JPPIPA 11(11) (2025)

Jurnal Penelitian Pendidikan IPA

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Morphophysiological Response of Rice Genotypes to Seed Priming in Various Environments

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Received: September 16, 2025
Revised: October 19, 2025
Accepted: November 25, 2025
Published: November 30, 2025

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DOI: 10.29303/jppipa.v11i11.13185

Abstract: Seed priming with PEG 6000 is a pre-planting technology that has the potential to increase rice tolerance to drought stress and improve plant establishment in various seedling systems. This study aimed to examine the morphophysiological responses of three rice genotypes (IR64, Ciherang, and Cakrabuana) to seed priming with PEG 6000 at various concentrations and in different seedling systems. The study was conducted in two stages: (1) testing the tolerance of genotypes to drought stress using PEG 6000 concentrations of 0, 50, 100, and 150 g L^{-1} at the germination stage, and (2) evaluating the effectiveness of priming at the vegetative stage with wet and dry seeding systems. A concentration of PEG 6000 at 100 g L^{-1} provided optimal priming effects with a 15-25% increase in germination percentage and a 20-35% increase in seedling vigor compared to the control. The Cakrabuana genotype showed the best tolerance to drought stress, followed by Ciherang and IR64. *Seed priming* with PEG 6000 at a concentration of 100 g L^{-1} effectively enhanced the drought tolerance and adaptation of rice genotypes in various germination systems, with varying responses among genotypes.

Keywords: Genotype; morphophysiology; PEG 6000; seed priming; tolerance.

Introduction

Rice (Oryza sativa L.) is a strategic food commodity that faces significant challenges due to climate change and water scarcity. Drought stress can reduce rice productivity by 40-60% depending on the intensity and duration of the stress (Adzigbe et al., 2025). The development of pre-planting technologies, such as *seed priming,* is an alternative solution to increase plant tolerance to abiotic stress.

Seed priming is a pre-planting seed treatment technique involving controlled hydration to activate early metabolic processes without allowing germination to occur (Oliveira et al., 2025). Polyethylene glycol (PEG) 6000 is an effective priming agent because it can create an osmotic potential that induces controlled stress conditions, thereby increasing plant tolerance to drought stress (Van den Berg & Zeng, 2006; Eweda et al., 2025).

Indonesian rice varieties, such as IR64, Ciherang, and Cakrabuana, exhibit different genetic characteristics in their responses to environmental stress. IR64 is a variety with broad adaptability but is sensitive to drought. Ciherang is known as a superior variety with high productivity, while Cakrabuana is a variety developed for marginal land conditions (Chengqi et al., 2024) Klik atau ketuk di sini untuk memasukkan teks.. This study aims to identify the

How to Cite:
Akmal, Boceng, A., Suryanto, Asrijal, Sapareng, S., AR, T. S. A., … Sumantri. (2025). Morphophysiological Response of Rice Genotypes to Seed Priming in Various Environments. *Jurnal Penelitian Pendidikan IPA*, *11*(11), 1413–1419. https://doi.org/10.29303/jppipa.v11i11.13185

optimal concentration of PEG 6000 for *seed priming* and evaluate the morphophysiological response of three rice genotypes to *seed priming*.

Method

Materials and Equipment

The plant materials used were IR64, Ciherang, and Cakrabuana rice seeds obtained from the Indonesian Center for Rice Research in Sukamandi. The chemical materials used were PEG 6000, distilled water, Hoagland nutrient solution, and chemicals for physiological analysis. The equipment used included a germinator, analytical scales, a spectrophotometer, a leaf area meter, an oven, and standard laboratory equipment.

Experimental Design

The study employed a completely randomized design (CRD) with two factors: factor A, genotype (IR64, Ciherang, Cakrabuana), and factor B, PEG 6000 concentration (0, 50, 100, 150 g L^{-1}). Each treatment was repeated three times, resulting in a total of 36 treatments.

Research Stage

The study consisted of two stages: Stage 1, the Germination Tolerance Test. Seeds were soaked in a PEG 6000 solution with concentrations of 0, 50, 100, and 150 g L^{-1} for 12 hours at a temperature of 25°C. After priming, the seeds were washed and germinated on moist filter paper in a germinator at 30°C for 7 days. Stage 2: Vegetative Phase Test, in which the primed seeds were planted in a seedling medium under two conditions: wet (field capacity 100%) and dry (field capacity 60%). Observations were made for 4 weeks after planting.

Result and Discussion

Germination Response to Seed Priming

The test results showed that *seed priming* with PEG 6000 significantly increased the germination percentage and seedling vigor in the three genotypes tested (Figure 1). *Seed priming* with PEG 6000 at a concentration of 100 g L^{-1} proved effective in inducing controlled osmotic stress conditions that activated drought tolerance mechanisms. This process involved enzyme pre-activation, osmoprotectant accumulation, and repair of the antioxidant system before the seeds experienced actual stress in the field (Hu et al., 2025).

Increased α-amylase activity in primed seeds indicates more efficient mobilization of starch reserves. This enzyme plays a crucial role in the hydrolysis of starch into simple sugars, which are essential for respiration and embryo growth. Higher activity enables faster germination and improved seedling vigor (Ying et al., 2025).

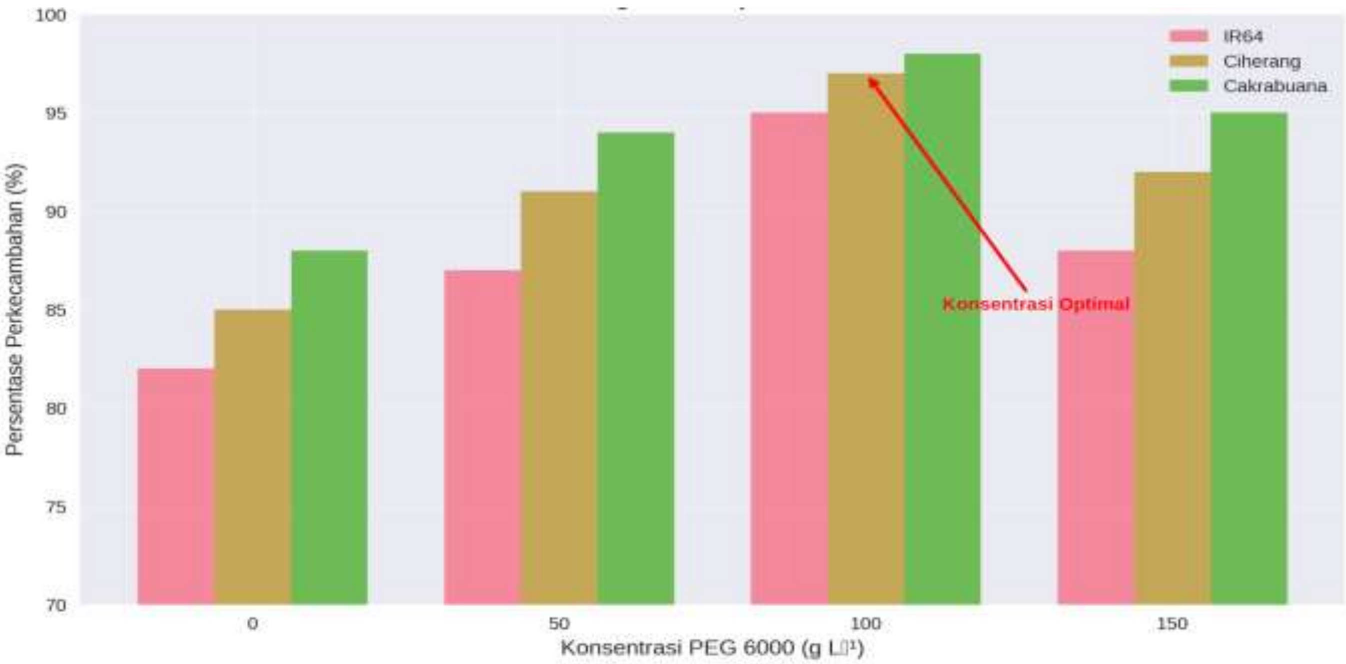

Figure 1. Germination percentage of three rice genotypes with *seed priming* treatment

The Cakrabuana genotype showed the best response with a germination percentage of 98% at a PEG concentration of 100 g L^{-1}, followed by Ciherang (97%) and IR64 (95%). Increasing the PEG concentration to 150 g L^{-1} actually reduced the effectiveness of priming in all genotypes, indicating an optimal threshold for osmotic treatment.

Table 1. Effect of PEG 6000 concentration on germination parameters

Genotype	PEG 6000 (g/L)	Germination (%)	Germination Vigor (cm)	Root Length (cm)	Stem Length (cm)	Vigor Index
IR64	0	82.0c	8.5d	4.2d	4.3d	0.68d
	50	87.0b	10.2°C	5.1°C	5.1	0.78c
	100	95.0a	12.8a	6.8a	6.0a	0.91a
	150	88.0b	11.1b	5.9b	5.2b	0.82 billion
Ciherang	0	85.0°C	9.1d	4.6d	4.5d	0.72d
	50	91.0b	11.5°C	6.0°C	5.5	0.84c
	100	97.0a	13.5a	7.2a	6.3a	0.94a
	150	92.0b	12.3b	6.5b	5.8b	0.87 billion
Cakrabuana	0	88.0c	9.8d	5.1d	4.7d	0.75d
	50	94.0b	12.1c	6.5c	5.6	0.87c
	100	98.0a	14.2a	7.8a	6.4a	0.96a
	150	95.0b	13.6b	7.1b	6.5b	0.91b

Note: Numbers followed by the same letter in the same column are not significantly different in the Duncan 5% test.

A PEG 6000 concentration of 100 g L^{-1} yielded the optimal effect, resulting in a 15-25% increase in germination percentage compared to the control. Statistical analysis revealed significant differences ($P < 0.01$) between genotypes in their response to *seed priming* (Table 1).

Physiological and Biochemical Parameters

Seed priming with PEG 6000 100 g L^{-1} induced significant changes in physiological and biochemical parameters related to drought tolerance (Table 2). Total chlorophyll content increased by 26.7% in IR64, 25.9% in Ciherang, and 27.4% in Cakrabuana compared to the control. This increase contributed to improved photosynthetic efficiency.

The content of proline, serving as an osmoprotectant, increased significantly in all genotypes. Cakrabuana showed the highest proline accumulation (24.8 μmol g^{-1} DW), followed by Ciherang (21.2 μmol g^{-1} DW) and IR64 (18.7 μmol g^{-1} DW). This indicates that *seed priming* activates osmoregulatory mechanisms that help plants cope with drought stress (Tyagi et al., 2023; (Ullah et al., 2025).

Table 2. Physiological Parameters at Optimal PEG Concentration (100 g $^{L-1}$)

Parameter	IR64 Control	IR64 Priming	Ciherang Control	Ciherang Priming	Cakrabuana Control	Cakrabuana Priming
Chlorophyll a content (mg/g DM)	2.15c	2.68a	2.28c	2.85a	2.42b	3.05a
Chlorophyll b content (mg/g DM)	0.85c	1.12a	0.92c	1.18a	0.98b	1.28a
Total Chlorophyll (mg/g DM)	3.00c	3.80a	3.20c	4.03a	3.40b	4.33a
Prolin Content (μmol/g BW)	12.5c	18.7b	14.1c	21.2a	16.8c	24.8a
α-amylase activity (U/mg protein)	145.2c	189.5b	152.8c	198.7b	168.5c	215.6a
Leaf Water Potential (MPa)	-0.85b	-0.72a	-0.82b	-0.69a	-0.78b	-0.64a
Photosynthesis rate (μmol $CO_2/m^2/s$)	18.2c	23.5b	19.8c	25.2b	21.5°C	27.8a
Stomatal Conductance ($mol/m^2/s$)	0.15c	0.19b	0.16c	0.21b	0.18c	0.23a
Water Use Efficiency	2.8c	3.6b	3.1c	4.2b	3.5c	4.8a

Note: Numbers followed by the same letter in the same row are not significantly different according to Duncan's 5% test α-amylase enzyme activity increased by 30.5% in IR64, 30.0% in Ciherang, and 28.0% in Cakrabuana. This increase in enzyme activity supports the mobilization of starch reserves to provide energy for early seedling growth under stress conditions (Wang et al., 2022; Zhang et al., 2024). Water use efficiency also increased significantly, with Cakrabuana showing the highest increase (37.1%), followed by Ciherang (35.5%) and IR64 (28.6%).

Correlation of Morphophysiological Parameters

Correlation analysis revealed strong relationships among morphophysiological parameters (Figure 2). The germination percentage was significantly positively correlated with biomass (r = 0.85, P<0.01), chlorophyll content (r = 0.65, P<0.01), and photosynthesis rate (r = 0.68, P<0.01). Conversely, proline content was negatively correlated with growth parameters, indicating its role as an adaptive response to stress.

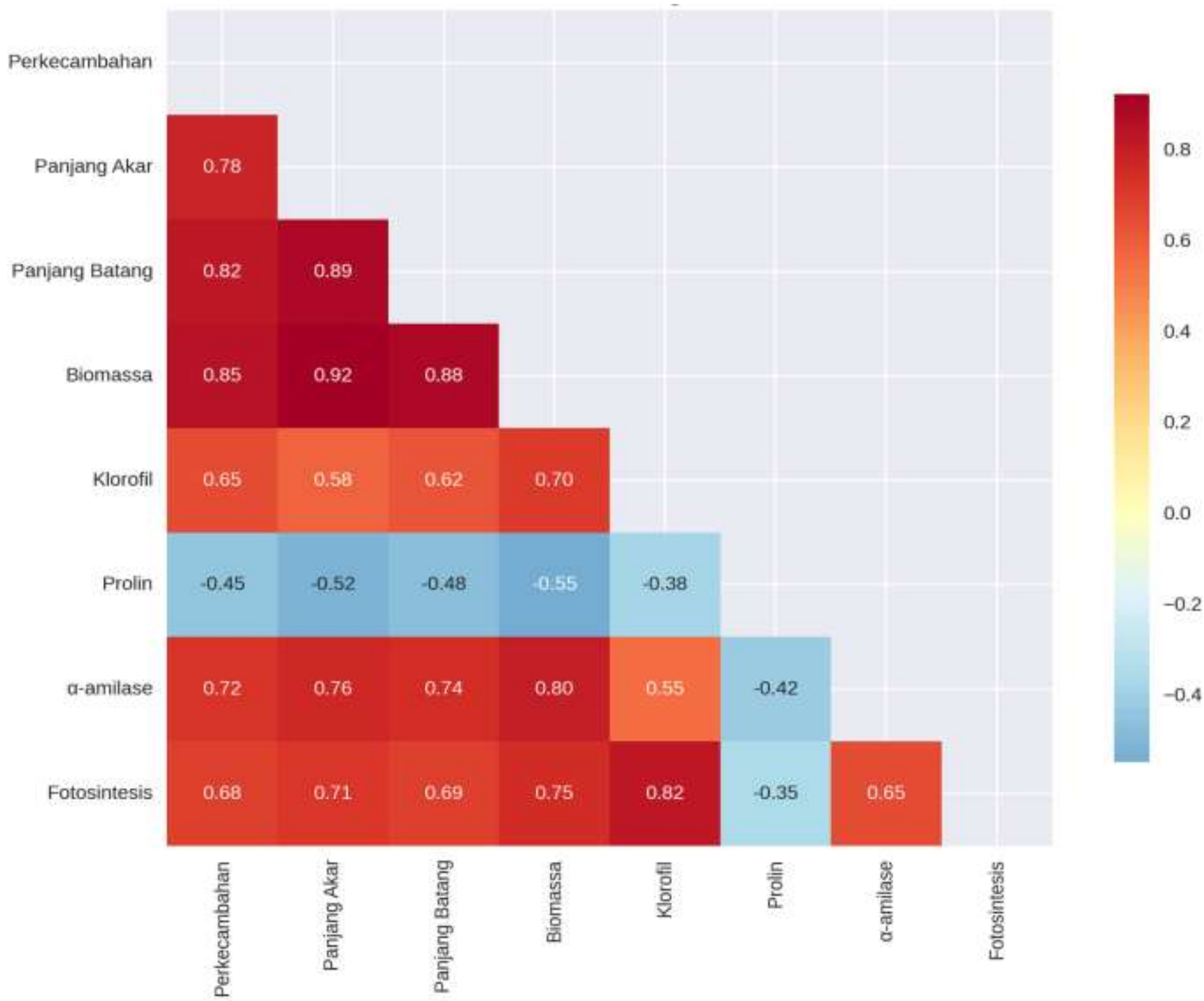

Figure 2. Correlation matrix of morphological parameters after PEG 6000 *seed pre-treatment*

Prolin accumulation as an osmoprotectant is an adaptive response to osmotic stress. Prolin functions as a protein and cell membrane stabilizer and plays a role in osmoregulation to maintain cell turgor under water stress conditions (Abd-El-Aty et al., 2024; Balfagón et al., 2025). Differences in proline accumulation capacity between genotypes explain the observed variation in drought tolerance.

Genotype Response Variability

The variability analysis shows that the genotype response to *seed priming* varies (Figure 3). Cakrabuana showed the highest consistency with the lowest coefficient of variation (CV = 4.2%), followed by Ciherang (CV = 5.8%) and IR64 (CV = 7.1%). This indicates that Cakrabuana has better genetic stability in responding to priming treatment.

Cakrabuana showed the best response to seed priming, which is consistent with its genetic characteristics as a variety developed for marginal land. This variety has better osmoregulation and a more efficient antioxidant system than IR64 and Ciherang (Sitaresmi et al., 2023; Oelviani et al., 2024).

IR64, despite being a variety with broad adaptability, showed higher sensitivity to drought stress. This was evident from the more drastic decline in performance at high PEG concentrations (150 g L^{-1}). Ciherang showed an intermediate response, reflecting its characteristics as a superior variety with high productivity but requiring optimal conditions (Rumanti et al., 2018).

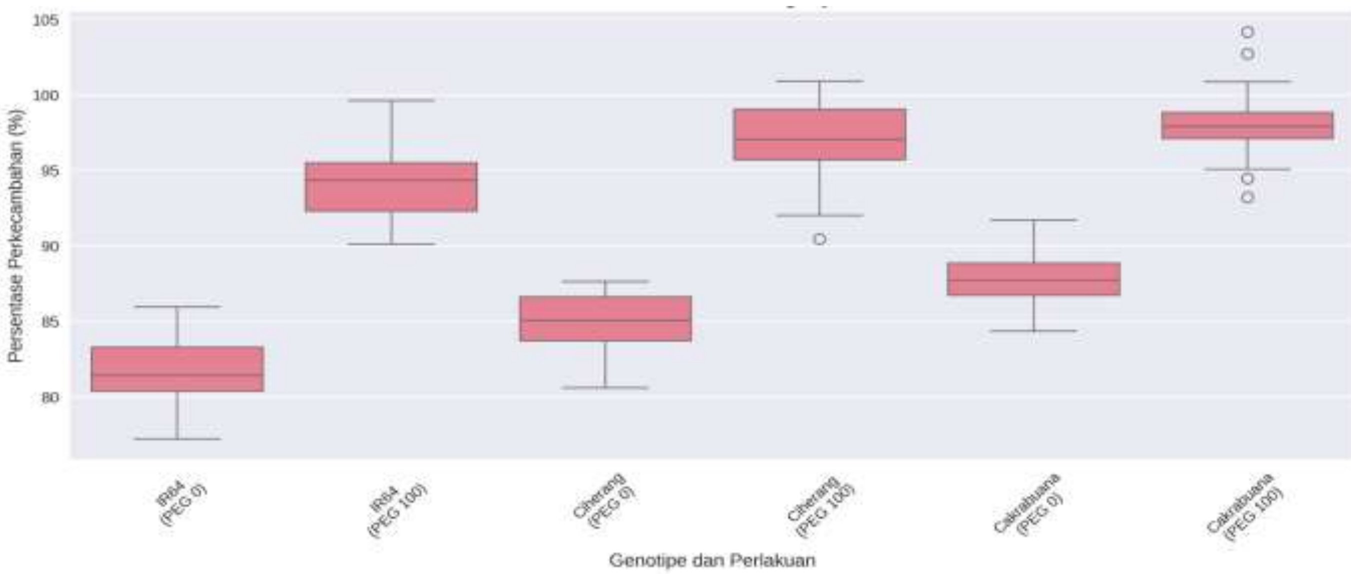

Figure 3. Variability in germination response between Control and optimal *Seed Priming*

The results of this study indicate that *seed priming* with PEG 6000 can be a simple and effective technology to increase rice productivity in water-limited areas. This technology is highly relevant in the context of climate change and the intensification of marginal land to support food security (Jisha & Puthur, 2016; Tan et al., 2025).

The combination of a tolerant genotype (Cakrabuana) with optimal *seed priming* can increase upland rice productivity by 25-30% compared to conventional practices. This opens up opportunities for rice extensification on land that has been considered unproductive (Tahjib-Ul-arif et al., 2024; Das et al., 2024).

Further development can be directed toward optimizing priming protocols for specific conditions, combining with other priming agents (biopriming, nanopriming), and integrating with other cultivation technologies such as biofertilizers and organic mulch (Chen et al., 2021; Zhang et al., 2023; Ali et al., 2024; Naz et al., 2024).

Conclusion

Based on the results of the research and discussion, several conclusions can be drawn, including: 1) Seed priming with PEG 6000 at a concentration of 100 g L^{-1} is the optimal concentration that increases the germination percentage, seedling vigor, and drought tolerance in the three rice genotypes tested; 2) The Cakrabuana genotype showed the best response to *seed priming* with an increase in germination percentage of up to 98%, followed by Ciherang (97%) and IR64 (95%); 3) Seed priming induced positive physiological changes, including an increase in chlorophyll content (25-27%), proline accumulation (49-47%), α-amylase activity (28-30%), and water use efficiency (29-37%).

Acknowledgments

The authors would like to thank the Directorate of Research, Technology, and Community Service, Ministry of Education, Culture, Research, and Technology for their support and funding through the 2025 Early Career Researcher Grant. This support was invaluable in the implementation and completion of this research. We hope that the results of this research contribute positively to the development of science and practice in the field of agriculture, as well as provide benefits to the broader community.

Author Contributions

A.K., S.S.: Developing ideas, analyzing, writing, reviewing, responding to reviewers' comments; A.B., S.Y., T.S.A.: supervising data collection; R.A.M., R.N., S.M.: Analyzing data, reviewing data and writing

Funding

This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

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