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RESEARCH ARTICLE Open Access

Agronomic Response of Various Rice Varieties Using Nano Priming ZnO Treatment on Seeds to Drought Stress

Anita Natalia[1], Iin Siti Aminah[1,*], and Neni Marlina[1]

Abstract

Rice (*Oryza sativa* L.) is a staple food crop that is being promoted as part of efforts to enhance national food security. The utilization of dry land in Indonesia is one mechanism that supports this food security initiative. Seed priming treatments have been shown to improve plant tolerance to drought stress conditions. This adaptation study aims to investigate the differences in seed viability among several rice varieties subjected to different concentrations of ZnO nanopriming, as well as the performance of selected seeds grown under drought stress conditions during the vegetative phase of the plants. The first phase of the research involved seed screening and variety selection, accompanied by seed priming treatments. The experimental design employed was a Factorial Randomized Block Design, with the first factor being ZnO concentrations of 0 ppm, 10 ppm, 15 ppm, and 20 ppm. The second factor involved the selection of four rice varieties: Ciherang, Inpari 32 HDB, Mekongga, and the expired Ciherang. The second phase of the research built upon the first phase, utilizing the best rice seeds identified from the screening results along with the most effective priming treatment. These seeds were subjected to drought stress treatments (without watering), which included 0 HTS, 5 HTS, 7 HTS, and 14 HTS. The results indicated that the seed priming treatment with ZnO nanoparticles (NPs) significantly increased the germination percentage of rice seeds across all varieties, with the optimal treatment combinations being 30 ppm for the Ciherang variety and 100 ppm for the Mekongga variety. Under drought stress conditions, the Ciherang variety at 30 ppm exhibited the best adaptation up to 14 HTS. Overall, the application of nano-priming with ZnO NPs enhanced seed germination and promoted plant growth.

Keywords: Drought Stress, Dry Land, Nano Priming, Rice, Seed Germination, ZnO NPs

1. Introduction

One of the primary causes of the decline in rice production in Indonesia is the reliance on traditional cultivation methods in rice fields. Meanwhile, the conversion of many agricultural lands for other uses has negatively impacted national rice production (Lutfiyani et al., 2025) .

Indonesia, with its tropical climate, has extensive dry lands that have not been optimally cultivated. To support efforts in achieving food security, various mechanisms are implemented to utilize these dry lands effectively. One such mechanism is enhancing the productivity of rice plants (*Oryza sativa* L.), which is a key staple food crop (Puspitaningrum & Salamah, 2023) .

The prolonged dry season is one of the threats that is likely to become more frequent in the future due to the effects of global warming (Rivero et al., 2007) Insufficient water availability can hinder overall plant growth, including the development of leaves, stems, and roots. Other physiological changes in plants may include wilting, yellowing, or drying of leaves, and in severe cases, plants may die. However, it is important to note that plants have developed various mechanisms to survive in conditions of water scarcity.

Climate change is the biggest threat to agricultural countries in the world, including Indonesia. El Nino is expected to affect crop yields in at least 25% of global agricultural land. Based on the review conducted, it is understood that climate change does significantly affect many aspects, especially in the agricultural sector (especially rice commodities) (Ramadhan, 2024) .

Various efforts have been made as tests or to improve

*Correspondence: iin_siti.aminah@yahoo.com
1) Universitas Muhammadiyah Palembang - Jl Jenderal Ahmad Yani 13 Ulu, Palembang, Sumatera Selatan, Indonesia

the condition of plants due to drought stress, including improving the structure and texture of the soil by providing organic materials and making rorak, or internal treatment such as providing ZPT or hormones, or by soaking (priming) seeds before planting (seed priming).

Seed priming with NPs induces electron exchange and enhances the surface reaction capabilities associated with plant cells and tissue components (Salam et al., 2022) . The main objectives of nanotechnology in agriculture are to reduce the use of chemicals, minimize nutrient losses during fertilization, and increase plant tolerance to biotic and abiotic stress, leading to increased yields (Chand Mali et al., 2020) .

The results of the study (Mazhar et al., 2022) showed that seed priming treatment with ZnO NPs (Zinc Oxide Nano Particles) with a concentration of 25 ppm helped increase resistance to drought stress in rice plants at the beginning of vegetative growth.

Other research results illustrate that ZnO NPs alone or combined with biochar increase corn plant height, number of leaves, dry biomass of shoots and roots, chlorophyll concentration and gas exchange attributes. (Rizwan et al., 2018) .

Dimkpa et al., (2019) in their research on sorghum plants stated that the emergence of flag leaves and grains was delayed by 6-17 days by drought, but this delay was reduced to 4-5 days by ZnO-NPs.

Drastic climate change over the years has posed environmental challenges to crops and yields due to fluctuating weather patterns across the globe. This has led to various types of stressors, which are responsible for the decline in plant life and biological productivity, with consequent food shortages, especially in areas threatened by desertification. Nanotechnology-based approaches have great potential in reducing environmental stressors, thereby promoting sustainable agriculture (Donia & Carbone, 2023) .

Nano-priming is an innovative seed priming technology that helps to improve seed germination, seed growth, and yield by providing resistance to various stresses in plants. Nano-priming is a much more effective method compared to all other seed priming methods because it develops enhanced electron exchange and surface reaction capabilities associated with various components of plant cells and tissues (Nile et al., 2022) .

Therefore, this study aims to see the effectiveness of the use of ZnO NPs at various treatment concentrations in several varieties of rice plants on seed viability and adaptation in the field to drought stress during the vegetative period of plants. Because previously there has been no research using the nano priming method using ZnO NpS, this study is expected to be a guideline in the development of rice in dry land in Indonesia.

2. Material and Methods

The research was conducted at an off-campus research facility in Muara Enim Regency, South Sumatra Province (3°42'08.6"S 103°48'13.1"E), with an altitude of about 45 meters above sea level. The research implementation time started from August to November 2024. This study used a Factorial Randomized Block Design consisting of 2 stages. The first stage, seed screening and selection of varieties that were given seed priming treatment. The first factor was ZnO NPs with concentrations of 0 ppm (P0), 10 ppm (P1), 20 ppm (P2), 30 ppm (P3) and 100 ppm (P4). The second factor was the selection of 4 rice varieties, namely Ciherang (V1), Mekongga (V2), Inpari 32 HDB (V3) and expired Ciherang (V4). Each treatment was repeated 3 times, resulting in a total of 60 treatment units. Each treatment contained 50 rice seeds.

The second stage is a continuation of the first study, using the best rice seeds from the screening results and the best priming treatment. The first factor is the selected varieties in the first stage of the study, namely 2 varieties (Ciherang 30 ppm and Mekongga 100 ppm). The second treatment factor is 4 drought stress treatments, including; P0 = control (watered every day), P1 = 5 days without watering (HTS), P2 = 7 days without watering (HTS) and P3 = 14 days without watering (HTS), which were repeated 4 times and each repetition contained 2 sample plants so that the total experimental unit was 64 plants. Observations only reached the vegetative phase of the plant (40 - 50 HST).

All data from seed germination and growth were analyzed using the STAR application version 2.0.1 and Microsoft Excel. The STAR (Statistical Tool for Agricultural Research) application version 2.0.1 is software used to analyze data, especially in the field of agriculture. This application is designed to help users analyze agricultural experimental data, such as completely randomized experimental design (CRD) and randomized block experimental design (RBD).

Analysis of variance was used to evaluate the significance between individual treatments and their interactions. Further tests conducted were Duncan at a significance level of 0.05 and BNT was conducted to see the smallest significant difference between varieties and priming in the data that had been collected.

In seed germination, the percentage of living sprouts, both normal and abnormal, is called Maximum Growth Potential (PTM), calculated using the formula:

$$\mathbf{PTM = \frac{\sum Living\ sprouts}{\sum Sprouting\ seeds}\ x\ 100\%}$$

(Taghfir et al., 2018)

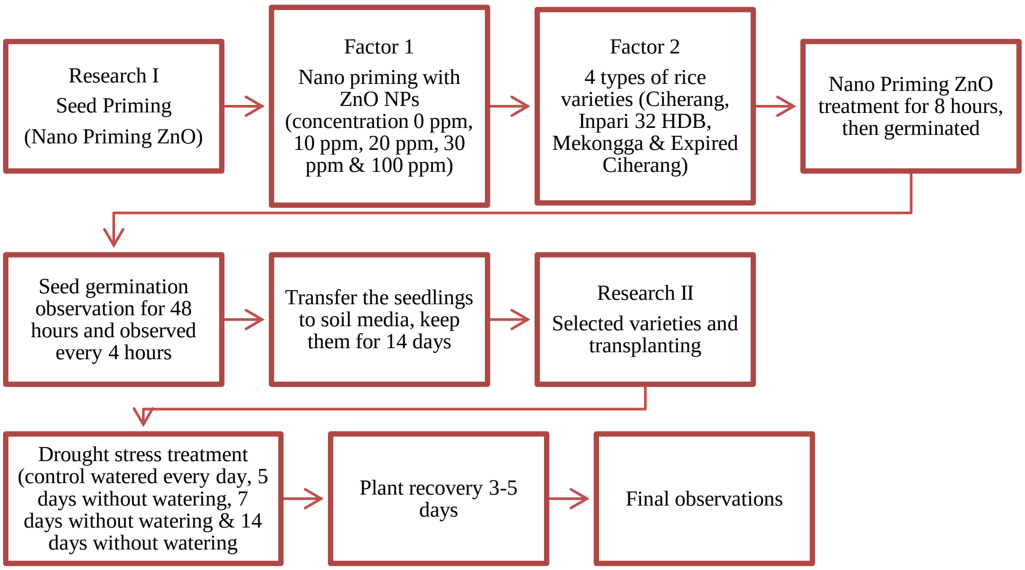

Figure 1. Research flow diagram

3. Results and Discussion

3.1. Phase I Research

The results of the research that has been conducted show that the treatment of seed immersion (priming) with ZnO NPs has a very significant effect on the treatment of varieties and treatment of immersion concentration and their interactions. Of the four seed varieties tested, 2 varieties were selected with the best seed immersion concentration, namely the Ciherang Variety 30 ppm and the Mekongga Variety 100 ppm.

In Ciherang variety, the first seed germinated in the 100-ppm treatment at 12 hours after the immersion application. However, the best seed germination fluctuation up to 48 hours was shown by the 30-ppm treatment. In this treatment, the number of seeds germinated continuously until reaching maximum germination with the highest percentage among other treatments. While in the 100-ppm treatment, the germination percentage decreased and even had the lowest value among the others. This was also followed by the variables of plant height and number of leaves when the seeds were sown. Seeds with 30 ppm immersion treatment showed the highest results among other immersion treatments.

In the Mekongga variety, the first seeds germinate at 8 hours with 30 ppm treatment. However, the best germination percentage increased until the maximum germination time of 48 hours, namely in the 100 ppm treatment. The fluctuation of germination in this variety is that the higher the dose of soaking treatment, the higher the germination percentage. This is also followed by the variables of plant height and number of leaves. Where the 100 ppm treatment has the highest plant height and number of leaves among other treatments.

Meanwhile, in the Inpari 32 variety, the highest germination percentage was in the 30-ppm treatment. The first seeds germinated at 16 hours, but the percentage of germinated seeds was very small when compared to the Ciherang and Mekongga varieties. The Inpari 32 variety used in this study was seeds that had expired for 1 month. However, the response to the ZnO NPs immersion given was quite good, seeing that in the 30-ppm treatment the seeds had germinated within 16 hours after the immersion application. Compared to the control without ZnO NPs immersion treatment, the seeds germinated within 32 hours.

Treatment of expired Ciherang variety seeds has the smallest germination percentage value among the others, both variety treatments and soaking treatments. This is because the expired Ciherang variety seeds have entered their 3-month expiration period.

Figure 2. The seed priming process uses the osmopriming method using PEG solution.

In terms of germination, it is seen that seed soaking (priming) treatment with ZnO NPs has the best germination percentage compared to no soaking treatment in all varieties. This is in line with what was stated by (Nawaz et al., 2013) that priming treatment can increase germination, accelerate seedling emergence time and increase stand formation.

Figure 3. Ciherang with ZnO nanopriming 10 ppm, 20 ppm and 30 ppm

Figure 4. Ciherang 0 ppm and 100 ppm, Mekongga 0 ppm and 100 ppm.

Figure 5. Mekongga 10 ppm, 20 ppm, 30 ppm.

Mahakham et al., 2017) stated that old rice seeds primed with phytosynthetically synthesized AgNPs at 5 and 10 ppm significantly increased germination performance and seedling vigor compared to unprimed controls, AgNO3 priming, and conventional hydropriming.

In addition, not all the same responses were shown by each variety to the treatment of various concentrations of ZnO NPs immersion. In the Ciherang variety, the best treatment was at a concentration of 30 ppm, the higher the dose, the germination decreased. In the Mekongga variety, the opposite happened, the best treatment was at a concentration of 100 ppm. While in the Inpari 32 variety, the best treatment was at a concentration of 30 ppm. And for the Ciherang Kda variety at 20 ppm.

Zinc oxide nanoparticles (ZnO NPs) have been shown to be biostimulants as well as remedies for environmental and biotic stress. Their administration at the early stage of sowing, i.e., seed priming, has been shown to be effective in increasing germination rates, seedling and plant growth and in improving plant welfare indicators (Donia & Carbone, 2023).

3.2. Phase II Research

In the second study or follow-up study, the best seeds from the soaking treatment (priming) were planted in the field with continued drought stress. The selected seeds were Ciherang 30 ppm and Mekongga 100 ppm. The results of this stage 2 study showed that the treatment of days without watering had a very significant effect on the variables of plant height, leaf wet weight and leaf and stem dry weight. Then it had a significant effect on root wet weight and root dry weight. However, in the variety treatment, all had no significant effect on the observed variables. This shows that each variety adapts to the stress or drought stress given.

The Mekongga variety has the highest and lowest plant height compared to the Ciherang variety. The highest value of the plant height variable is in the Mekongga variety with a treatment of 0 days without watering, while the lowest is in the Mekongga variety with a treatment of 14 days without watering. For the Ciherang variety, plant adaptation tends to be stable according to the drought stress treatment.

The assimilation process (nutrient absorption) in the Mekongga variety at the beginning of growth runs quickly, shown by better growth than the Ciherang variety. While in the Ciherang variety, the assimilation process tends to run slowly but its growth is gradual and continuous. As a result, the Ciherang variety has a reserve of nutrients, so that when the drought stress process occurs, the Ciherang variety plants survive. This is indicated by the condition of the Ciherang variety plants being more adaptive for up to 14 days without watering.

Based on the leaf rolling score, both Ciherang and Mekongga variety plants can still survive up to 7 days without watering. The critical period of the plant begins to appear when entering the 8th day without watering. The highest value of the plant with a perfect leaf rolling score is on the 11th day without watering. Physically, it can be seen that the Ciherang 30 ppm variety plant survives more against drought stress, reaching 14 and 15 days without watering. Meanwhile, the Mekongga variety plant only lasts up to a period of 11-12 days without watering.

Leaf rolling is the initial response of rice plants to drought stress followed by leaf drying. Leaf rolling is a mechanism for avoiding drought (drought avoidance) which is related to the ability to adjust the transpiration rate to maintain high leaf water potential in drought conditions. (Nio & Lenak, 2014).

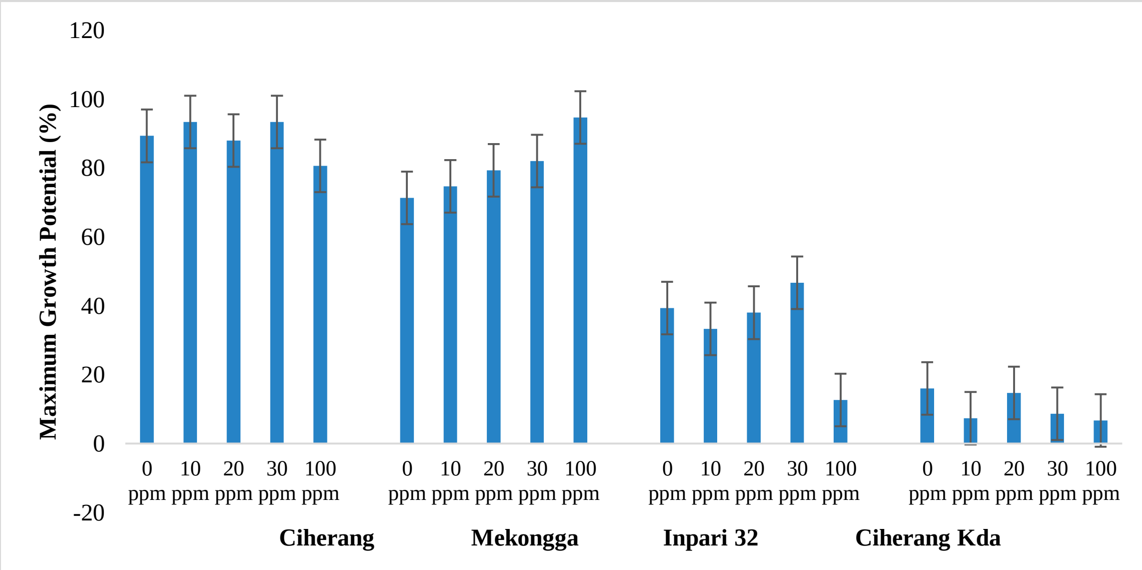

Figure 6. Maximum growth potential (%) of various rice varieties

Observed variables	*F Hit*			*KK*
	Varieties	**P. Day**	**VxP Interaction**	
Plant height (cm)	tn	**	*	12.45
J.A.	tn	tn	tn	29.98
BB Root (g)	tn	*	tn	40
Root BK(cm)	tn	*	tn	47.52
Root Length (cm)	tn	tn	tn	22.89
BB Bar (g)	tn	tn	tn	37.88
BB Leaf (g)	tn	**	tn	36.86
BK Stem & Leaf (g)	tn	**	tn	40.96
f table 0.05	10.13	9.28	9.28	
f table 0.01	34.12	29.46	29.46	

Figure 7. Ciherang variety (Pink Label) drought stress treatment of days without watering 0 days, 5 days, 7 days and 14 days (sequentially from left to right)

In both varieties, both Ciherang and Mekongga experienced relatively fast recovery, namely 1 day of watering, the plant leaves had reopened. This is in line with the opinion of Rizwan et al., 2018) that the defense mechanisms developed as a result of seed priming also form a 'primary memory' that helps achieve greater tolerance to abiotic stress on subsequent exposure.

Figure 8. Mekongga variety (Blue Label) drought stress treatment days without watering 0 days, 5 days, 7 days and 14 days (sequentially from left to right)

According to Nio & Lenak, 2014) Plant resistance to water shortages can be in the form of drought escape (resistant to drought), drought avoidance (avoiding drought), drought tolerance (tolerance to drought) and drought recovery (recovery from drought). The type of drought resistance mechanism that can be carried out is an innate factor of each plant genotype.

Figure 9. Ciherang (pink label) 14 days without watering (leaves roll up perfectly)

Figure 10. Ciherang (pink label) recovery 1 day (after rolling perfectly, water it again)

Priming is an important approach to address biotic and abiotic stresses (Hossain et al., 2015) . Rice seeds primed with seaweed-based biogenic ZnO nanoparticles at 10 mg/L showed an increase in seed germination (100%), shoot length (100 mm), shoot width (1.0 mm), root length (185.0 mm), root width (0.5 mm), seedling length (216 mm), leaf length (33.0 mm), leaf width (2.0 mm), seedling vigor (vigor index 28,500) and dry matter production (DMP) compared to conventional hydropriming (Itroutwar, PD, 2020).

Figure 11. Mekongga (blue label) 11 days without watering, the leaves have rolled up perfectly

Figure 12. Mekongga (blue label) recovery 1 day

The results of the study (Nawaz et al., 2013) increased plant height, total chlorophyll content, fresh and dry weight of plants were obtained by using seed priming with ZnO NPs. The analysis illustrated that 25 ppm was found to be more suitable for increasing the weight of 1000 grains of rice plants both in good irrigation conditions and water shortages.

In this study, it is also seen that plants adapt to drought stress by elongating plant roots. Rice that has the ability to survive in conditions of water shortage has large and long roots, so that with this root system, rice plants can penetrate deeper soil layers to maintain water status in plant tissues (Torey et al., 2014) .

Figure 13. Root length 14 days without watering Mekongga variety (blue label)

According to Nasrudin & Firmansyah, 2020) drought stress treatment significantly affected leaf area, root length, dry weight of the crown, and plant biomass but did not significantly affect plant height, total number of tillers, leaf color, and dry weight of the root. Increasing drought stress by 30-50% of field capacity decreased leaf area, dry weight of the crown, and plant biomass but increased root length.

Seed priming with ZnO NPs resulted in 53% reduction in MDA content in water-stressed rice plants. Drought stress caused a reduction in plant height by 31%, plant fresh weight by 22%, and plant dry weight by 28%. Priming with ZnONPs further increased the levels of amino acid proline which facilitated the plants to cope with water stress. Increased antioxidant enzyme activities and decreased levels of oxidative stress indicators were found after seed priming with ZnO NPs.

The study (Wu et al., 2021) explained the role of zinc oxide nanoparticles (ZnO NPs) in reducing arsenic (As) stress on rice (*Oryza sativa*) germination and early seedling growth. Rice seeds were prepared with different concentrations (10, 20, 50, 100, and 200 mg L^{-1}) of ZnO NPs and As (0, and 2 mg L^{-1}) for 12 days in a petri dish. ZnO NPs (10–100 mg L^{-1}) increased germination rate (2.3–8.9%), shoot weight (18.2–42.4%), root weight (5.2–23.9%), and chlorophyll content (3.5–40.1%), while increasing SOD (2.2–22.8%) and CAT (7.2–60.7%) activities and reducing MDA content (17.5–30.8%)

Research on wheat plants using ZnO NPs as priming resulted in 100% successful seed germination compared to the control group. The physiological factors examined, such as shoot length, root length, and fresh and dry weight of leaf and root tissues, all showed significant increases. However, beyond this threshold, at 500 ppm, these parameters decreased (Pandya et al., 2024).

The increase in photosynthetic activity of wheat plants is associated with the content of ZnO NPs in plant biomass, namely in the fresh weight of wheat shoots and roots which increased by 57% and 88% from the control treatment (Pandya et al., 2024). However, when treated with 800 ppm ZnO NPs, the fresh biomass of shoots and roots decreased significantly in a previous report (Saber et al., 2021).

4. Conclusion

Seed soaking treatment (nano-priming) with ZnO nanoparticles significantly increased the percentage of seed germination in all tested varieties compared to the control group, which received only water soaking. The optimal combination of priming treatments was Ciherang at 30 ppm and Mekongga at 100 ppm. The Ciherang variety, when subjected to 14 days of treatment without watering, demonstrates the most effective combination of treatments for combating drought stress.

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