice system and proposed that the bioenrichment factor (BCF) of Cd has a relatively high migration rate. That is to say, compared with other heavy metals, the same concentration of Cd in the soil is more easily absorbed by rice and accumulates in it. Even if the Cd pollution level in the soil only slightly exceeds the standard, due to its high enrichment capacity, the Cd content in rice may easily exceed the food safety standard, resulting in "Cd-contaminated rice", which directly threatens food security and human health [1]. The discovery of "Itai-Itai Disease" in Japan in the late 1960s was due to local residents consuming "Cd rice" produced from soil contaminated with Cd. Research has found that consuming plants containing Cd in can lead to osteoporosis, increased susceptibility to cancer, liver and kidney dysfunction, and hypertension in the human body [2]. This indicates the necessity of studying how Cd affects the growth of crops. Therefore, this paper studies the effects of different levels of Cd treatment on rice seed germination, and uses Microsoft Excel 2003 and SPSS statistical analysis software for One-Way Analysis of variance and Duncan method for significance test of differences. The effects of Cd on rice germination are studied from the perspectives of energy supply and plant defense. And outline the relevant measures to address Cd stress.

2. RESEARCH STATUS

Different scholars have conducted research from various perspectives, including the impact of Cd on the germination of crop seeds and the growth of seedlings, the influence of Cd on the photosynthesis of crops, the effect of Cd on the material metabolism of crops, and the influence of Cd on the permeability of crop cell membranes and protective enzymes. Wang Yusuo et al. (2021) revealed the relationship between the degree of heavy metal pollution and the emergence rate and growth of corn through soil gradient pollution experiments. He found that as the concentration of heavy metals in the soil increased, the germination rate of corn showed a significant downward trend. When corn grows, the growth status indicators such as the number of leaves, leaf length, leaf width and plant height are all inhibited. Moreover, under highly polluted conditions, the leaves show yellowing. This indicates the universal toxic effect of Cd on corn seedlings, as Cd interferes with multiple physiological metabolic processes of corn [3].

The research of Yang Jurong et al. (1995) indicated that the chloroplast content in the leaves of several plants treated with Cd decreased [4]. The research of Liao et al. (2024) showed that Cd stress would lead to a significant decrease in the contents of chlorophyll A, chlorophyll b and total chlorophyll in rice leaves. Cd may weaken the photosynthetic capacity of rice by replacing magnesium ions in chlorophyll molecules or by inducing reactive oxygen species (ROS) to damage the chloroplast structure, thereby hindering the growth of complete plants and causing yellowing leaves [5].

Wang Yongrui et al. (1999) confirmed that the decrease in soluble sugar content in post-flowering flag leaves and grains of wheat under copper and Cd stress was due to the dual inhibition of heavy metals on the transport of photosynthetic products and the self-metabolism of the grain bank, which led to the obstruction of grain filling and a decline in wheat yield [6]. The research by Hong Renyuan et al. (1991) revealed the two-stage effects of Cd stress on the physiological metabolism of wheat. In the long run, Cd stress significantly inhibited the activity of key nitrogen metabolism enzymes (NR) in post-flowering flag leaves, leading to a decrease in soluble protein content, accelerating leaf senescence and shortening the photosynthetic function period. Eventually, due to insufficient carbohydrate accumulation, the grain yield decreased [7].

The above-mentioned research provides a reference for the indicators (germination rate, plant height, root length) and measurement methods of rice growth in this study.

3. STUDY ON THE EFFECTS OF CD STRESS ON RICE SEED GERMINATION

In this section, a seed germination experiment was conducted to investigate the changes in germination rate, germination index, root length, shoot length, and amylase activity during the germination process of rice seeds under different Cd concentrations, aiming to explore the impact of Cd on rice seed germination.

3.1. Experimental Methods

3.1.1. Test Materials

(1) Heavy Metal Tested: ($CdCl_2 \cdot 2.5H_2O$) of chemical purity. Distilled water was used as the control group: CK (control): 0 μmol/L Cd^{2+} (distilled water). The Cd concentrations were set as T1: 25 μmol/L Cd^{2+}, T2: 50 μmol/L Cd^{2+}, T3: 100 μmol/L Cd^{2+}, and T4: 200 μmol/L Cd^{2+}.

Each treatment was set up with three replicates, and each replicate used 40 seeds to ensure the statistical significance of the data.

(2) Oryza Sativa: The rice variety used was Jinggeng 1, bred by the Beijing Academy of Agriculture and Forestry Sciences. It is a high-quality, high-yield, and multi-resistant variety widely cultivated in Beijing and North China. Its extensive cultivation facilitates the exclusion of climatic and other factors in the study, making the research more applicable and valuable.

(3) Preparation of Enzyme Extract:

The phosphate buffer, ethylenediaminetetraacetic acid, and polyvinylpolypyrrolidone were combined in a ratio of 50 mM PBS (pH 7.8) + 1% PVPP + 0.1 mM EDTA. The mixture was homogenized in an ice bath at 0-4°C, followed by grinding and centrifugation at 4°C and 12,000 rpm for 20 minutes. The supernatant was collected as the crude enzyme extract and stored under freezing conditions.

3.1.2. Experimental Design and Treatment

Rice seeds were sun-dried for half a day, repeatedly rinsed with clean water, and shriveled grains floating on the water surface were removed. The seeds were then disinfected by soaking in a 2% formalin solution for 2 hours, followed by multiple rounds of rinsing with clean water to ensure no residual formalin remained. Afterward, the seeds were soaked in distilled water.

The seeds were placed in a well-ventilated area, and during the soaking period, the water was changed twice daily. Soaking continued for 36–48 hours (until the rice seeds appeared semi-transparent). After soaking, 40 plump and uniformly sized rice seeds were selected and placed in a 9×9 cm culture dish. The bottom of the dish was lined with 2–3 layers of moist filter paper to maintain humidity.

Each dish was treated with 8 ml of treatment solution, while the control group received distilled water. The seeds were then transferred to a light incubator set at 26°C with 75% humidity. Under a 12-hour light cycle, the seeds were cultivated for 7 days before subsequent indicators were measured.

3.1.3. Measurement Methods

This study integrates "energy metabolism indicators (amylase)", "oxidative stress indicators (SOD, POD, MDA)", and "growth morphological indicators (root length, shoot length, etc.)" to investigate the effects of Cd on rice germination from the perspectives of energy supply and plant defense.

(1) Measurement of Rice Seed Germination

Germination Rate: A seed is considered effectively germinated when the radicle length equals the seed length and the coleoptile length reaches half of the seed length.

Germination Potential: The number of germinated seeds is recorded on the 4th day.

Germination Potential (%) = (Number of seeds germinated on Day 4 / Total number of tested seeds) × 100%

Germination Rate: The number of germinated seeds is recorded on the 7th day.

Germination Rate (%) = (Number of seeds germinated on Day 7 / Total number of tested seeds) × 100%

Root Length and Shoot Length: Measured using a ruler with a minimum scale of 0.1 cm, from the base of the root or shoot to the tip of the root or shoot.

(2) Energy Metabolism Indicators:α-amylase and β-amylase activity, Determined using the 3,5-dinitrosalicylic acid (DNS) method to measure maltose content. Enzyme activity is expressed as the amount of maltose consumed per gram of fresh plant material per minute.

Storage Material Consumption Rate (%) = [(Seed weight before germination - Total dry weight of germinated seed parts after 7 days) / Seed weight before germination] × 100%. This directly reflects the activity of amylase and represents the final outcome.

Storage Material Transport Efficiency (%) = [Dry weight of seedling (shoot + root) / Total dry weight of seedling parts (shoot + root + grain)] × 100%.

This efficiency measures how much of the decomposed material is successfully transported and utilized for the development of the coleoptile and radicle.

(3) Oxidative Stress Indicators

Superoxide Dismutase (SOD) Activity: Determined using the nitro-blue tetrazolium (NBT) photochemical reduction method. One unit of enzyme activity (U) is defined as the amount of enzyme required to inhibit 50% of NBT photochemical reduction.

Peroxidase (POD) Activity: Measured using the guaiacol method. One unit of enzyme activity (U) is defined as a change of 0.01 in OD470 per minute.

Malondialdehyde (MDA) Content: Determined using the thiobarbituric acid (TBA) method.

3.2. Data Processing

The collected data were analyzed using SPSS software through One-Way Analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of differences among treatments. Further Pearson correlation analysis was conducted to reveal the intrinsic relationship between impaired energy metabolism and oxidative damage. This comprehensive approach elucidates the dual mechanism of Cd stress affecting rice germination from both energy supply and physiological defense perspectives.

3.3. Results and Analysis

3.3.1. Research on the Effect of Cd on Rice Seed Germination

Table 1. Effects of Cd Stress on Rice Seed Germination

Groups	Germination Rate (%)	Germination Potential (%)	Root Length (cm)	Shoot Length (cm)
CK	98.3±1.9a	95.2±2.8a	5.74±0.29a	3.67±0.19a
T1	93.4±2.9a	89.2±3.2a	4.91±0.22b	3.37±0.26ab
T2	82.5±3.3b	79.6±5.9b	4.01±0.31b	2.61±0.29bc
T3	60.8±2.7C	54.4±5.3b	2.13±0.26c	2.33±0.31c
T4	53.7±4.1d	40.1±2.5d	1.06±0.21d	1.54±0.17c
F	234.56**	193.78**	201.39**	102.56**

Note: Different lowercase letters following data in the same column indicate significant differences between treatments at *p* < 0.05 (one-way ANOVA and Duncan's test). ** indicates *p* < 0.01, representing highly significant differences.

As shown in Table 1, under low-concentration Cd stress (T1, 25 μmol/L), the germination rate and germination potential of rice seeds showed no significant decrease compared to the control group (CK). This indicates that low-concentration Cd stress has minimal impact on the initial germination process of the seeds. However, under medium to high concentrations of stress (T2-T4), starting from T2 (50 μmol/L), both germination rate and germination potential exhibited significant differences. The effects of 50 μmol/L Cd^{2+}, 100 μmol/L Cd^{2+}, and 200 μmol/L Cd^{2+} on the germination rate and germination potential of rice seeds demonstrated notable variations. As the Cd concentration increased, the germination rate and germination potential declined.Furthermore, observations revealed that seeds subjected to Cd stress exhibited browning of the coleoptile and radicle. Some seeds developed mold or decay during the germination process, while others failed to germinate altogether. These findings suggest that Cd stress inhibits rice seed germination, with the inhibitory effect strengthening as the concentration increases.

Furthermore, Cd stress also exhibits an inhibitory effect on seedling growth, with a more pronounced impact on the root system. As shown in table 1, at the lowest concentration of 25 μmol/L, root length significantly decreased compared to the CK group. As Cd concentration increased, the inhibition of root length intensified sharply, with significant differences observed among all treatment groups. This indicates that one of the toxic effects of Cd on rice seeds is the suppression of root growth and development.

In contrast, the inhibition of shoot length was relatively delayed: at 25 μmol/L, shoot length showed no significant difference compared to CK, and a significant decreasing trend was not observed until the concentration reached 50 μmol/L. This suggests that shoots are less sensitive to Cd than roots. However, under high concentrations (T3, T4), shoot growth was also severely inhibited.

Overall, Cd stress significantly suppresses both rice seed germination and seedling growth, with the effects demonstrating a clear concentration-dependent relationship.

3.3.2. Effects of Cd on Energy Metabolism in Rice Seeds

Seed germination depends on the efficient mobilization and transport of stored substances from the endosperm. Starch, as the primary energy reserve in rice seeds, is broken down to provide the necessary energy for germination. This section investigates the toxic effects of Cd on two key enzymes and explores how Cd "cuts off" the energy supply for seeds.

Table 2. Effects of Cd on Energy Metabolism in Rice Seeds

	α-Amylase (mgMaltose/g FW·min)	β-Amylase Activity (mgMaltose/g FW·min)	Storage Material Consumption Rate (%)	Storage Material Transport Efficiency (%)
CK	36.8 ± 1.4 a	28.4 ± 1.2 a	69.4 ± 2.1 a	72.3 ± 1.8 a
T1	33.1 ± 1.8 a	25.9 ± 1.5 ab	67.2 ± 2.3 a	69.7 ± 2.2 ab
T2	26.6 ± 2.1 b	21.3 ± 1.4 bc	56.9 ± 3.4 b	60.5 ± 2.7 bc
T3	20.2 ± 1.4 c	15.7 ± 1.1 cd	48.3 ± 2.8 c	52.8 ± 3.1c
T4	15.5 ± 1.1 d	9.2 ± 0.9 d	35.7 ± 3.5 d	45.2 ± 3.5d
F	195.47**	132.86**	98.24**	86.74**

Note: Different lowercase letters after data in the same column indicate significant differences between treatments at *p* < 0.05 (one-way ANOVA and Duncan's test). ** denotes *p* < 0.01, indicating highly significant differences.

As shown in Table 2, regarding the effect of Cd stress on starch decomposition in rice seeds, the activities of both α-amylase and β-amylase exhibited a concentration-dependent decline with increasing Cd concentrations (F-values = 195.47** and 132.86**, respectively). At the highest concentration, the activities of the two amylases decreased to 42.1% and 32.4% of the control levels, respectively. Specifically, starting from T2 (50 μmol/L), α-amylase activity showed a significant difference compared to the control group, while β-amylase activity exhibited a significant difference under T3 (100 μmol/L) treatment. In the respiratory metabolism during the early stages of rice seed germination, α-amylase and β-amylase jointly catalyze the hydrolysis of stored starch in the endosperm, producing soluble sugars such as glucose, which provide substrates for the glycolytic pathway and thereby supply energy for seed germination and early seedling establishment. The results of this study indicate that Cd stress significantly inhibits amylase activity, which explains the obstruction of rice seed germination from the perspective of energy metabolism. Furthermore, both the storage material consumption rate and the storage material transport efficiency were significantly suppressed by Cd stress. Starting from the T2 (50 μmol/L) concentration, the consumption rate showed a significant decline, indicating that the decomposition and utilization of stored substances in the endosperm were markedly slowed. This result is entirely consistent with the declining trend in amylase activity, confirming that Cd stress disrupts the primary supply of energy and "carbon skeletons" required for germination. Similarly, starting from the T2 (50 μmol/L) concentration, transport efficiency significantly decreased, demonstrating that Cd stress had a highly significant impact on storage material transport efficiency (F = 86.74**), with further deterioration under T3 and T4 treatments. At the T4 concentration, transport efficiency dropped to 62.5% of the control level. Thus, the experimental results demonstrate that Cd stress significantly inhibits amylase activity in rice seeds, slows the decomposition of stored substances, and severely impairs the allocation process of materials from the endosperm to the developing seedling. This indicates that the toxic effects of Cd permeate two core stages of energy metabolism: decomposition and transport. This also explains why rice under Cd stress exhibits symptoms such as poor root development, seed rot, and necrosis.

Therefore, the experimental results demonstrate that Cd stress significantly inhibits amylase activity in rice seeds, slows the decomposition of stored reserves, and severely disrupts the allocation of materials from the endosperm to developing seedlings.This indicates that the toxic effects of Cd permeate two critical stages of energy metabolism: decomposition and transport. These findings also explain why Cd-stressed rice plants exhibit symptoms such as poor root development, seed rot, and tissue necrosis.

3.3.3. Effects of Cd Stress on Enzyme Activities in Rice Seeds

Superoxide dismutase (SOD) is a key protective enzyme in organisms, associated with plant stress tolerance and the scavenging of reactive oxygen species (ROS) induced under adverse conditions. Peroxidase (POD) activity reflects the strength of a plant's antioxidant capacity and the severity of toxin-induced damage, as it catalyzes the decomposition of toxic substances within a certain range. Malondialdehyde (MDA), a product of the thiobarbituric acid (TBA) reaction, is widely used as an indicator of membrane lipid peroxidation damage. An increase in MDA content indicates intensified membrane lipid peroxidation, manifested as increased membrane permeability. Studying these indicators helps reveal the toxic mechanisms of Cd stress at the cellular level.

Table 3. Study on the Effects of Cd Stress on Oxidative Stress Indicators of Rice

Group	SOD (U|g fw)	POD (U|g.min)	MDA
CK	142	14	1.6
T1	167	21	2.4
T2	182	29	2.9
T3	135	12	4.3
T4	107	8	4.9

As shown in Table 3, under Cd stress, the activities of SOD and POD in rice seeds exhibited consistent trends: under low to moderate Cd stress (T1, T2), the activities of SOD and POD were significantly induced and increased. This indicates that when rice seeds encounter Cd stress, they actively upregulate the antioxidant enzyme system to eliminate the large amounts of superoxide anion radicals and peroxides produced by Cd stress. However, once the Cd concentration reaches a certain level, it leads to the collapse of the rice defense system. When the Cd concentration increases to T3 and T4, the activities of both enzymes significantly decline, suggesting that the high concentration of Cd has exceeded the regulatory capacity of the rice seeds, causing irreversible damage to the enzyme proteins themselves. Additionally, membrane lipid peroxidation damage in rice seeds continuously worsened with increasing stress intensity, as indicated by the gradual rise in MDA content with higher Cd concentrations. This signifies severe oxidative damage to the cellular membrane system, increased membrane permeability, and disruption of structure and function, ultimately leading to metabolic disorders and even cell death.

Thus, when rice seeds are exposed to low concentrations of Cd stress, they activate the antioxidant enzyme system for effective resistance. SOD converts superoxide anions ($O_2^{\cdot -}$) into hydrogen peroxide (H_2O_2), which is then decomposed by POD into harmless water and oxygen. At this stage, although slight membrane damage occurs (as indicated by a slight increase in MDA), the defense system operates effectively. However, when the concentration exceeds a certain threshold, the rate of reactive oxygen species (ROS) production far exceeds the scavenging capacity of the enzymes. Excessive ROS attacks and inactivates enzyme proteins such as SOD and POD. Coupled with the direct toxic effects of high Cd concentrations, the antioxidant system of the rice seeds becomes inactivated. The explosive increase in MDA content indicates catastrophic damage to the cellular membrane structure and severe impairment of physiological functions.

4. CONCLUSION

This study investigated the effects of different Cd concentrations (T1: 25 μmol/L Cd^{2+}, T2: 50 μmol/L Cd^{2+}, T3: 100 μmol/L Cd^{2+}, T4: 200 μmol/L Cd^{2+}) on rice seed germination. Experimental observations revealed that under medium to high Cd stress, both germination rate and germination potential showed a declining trend. Additionally, Cd stress exhibited a more pronounced inhibitory effect on the root system of rice seeds. This finding provides the most sensitive indicator for early warning of Cd pollution and supports the development of strategies such as applying passivating agents or biochar to protect the root system of rice seeds and reduce Cd uptake. Such approaches could achieve synergistic benefits in ensuring both yield and grain safety with enhanced efficiency.

This study further investigated the toxic effects and mechanisms of Cd stress on rice seeds from the perspectives of energy metabolism and antioxidant response. It was concluded that Cd stress disrupts the energy supply system during rice seed germination through a dual mechanism of "inhibiting starch catabolism" and "impeding reserve substance translocation," ultimately leading to impaired seedling growth.Specifically, at the starch catabolism level, Cd ions significantly suppress the activities of α-amylase and β-amylase, severely hindering the conversion of stored starch in the endosperm into soluble sugars (maltose, glucose), thereby reducing the consumption rate of reserve substances. Simultaneously, Cd induces intense oxidative stress, characterized by compensatory increases in antioxidant enzyme (SOD, POD) activities at low concentrations, followed by suppression at high concentrations, along with a sharp rise in the content of malondialdehyde (MDA), a marker of membrane lipid peroxidation. This oxidative damage is likely one of the key reasons for the inactivation of critical metabolic enzymes such as amylases.

Furthermore, at the level of "reserve substance translocation," even when partial decomposition of reserves occurs, Cd stress still leads to a significant decline in "reserve substance translocation efficiency." This indicates severe disruption in the transport of hydrolyzed soluble sugars from the endosperm (source) to the seedling growth sites (sink). This impairment likely stems from two primary mechanisms: first, oxidative damage to cellular membrane systems caused by Cd compromises membrane integrity and selectivity, thereby impeding transmembrane transport functions; second, as substance translocation is an active process requiring energy, Cd-induced mitochondrial dysfunction leads to insufficient ATP supply, depriving the transport process of necessary energy. Ultimately, these disruptions adversely affect the germination and growth of rice seeds.

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