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Effects of zinc application on seed physiological quality in Ciherang rice (Oryza sativa L.) cultivar

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Abstract

Most research related to the role of zinc in improving the physiological quality of rice seeds still focuses on the germination aspect, while studies utilizing molecular approaches are still relatively limited. The purpose of this study was to determine the effect of zinc application on the physiological and molecular quality of Ciherang rice seeds. The experiment was conducted using a randomized block design with seven treatments: Priming with distilled water (control); priming with solution (PS) 0.07% ZnSO4; PS 1% Urea + 0.5% ZnSO4; spraying with solution (SS) 0.07% ZnSO4; SS 1% Urea + 0.5% ZnSO4; PS 0.07% ZnSO4 + SS 0.07% ZnSO4; and PS 1% Urea + 0.5% ZnSO4 + SS 1% Urea + 0.5% ZnSO4. The results showed that zinc treatment did not cause a significant increase in germination percentage or germination rate compared to the control, but had a positive effect on seed vigor, particularly on vigor index and plumule length. The combination of zinc and urea treatments produced a more consistent improvement in seedling vigor, indicating a synergistic interaction between zinc and nitrogen.

Keywords: Biofortification, enhancement, molecular, paddy, vigor.

Introduction

High-quality seeds are true-to-type, possess good physical characteristics, germinate rapidly and uniformly, and are free from pests and disease-causing pathogens (Finch-Savage & Bassel., 2016; Klaedtke et al., 2022). Rapid and uniform germination is crucial for successful rice cultivation because it promotes improved plant growth and development and can ultimately increase yield. Germination is a key component of seed physiological quality, defined as the set of seed properties that determine the potential activity and performance of a seed lot, including its ability to germinate and establish under diverse environmental conditions (Finch-Savage & Bassel., 2016).

One factor influencing seed physiological quality is the availability of nutrient reserves within the seed. The major components determine seed quality, such as: carbohydrates, proteins, and lipids, but the micronutrient especially zinc (Zn) is also essential for germination. Zinc functions as a cofactor for many enzymes and as a regulator of gene expression, participating in multiple physiological processes important for early seedling growth. Biofortification involves the enhancement of nutrient availability in plants by increasing the concentration of essential elements within plant tissues. Common approaches to biofortification include agronomic (fertilization-based) and genetic (plant breeding) interventions (De

Steur et al., 2017). Several studies have demonstrated that zinc biofortification can improve both the physiological quality and zinc content of harvested seeds. For instance, Tuiwong et al. (2022) reported that the application of urea and zinc fertilizers to rice increased seed germination, seed vigor, grain yield, and Zn concentration in grains of the SPT1 variety. Similarly, the combined application of zinc and urea enhanced the zinc content in Ciherang rice grains (Hidayat et al., 2025); however, its effect on the physiological and molecular quality of Ciherang rice seeds remains unknown.

Results

Vigor index

The results showed that priming, spraying, and the combination of both zinc treatments generally increased the vigor index of Ciherang rice seedlings compared to the untreated control. This increase in vigor indicates that zinc plays a crucial role in stimulating early germination metabolism, functioning as a cofactor for various enzymes and as a membrane stabilizer. However, priming at a low concentration (0.07% ZnSO4) no different from control, suggesting that this dosage was likely insufficient to stimulate optimal physiological activity (Table 1). These findings imply that the plant response to ZnSO4 was dose-dependent, making the application at an appropriate concentration or through combined methods, such as priming with a 1% urea solution + 0.5% ZnSO4, spraying with a 0.07% ZnSO4 solution, spraying with a 1% urea solution + 0.5% ZnSO4 solution, priming with a 0.07% ZnSO4 solution + spraying with 0.07% ZnSO4, priming with a 1% urea solution + 0.5% ZnSO4 solution + spraying with 1% urea + 0.5% ZnSO4 solution was effective in increasing vigor. Therefore, the results highlight the importance of optimizing the concentration and method of ZnSO4 application to maximize its physiological benefits during rice seed germination.

Table 1. Effect of treatment application on the vigor index of Ciherang rice seedlings

Treatment	**Vigor Index (%)***
Priming with distilled water (control)	87.00 c
Priming with 0.07% ZnSO4 solution	84.25 c
Priming with 1% urea + 0.5% ZnSO4 solution	92.25 ab
Spraying with 0.07% ZnSO4 solution	91.00 b
Spraying with 1% urea + 0.5% ZnSO4 solution	95.50 a
Priming with 0.07% ZnSO4 solution + spraying with 0.07% ZnSO4	92.25 ab
Priming with 1% urea + 0.5% ZnSO4 solution + spraying with 1% urea + 0.5% ZnSO4	92.50 ab
LSD 0.05	3.82

**The mean followed by the same lowercase letter in the column do not differ by the LSD test (p< 0.05).*

Seedling viability

The results showed that priming and spraying with ZnSO4, either individually or in combination, on the Ciherang rice variety did not significantly increase germination compared to the control seeds without ZnSO4 treatment (Table 2). Although zinc plays an important role in various physiological processes, including cell division, protein synthesis, and membrane stabilization, its application in the form of priming or spraying under the conditions of this study was not sufficiently effective to improve seed physiological quality. Another possible explanation is that the germination capacity of the Ciherang seeds used was already relatively high at the outset, thereby limiting the potential for further improvement through ZnSO4 treatment (a ceiling effect). Furthermore, the effectiveness of zinc may also be strongly influenced by factors such as concentration, application method, and the physiological condition of the seeds prior to treatment. Thus, these results indicate that the role of zinc is more pronounced in enhancing early growth vigor (e.g., plumule length or vigor index) rather than directly increasing germination capacity.

Table 2. Effect of different treatments on the viability of Ciherang rice seedlings.

Treatment	Seedling Viability (%)*
Priming with distilled water (Control)	98.50 a
Priming with 0.07% ZnSO4 solution	93.75 b
Priming with 1% urea + 0.5% ZnSO4 solution	98.35 a
Spraying with 0.07% ZnSO4 solution	99.25 a
Spraying with 1% urea + 0.5% ZnSO4 solution	100.00 a
Priming with 0.07% ZnSO4 solution + spraying with 0.07% ZnSO4	99.25 a
Priming with 1% urea + 0.5% ZnSO4 solution + spraying with 1% urea + 0.5% ZnSO4	98.25 a
LSD 0.05	2.00

**The mean followed by the same lowercase letter in the column do not differ by the LSD test (p< 0.05).*

Germination speed

The results showed that zinc application, whether through priming, spraying, or a combination of both, did not increase the germination rate of Ciherang rice seeds compared to the untreated control (Table 3). The addition of zinc at the early stage does not necessarily accelerate the emergence of the radicle and plumule, even though zinc functions as a cofactor for enzymes involved in germination metabolism, also the physiological condition of the Ciherang rice seeds used was already of relatively high initial quality, thereby limiting their response to additional treatments in terms of germination rate.

Table 3. Effect of treatment application on the germination rate of Ciherang rice seedlings.

Treatment	Germination Speed (%)*
Priming with distilled water (Control)	21.70 a
Priming with 0.07% ZnSO4 solution	19.75 c
Priming with 1% urea + 0.5% ZnSO4 solution	21.17 ab
Spraying with 0.07% ZnSO4 solution	20.20 bc
Spraying with 1% urea + 0.5% ZnSO4 solution	21.71 a
Priming with 0.07% ZnSO4 solution + spraying with 0.07% ZnSO4	21.85 a
Priming with 1% urea + 0.5% ZnSO4 solution + spraying with 1% urea + 0.5% ZnSO4	22.13 a
LSD 0.05	1.41

**The mean followed by the same lowercase letter in the column do not differ by the LSD test (p< 0.05).*

Plumule length

The analysis showed that ZnSO4 application, whether through priming, spraying, or a combination of both, increased the plumule length of Ciherang rice seedlings compared to the control (Table 4). Treatments that produced positive responses included priming with a 0.07% ZnSO4 solution, spraying with a 1% urea + 0.5% ZnSO4 solution, a combination of priming with 0.07% ZnSO4 followed by spraying, and a combination of priming with 1% urea + 0.5% ZnSO4 followed by spraying. This increase in plumule length indicates that Zn plays an active role in supporting early seedling growth, primarily through its function as a cofactor for enzymes involved in carbohydrate metabolism and protein synthesis, as well as in maintaining cell membrane integrity. A synergistic effect was also observed when ZnSO4 was combined with urea, likely due to the readily available nitrogen source provided by urea, which supports cell division and elongation. These findings confirm that although ZnSO4 treatment does not always significantly affect germination strength or germination rate, its application can enhance advanced vigor parameters such as plumule length. Therefore, ZnSO4 has the potential to be more effective when applied during the early growth phase to stimulate vegetative development in rice, although its effectiveness remains highly dependent on the dosage and application method used.

Table 4. Effect of treatment application on the length of plumule of Ciherang rice seedlings.

Treatment	Plumule Length (cm)*
Priming with distilled water (Control)	22.75 c
Priming with 0.07% ZnSO4 solution	24.13 ab
Priming with 1% urea + 0.5% ZnSO4 solution	23.95 abc
Spraying with 0.07% ZnSO4 solution	23.62 bc
Spraying with 1% urea + 0.5% ZnSO4 solution	25.02 a
Priming with 0.07% ZnSO4 solution + spraying with 0.07% ZnSO4	24.70 ab
Priming with 1% urea + 0.5% ZnSO4 solution + spraying with 1% urea + 0.5% ZnSO4	25.10 a
LSD 0.05	1.32

**The mean followed by the same lowercase letter in the column do not differ by the LSD test (p< 0.05).*

Discussion

The results showed that zinc application, whether through priming, spraying, or a combination of both, did not consistently increase the germination capacity or germination rate of Ciherang rice seeds. However, it did enhance vigor-related parameters, particularly the vigor index and plumule length (Tables 1 and 4). These findings confirm that zinc plays a more significant role in strengthening the early post-germination growth phase rather than directly affecting the percentage of germinated seeds. The observed increase in vigor index and plumule length reflects an improvement in seed physiological quality beyond germination capacity and germination rate, resulting in treated seeds exhibiting better field establishment potential than untreated controls (Farooq et al., 2019; Mitra et al., 2022). This enhancement in vigor can be attributed to zinc's function as a cofactor for numerous enzymes involved in carbohydrate metabolism, protein synthesis, and antioxidant defense mechanisms that mitigate membrane damage during imbibition and early seedling growth. This mechanism has often been associated with increased vigor parameters, such as coleoptile or plumule length and vigor index, following Zn nutripriming or foliar Zn application (Sami et al., 2021).

Several reviews and experimental studies have demonstrated that Zn nutripriming enhances hydrolytic enzyme activity and antioxidant capacity, thereby accelerating reserve mobilization and supporting early seedling development (Macdonald & Mohan, 2025). According to Veena & Puthur (2022), even in high-quality seeds, increases in germination percentage are often negligible. This suggests that Zn tends to enhance quantitative physiological traits (vigor) more markedly than binary or quasi-binary parameters such as germination percentage. The increase in plumule length in zinc-treated seeds can also be explained by zinc's role in physiological regulation, particularly as a cofactor for enzymes involved in auxin metabolism and protein synthesis that promote cell elongation (Mousavi, 2011; Alloway, 2009). Foliar application of ZnSO4, even at low concentrations, can increase Zn availability on the seed surface, thereby influencing the mobilization of stored reserves and promoting cell division during germination. These findings align with previous reports indicating that zinc application enhances early seedling growth and plumule elongation in several crop species by increasing enzyme activity and maintaining membrane stability during imbibition (Cakmak, 2008; Broadley et al., 2012).

Combinations involving fast-absorbing nitrogen sources (urea) or dual applications (priming + foliar spraying) can exhibit synergistic effects because nitrogen supports protein synthesis and cell division, while zinc enhances enzymatic activity. This finding aligns with the observed increase in plumule length under urea + Zn treatments (Gonzalez et al., 2019). Nitrogen derived from urea facilitates protein and amino acid synthesis, which supports reserve mobilization and energy production during the early stages of germination. The Zn–N synergy achieved through priming and foliar application is thought to enhance metabolic efficiency, thereby contributing to the increased vigor index (Farooq et al., 2019). However, the effectiveness of such treatments is largely determined by factors including concentration, application method, priming duration, and storage conditions. Uncontrolled storage environments, in particular, may induce osmotic stress or accelerate metabolic aging (Paparella et al., 2015). From a practical standpoint, the combination of priming and urea–zinc spraying shows promise as a strategy for improving rice seed quality. Nevertheless, widespread implementation requires protocol optimization and validation across multiple rice varieties and under diverse tropical environmental conditions. Additional physiological measurements, such as antioxidant enzyme activity (superoxide dismutase [SOD], catalase, peroxidase [POD]), malondialdehyde (MDA) content, electrolyte leakage, and seed Zn concentration would strengthen the mechanistic evidence and help ensure that the observed increase in vigor index results from genuine physiological improvements rather than environmental or procedural variations (Paparella et al., 2015).

Zinc priming can modulate the reactive oxygen species (ROS) balance and enhance the activity of antioxidant enzymes such as SOD, catalase (CAT), and POD, thereby reducing lipid and protein oxidation during imbibition. At the same time, zinc can increase amylolytic enzyme activity, accelerating the breakdown of starch into soluble sugars that support radicle and plumule growth. This mechanism helps explain the observed increase in seed vigor without a corresponding rise in germination percentage, suggesting that the initial stages of imbibition and enzymatic activation become more efficient, even though the total proportion of germinated seeds remains unchanged (Donia et al., 2023; Macdonald & Mohan, 2025). Therefore, the success of zinc application in improving seed quality is not solely determined by the dose and method of application but is also influenced by the genetic diversity of the variety or even the seed population.

Materials and Methods

Plant material and experimental design

This research was conducted from September 2024 to February 2025 at the Seed and Plant Breeding Laboratory, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia. The research was arranged in a non-factorial randomized block design consisting of seven treatments of zinc application, namely: soaking with distilled water (control), priming with 0.07% ZnSO4, priming with 1% urea + 0.5% ZnSO4, spraying with 0.07% ZnSO4, spraying with 1% urea + 0.5% ZnSO4, priming with 0.07% ZnSO4 + spraying with 0.07% ZnSO4, and priming with 1% urea + 0.5% ZnSO4 + spraying with 1% urea + 0.5% ZnSO4. Each treatment was repeated four times, with each unit of experimental consist of 2 plant pots.

Treatment

Priming was applied by soaking the seeds in a control solution (distilled water), a 0.07% $ZnSO4 \cdot 7H_2O$ solution, and a combined solution of 1% urea + 0.5% $ZnSO4 \cdot 7H_2O$. Seed priming was carried out for 24 hours (Tuiwong et al., 2022). Spraying with 0.07% $ZnSO4 \cdot 7H_2O$ and 1% urea + 0.5% $ZnSO4 \cdot 7H_2O$ solutions was conducted four times: during the tillering phase (35 days after planting), the booting phase (45 days after planting) (Tuiwong et al., 2022), the flowering phase (75 days after planting), and one week after the application on the 75th day (82 days after planting) (Boonchuay et al., 2013). The observation parameters in this study were the physiological and molecular qualities of the seeds, which are presented in the following variables.

Vigor index

Vigor Index observations were conducted on normal seedlings during the first count, at seven days after sowing. The vigor index was calculated following the ISTA (2020) formula, as follows:

Germination percentage

Germination percentage (GP) was determined by recording the number of normal seedlings that emerged on the fifth and seventh days after sowing. The germination percentage was calculated following the ISTA (2020) formula, as follows:

Germination speed

The germination speed (GS) was calculated by recording the number of germinated seeds, indicated by the emergence of the radicle, from the first day until the fifth day after sowing. Germination speed was calculated based on the formula of Haq et al. (2023), as follows:

where:
etmal = converted from observation time everyday
N = percentage of normal germination at each observation time, and
t_n = final observation time (day 5).

Seedling length

The measured seedling length included both the length of the shoot and the total seedling length. Seedling length was measured from the tip of the longest root to the tip of the shoot apex, expressed in centimeters.

Data analysis

Seed physiological quality data were analyzed using analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test at the 5% significance level to determine differences among treatments. The data were analyzed using the RStudio statistical software.

Conclusions

This study demonstrated that zinc application through priming, spraying, or their combination did not significantly enhance the viability of Ciherang rice seeds, as indicated by germination percentage and germination rate. However, it effectively improved vigor-related parameters, particularly plumule length and vigor index, especially when combined with urea, which synergistically supports early growth metabolism. Therefore, zinc application through spraying with 1% urea + 0.5% ZnSO4, priming with 0.07% ZnSO4 followed by spraying with 0.07% ZnSO4, and priming with 1% urea + 0.5% ZnSO4 followed by spraying with 1% urea + 0.5% ZnSO4 can be considered promising strategies to enhance the vigor of Ciherang rice seeds.

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