



## Effect of Hormonal Priming on Seed Germination and Initial Growth of Cambodian Rice in Salt Stress Condition

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### ABSTRACT

This experiment aimed to investigate the effect of hormonal priming with cytokinins and gibberellin on seed germination under salt stress conditions and its subsequent impact on the initial growth of *Oryza sativa* cv. Phka Rumduol. The experiment was divided into two parts: Part one examined the effect of hormonal priming (cytokinins, gibberellin) on seed germination of rice under salt stress (150mM NaCl solution). This part was arranged in a completely randomized design (CRD), and data collection included seed germination percentages, germination index (GI), and root and shoot length of rice seedlings. Part two investigated the effect of hormonal priming (cytokinins and gibberellin) on seed germination and initial growth of rice seedlings in nursery conditions. Similarly, this part was conducted in a CRD, with data collection spanning 30 days. Parameters assessed included seed germination percentage at 10 days after sowing (DAS), as well as root and shoot length, fresh and dry weight at 10, 20, and 30 DAS. Priming rice seeds with cytokinins and gibberellin induced seed germination under salt stress conditions and promoted rice seedling growth in the nursery compared to the control treatment of distilled water priming only. The overall results indicated that gibberellin at concentrations of 50ppm and 100ppm exhibited the greatest potential for promoting rice seed germination and initial seedling growth.

## **INTRODUCTION**

Rice holds profound significance for Cambodia, not only as a staple food but also as an integral part of its cultural, social, and economic fabric. As the main dietary staple, rice plays a central role in Cambodian cuisine, shaping culinary traditions and daily meals across the country (Shams, 2007). Rice cultivation forms the backbone of Cambodia's agricultural sector, employing a significant portion of the population and contributing substantially to the national economy (Kea et al., 2020). In 2023, Cambodia produced rice totaling 5.93 million tons (US Department of Agriculture, 2024). Unfortunately, rice cultivation in Cambodia consistently faces challenges due to climate change. Climate change poses significant challenges to rice production globally. Rising temperatures and changes in precipitation patterns can disrupt the delicate balance of water availability crucial for rice cultivation, leading to decreased yields and increased vulnerability to pests and diseases (Wassmann et al., 2009; Naylor et al., 2007). Aside from increasing temperature stress for rice production, salt stress is a significant problem for Cambodian farmers (Bell & Seng, 2003; Ly et al., 2016). Salty soil poses a significant challenge to rice seed germination, as high levels of salt can disrupt the delicate balance of water and nutrients essential for seed germination (Rengasamy, 2010). When rice seeds are planted in salty soil, the excess salt can draw water away from the seeds through osmosis, leading to dehydration and inhibited germination (Shaaban et al., 2013). Additionally, salt accumulation in the soil can directly inhibit enzyme activity crucial for seedling development. Consequently, rice seeds struggle to absorb water and nutrients, leading to delayed or failed germination. Furthermore, even if seeds do manage to germinate, seedlings may exhibit stunted growth and reduced vigor, ultimately impacting crop yield and quality (Carmona et al., 2010).

In response to these challenges, scientists are exploring solutions to enhance rice survival and yield in saline soil conditions, particularly focusing on seed priming. Seed priming is a pre-sowing technique used to enhance seed germination and early seedling growth, involving controlled hydration and dehydration cycles with specific nutrients, hormones, or other beneficial compounds (Paparelle et al., 2015; Nawaz et al., 2013). Waqas et al. (2019) categorized seed priming into conventional methods such as hydropriming, osmo-priming, and hormonal priming, as well as advanced methods involving nano-particles and physical agents. Hormonal priming involves soaking rice grains with plant hormones or plant growth regulators (PGRs) to induce germination and growth (Sneideris et al., 2015). This method has shown promise in alleviating salt stress in maize and wheat cultivation (Afsal et al., 2005; Afsal et al., 2006). Considering these challenges and potential solutions, this experiment aimed to investigate the effect of hormonal priming on seed germination under salt stress conditions using the Cambodian rice cultivar Phka Rumduol, employing two PGRs, cytokinin and gibberellin. Additionally, the experiment evaluated rice's initial growth in the nursery setting.

## LITERATURE REVIEW

The objective of seed priming is to activate specific physiological processes within the seed without inducing premature germination. By priming seeds prior to planting, growers can enhance the speed, uniformity, and vigor of germination, leading to improved crop establishment and ultimately higher yields (Lutts et al., 2016). Seed priming encompasses two main types: conventional seed priming methods and advanced methods (Waqas et al., 2019). Conventional methods are further categorized into hydro-priming, osmo-priming, nutrient priming, chemical priming, bio-priming, hormonal priming, and plant extract priming. Meanwhile, advanced methods include priming with nanoparticles and priming with physical agents (Sher et al., 2019; Pawar & Laware, 2018). Plant hormones, also referred to as phytohormones, are naturally occurring chemical substances produced by plants that regulate various physiological processes (Blazquez et al., 2020). These hormones play critical roles in plant growth, development, and response to environmental stimuli. Plant hormones coordinate processes such as seed germination, root growth, stem elongation, flowering, fruit ripening, and response to stress factors such as drought, pathogens, and light (Miransiri & Smith, 2014). There are several major classes of plant hormones, including auxins, cytokinins, gibberellins, abscisic acid, ethylene, and brassinosteroids, each with specific functions and effects on plant growth and development (Soliman, 2019).

Gibberellins constitute a class of plant hormones that play a crucial role in regulating various aspects of plant growth and development. These hormones are involved in processes such as stem elongation, seed germination, flowering, and fruit development. Gibberellins stimulate cell elongation by promoting the breakdown of growth-limiting factors in the cell wall, enabling cells to expand (Yamaguchi, 2008; Hedden, 2020). Xia et al. (2020) revealed that hormonal priming with gibberellin could induce cotton growth in low-temperature conditions. Additionally, hormonal priming with gibberellin increases plant growth and yields of rapeseed under drought stress conditions (Khan et al., 2020). Cytokinins are plant hormones that play a pivotal role in regulating various aspects of plant growth and development. These hormones are known for their influence on cell division, shoot formation, and overall plant morphology (Werner & Schmulling, 2009; Wybouw & De Rybel, 2019). Cytokinins are involved in breaking seed dormancy, promoting radicle emergence, and initiating seedling growth. They interact with other hormones, particularly abscisic acid (ABA), to regulate the delicate balance between dormancy and germination. Cytokinins inhibit the inhibitory effect of ABA on germination, allowing seeds to proceed with the germination process (Wang et al., 2011). Nawaz et al. (2013) demonstrated that seed priming with 10 ppm of kinetin increased the final germination percentage, germination index, shoot length, and seedling fresh weight of both tomato cultivars compared to untreated seeds. Cytokinins also increase wheat seed germination under salt stress conditions (Iqbal et al., 2006).

## **METHODOLOGY**

### ***Experimental design***

The experiment was conducted in the laboratory and nursery of the Department of Agronomy, Kampong Speu Institute of Technology, Kampong Speu province, Cambodia, at latitude 11.82832 and longitude 104.28627. The experiment was divided into two parts. The first experiment investigated the effect of hormonal priming (cytokinins and gibberellin) on the seed germination of Cambodian rice, *Oryza sativa* cv. Phka Rumduol, under salt stress conditions. The second part investigated the effect of hormonal priming (cytokinins and gibberellin) on the initial growth of Cambodian rice, *Oryza sativa* cv. Phka Rumduol, in nursery conditions.

The rice seeds were obtained from the Department of Agriculture, Forestry, and Fishery, Kampong Speu province. Cytokinins (6-benzyladenine) and gibberellin (Gibberellic acid: GA3) were purchased from Thailand. NaCl was used as the salt stress in this experiment. The rice seeds were carefully selected, and 100 seeds were counted and packed in white robes. The treatment consisted of 7 concentrations of cytokinins and gibberellin, with T1 being the control treatment using only distilled water. Treatments T2 to T4 were prepared using cytokinin solutions at concentrations of 10, 50, and 100 ppm, respectively, while treatments T5 to T7 utilized gibberellin solutions at concentrations of 10, 50, and 100 ppm, respectively. Subsequently, each pack of rice seeds was soaked in its respective solution (T1-T7) for 20 hours with continuous oxygen injection by an air pump at room temperature. Following soaking, the rice seeds were collected, dried at room temperature, and then stored in a desiccator filled with silica gel, awaiting the next step of the experiment.

The first experiment was investigated seed germination, the method was followed by ISTA (2020). The process was involved preparing plastic boxes measuring 22cm x 14.5cm x 9.5cm, each equipped with a cover. Germination papers were placed to cover the bottom of each box and then moistened by spraying with distilled water. Primed rice seeds were arranged in rows within the boxes, with 100 grains per box and four boxes per treatment. Subsequently, 150mM of NaCl solution was sprayed onto the rice seeds in each box until they were moistened, and the boxes were covered and kept at room temperature with daily data collection conducted for one week. The second experiment commenced by preparing the growing media using a mixture of soil, rice husk ash, and cow manure in a 1:1:1 ratio. This mixture was then filled into plastic baskets measuring 30cm x 40cm x 10cm. Primed rice seeds were planted in this growing media, with 100 seeds per basket and four baskets per treatment. The baskets were subsequently placed in a nursery under direct sunlight.

### ***Data Collection***

For Experiment One, data collection included germination percentages (%), germination index, root length, and shoot length. Shoot and root lengths of rice seedlings were measured by randomly selecting 10 out of 100 seedlings at 7 days after sowing. Germination percentages were assessed 7 days after moistening the rice seeds and calculated following the formula described by Ruttanaruangbowon et al. (2017) in equation (1). The germination index, or speed

of germination, was determined by daily counting the rice seeds that germinated and calculated following the method outlined by Ali et al. (2020), as shown in equation (2).

$$\text{Seed germination (\%)} = (\text{Germinated seeds} \times 100) / \text{all seed in box} \quad (1)$$

$$\text{GI} = (\text{NGS} / \text{n1}) + (\text{NGS} / \text{n2}) + \dots + (\text{NGS} / \text{nx}) \quad (2)$$

Information:

GI = germination index

NGS = number of germinated seed

N1, n2, nx = day of germinated seed counting

For Experiment Two, data collection included seed germination percentages at 10 days after sowing (DAS), which were calculated using equation (1). Subsequently, ten rice seedlings were randomly selected to measure their shoot length, root length, fresh weight, and dry weight at 10, 20, and 30 DAS.

### *Data Analysis*

The data were computed using Microsoft Excel and analyzed using analysis of variance (ANOVA) to observe significant differences among treatments. Treatments showing significant differences were further analyzed using the Least Significant Difference (LSD) test to identify variations where  $P > 0.05$ .

## **RESEARCH RESULTS**

In the laboratory setting, rice seeds were subjected to a salt condition of 150mM, aided by cytokinin and gibberellin. Regarding germination percentages seven days post-sowing, the results indicated no significant difference between treatments. However, in terms of the germination index (GI) or speed of germination, a notable contrast among treatments was observed. Specifically, the use of 100ppm cytokinin prompted a faster rice germination index compared to other treatments, although the variance was not statistically significant when compared to treatments involving 50ppm cytokinins and gibberellin concentrations ranging from 10 to 100ppm. Notably, germination with only distilled water exhibited the slowest rate among rice seeds.

The root length of rice seedlings germinated under salt stress conditions exhibited significant differences among treatments. Gibberellin at a concentration of 50ppm induced the highest root growth, measuring 3.66cm, but did not show a significant difference from the gibberellin solution at 100ppm, which yielded a length of 3.24cm. The lowest root growth was observed when utilizing cytokinin at concentrations of 10ppm and 100ppm. Additionally, there was a significant difference in shoot growth. Gibberellin solutions at concentrations of 100ppm and 50ppm demonstrated the highest shoot growth, measuring 5.73cm and 5.11cm, respectively. In contrast, treatment with only distilled water exhibited the lowest shoot growth, measuring 0.66cm (table 1).

Table 1. Germination percentages, Germination index, root and shoot length of *Oryza sativa* cv. Phka Rumduol in salt stress condition

Treatments	Germination (%)	Germination index	Root length (cm)	Stem length (cm)
Distilled water	78.00	30.31 <sup>c/1</sup>	2.16 <sup>cd</sup>	0.66 <sup>e</sup>
Cytokinin 10ppm	85.00	73.46 <sup>b</sup>	1.24 <sup>d</sup>	2.10 <sup>de</sup>
Cytokinin 50ppm	88.75	81.79 <sup>ab</sup>	1.52 <sup>cd</sup>	3.48 <sup>cd</sup>
Cytokinin 100ppm	90.75	85.60 <sup>a</sup>	1.38 <sup>d</sup>	4.02 <sup>bc</sup>
Gibberellin 10ppm	89.00	76.59 <sup>ab</sup>	2.54 <sup>bc</sup>	3.26 <sup>cd</sup>
Gibberellin 50ppm	88.75	79.05 <sup>ab</sup>	3.66 <sup>a</sup>	5.11 <sup>ab</sup>
Gibberellin 100ppm	85.75	78.04 <sup>ab</sup>	3.24 <sup>ab</sup>	5.73 <sup>a</sup>
F-test	ns	*	*	*

<sup>/1</sup> In the same column, different letter showed significant difference P > 0.05

In the nursery conditions, all rice seeds were not treated with a salt solution; they were simply allowed to grow under normal conditions. Cytokinins and gibberellin were applied to rice seeds to observe their germination and growth. All treatments involving cytokinins and gibberellin showed the highest germination percentages, which were significantly different from the treatment involving only distilled water. Root length was measured three times: at 10, 20, and 30 days after sowing (DAS). At ten days DAS, the results indicated that all treatments involving gibberellin at various concentrations exhibited the highest root lengths, followed by treatments involving cytokinins and those treated with distilled water. This trend was consistent at twenty DAS. By thirty DAS, the treatment treated with gibberellin at 10ppm demonstrated the highest root length, measuring 11.34cm, but did not show a significant difference from treatments involving cytokinins and gibberellin at 10ppm and 100ppm, respectively. Conversely, the treatment involving distilled water continued to display the lowest root length (Table 2).

Table 2. Germination percentages and root length of *Oryza sativa* cv. Phka Rumduol at nursery condition

Treatments	Germination (%)	Root length (cm)		
		10 days	20 days	30 days
Distilled water	58.00 <sup>b</sup>	2.48 <sup>c/1</sup>	3.69 <sup>c</sup>	5.31 <sup>c</sup>
Cytokinin 10ppm	77.75 <sup>a</sup>	3.64 <sup>b</sup>	7.03 <sup>b</sup>	10.24 <sup>ab</sup>
Cytokinin 50ppm	88.25 <sup>a</sup>	4.03 <sup>b</sup>	7.20 <sup>b</sup>	9.25 <sup>b</sup>
Cytokinin 100ppm	83.00 <sup>a</sup>	4.19 <sup>b</sup>	7.19 <sup>b</sup>	8.97 <sup>b</sup>
Gibberellin 10ppm	86.75 <sup>a</sup>	6.93 <sup>a</sup>	10.07 <sup>a</sup>	11.34 <sup>a</sup>
Gibberellin 50ppm	78.75 <sup>a</sup>	6.79 <sup>a</sup>	8.79 <sup>a</sup>	9.54 <sup>b</sup>
Gibberellin 100ppm	78.50 <sup>a</sup>	6.68 <sup>a</sup>	8.87 <sup>a</sup>	10.26 <sup>ab</sup>

F-test	*	*	*	*
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<sup>/1</sup> In the same column, different letter showed significant difference P > 0.05

Hormonal priming with cytokinins and gibberellin also affects the shoot growth of rice seedlings in the nursery. At ten DAS, seeds primed with gibberellin at a concentration of 100ppm exhibited the highest shoot growth, measuring 10.93cm, followed by gibberellin at a concentration of 50ppm, which measured 10.03cm. Priming seeds with cytokinins showed no effectiveness during this period. By twenty DAS, rice seeds primed with gibberellin at concentrations of 50ppm and 100ppm displayed the highest shoot growth, measuring 18.89cm and 18.62cm, respectively, but did not show a significant difference from seeds primed with cytokinins at a concentration of 10ppm, which measured 17.10cm. The lowest shoot growth still occurred in the treatment treated with only distilled water. At thirty DAS, seeds primed with gibberellin at a concentration of 100ppm exhibited the highest shoot growth, measuring 32.14cm, but did not show a significant difference from seeds primed with cytokinins and gibberellin at concentrations of 10ppm and 50ppm, respectively, measuring at 30.29cm and 32.24cm, respectively (Table 3).

Primed rice seeds with gibberellin at all concentrations gained more weight in terms of rice seedling fresh weight at 10 DAS, while those primed with cytokinins ranked second highest, and seeds primed with distilled water exhibited the lowest fresh weight. By twenty DAS, seeds primed with 10ppm and 50ppm of gibberellin displayed the highest fresh weights, measuring 1.60g and 1.41g, respectively, but did not show a significant difference from seeds primed with cytokinins at 10ppm and 50ppm, as well as gibberellin at 100ppm, which measured 1.33g, 1.36g, and 1.28g, respectively. Priming with distilled water continued to yield the lowest fresh weight. At 30 DAS, seeds primed with gibberellin at a concentration of 50ppm exhibited the highest fresh weight, measuring 5.58g.

Table 3. Shoot length of *Oryza sativa* cv. Phka Rumduol in Nursery Condition

Treatments	Shoot length (cm)		
	10 days	20 days	30 days
Distilled water	5.55 <sup>e/1</sup>	11.14 <sup>c</sup>	17.38 <sup>d</sup>
Cytokinin 10ppm	7.31 <sup>d</sup>	17.10 <sup>ab</sup>	30.29 <sup>ab</sup>
Cytokinin 50ppm	7.25 <sup>d</sup>	15.68 <sup>b</sup>	31.89 <sup>a</sup>
Cytokinin 100ppm	7.35 <sup>d</sup>	15.60 <sup>b</sup>	28.54 <sup>bc</sup>
Gibberellin 10ppm	8.51 <sup>c</sup>	16.18 <sup>b</sup>	26.30 <sup>c</sup>
Gibberellin 50ppm	10.03 <sup>b</sup>	18.89 <sup>a</sup>	32.14 <sup>a</sup>
Gibberellin 100ppm	10.93 <sup>a</sup>	18.62 <sup>a</sup>	30.72 <sup>ab</sup>
F-test	*	*	*

<sup>/1</sup> In the same column, different letter showed significant difference P > 0.05

Regarding the dry weight of rice seedlings, at 10 DAS, no significant difference was observed among treatments. At twenty DAS, priming rice seeds

with gibberellin at concentrations of 10ppm and 50ppm, respectively, and cytokinins at 10ppm showed the best dry weights, measuring 0.34g, 0.32g, and 0.34g, respectively. Priming with distilled water continued to yield the lowest dry weight at 0.24g. At 30 DAS, rice seeds primed with gibberellin at 50ppm and 100ppm demonstrated the highest dry weights, measuring 1.11g and 1.12g, respectively. Priming rice seeds with distilled water still resulted in the lowest seedling dry weight (Table 4).

Table 4. Fresh and dry weight of *Oryza sativa* cv. Phka Rumduol in nursery condition (10 seedling)

Treatments	Fresh weight (g)			Dry weight (g)		
	10 days	20 days	30 days	10 days	20 days	30 days
Distilled water	0.61 <sup>c/1</sup>	0.73 <sup>c</sup>	1.37 <sup>d</sup>	0.24	0.24 <sup>c</sup>	0.38 <sup>c</sup>
Cytokinin 10ppm	0.81 <sup>b</sup>	1.33 <sup>ab</sup>	3.64 <sup>c</sup>	0.24	0.34 <sup>ab</sup>	0.58 <sup>c</sup>
Cytokinin 50ppm	0.84 <sup>b</sup>	1.36 <sup>ab</sup>	4.78 <sup>ab</sup>	0.24	0.31 <sup>b</sup>	1.03 <sup>ab</sup>
Cytokinin 100ppm	0.78 <sup>b</sup>	1.08 <sup>b</sup>	3.57 <sup>c</sup>	0.23	0.30 <sup>b</sup>	0.68 <sup>bc</sup>
Gibberellin 10ppm	1.00 <sup>a</sup>	1.60 <sup>a</sup>	3.87 <sup>bc</sup>	0.24	0.34 <sup>a</sup>	0.69 <sup>bc</sup>
Gibberellin 50ppm	1.07 <sup>a</sup>	1.41 <sup>a</sup>	5.58 <sup>a</sup>	0.23	0.32 <sup>ab</sup>	1.11 <sup>a</sup>
Gibberellin 100ppm	0.98 <sup>a</sup>	1.28 <sup>ab</sup>	4.57 <sup>abc</sup>	0.26	0.30 <sup>b</sup>	1.12 <sup>a</sup>
F-test	*	*	*	ns	*	*

<sup>/1</sup> In the same column, different letter showed significant difference P > 0.05

## DISCUSSION

Seed priming plays a vital role in field crop germination, especially during climate challenges. It can induce germination in field crop seeds under severe conditions such as heat stress, salt stress, or exposure to sunlight (Paparella et al., 2015; Singh et al., 2020). Practicing seed priming, including the application of plant hormones like cytokinins and gibberellin, should be a good option for growers. Cytokinins and gibberellin assist field crop seeds in coping with changing environments and encourage better growth and yield (Eisvand et al., 2011; Khan et al., 2020). In this experiment, we observed that the use of cytokinins and gibberellin induced better germination and initial growth in rice compared to treatments with distilled water. However, upon examining the overall results, it becomes evident that utilizing gibberellin at concentrations of 50ppm or 100ppm is most effective for inducing rice germination in salty conditions and promoting initial growth in nursery conditions. Gibberellins, a class of plant hormones, are crucial for various aspects of plant growth and development (Yamguchi, 2008). They regulate processes such as seed germination, stem elongation, leaf expansion, and flowering (Mutasa-Gottgens & Hedden, 2009; De Lucas et al., 2008). Gibberellins play a pivotal role in promoting seed germination by breaking seed dormancy and initiating the growth process (Urbanova & Leubner-Metzger, 2016). Upon encountering suitable environmental conditions such as moisture and temperature, dormant seeds produce gibberellins, triggering a cascade of biochemical reactions within the seed (Vishal & Kumar, 2018). Gibberellins stimulate the synthesis of enzymes,

particularly  $\alpha$ -amylase, which breaks down starch stored in the endosperm into simple sugars. These sugars serve as energy sources for the developing embryo, facilitating its growth and emergence from the seed coat (Ravindran & Kumar, 2019). Additionally, gibberellins regulate the expression of genes involved in cell elongation and expansion, enabling the radicle to penetrate the soil and initiate root growth.

Gibberellin also induce plant to live in severe conditions. It serves as vital regulators in helping plants cope with adverse environmental conditions such as drought, high temperatures, and salty stress (Niharika et al., 2021). During periods of drought stress, gibberellins play a crucial role in regulating plant water uptake and distribution, aiding in maintaining cellular turgor pressure and preventing wilting (Maleki et al., 2020). Moreover, gibberellins can modulate the expression of stress-responsive genes, enabling plants to activate defense mechanisms against high temperatures and oxidative stress (Bawa et al., 2020). In conditions of salty stress, gibberellins assist in mitigating the negative effects of excess salts on plant growth by regulating ion transport and osmotic adjustment, thus enhancing plant tolerance to saline environments (Shi et al., 2019). Overall, gibberellins play a multifaceted role in enabling plants to adapt and thrive in unfavourable environmental conditions, ultimately contributing to their survival and resilience.

Xia et al. (2023) revealed that priming cotton seeds with gibberellin could increase their germination potential, germination index, and germination time in low-temperature conditions. Germination of rice seeds under salt stress conditions could induce  $\text{Na}^+/\text{K}^+$  ratio, increase proline and  $\text{H}_2\text{O}_2$  content, but priming seeds with gibberellin could mitigate these effects and induce rice seed germination and growth under such severe conditions (Chunthaburee et al., 2014). Gibberellin has been shown to mitigate salt stress in *Pisum sativum* production by up-regulating antiporter genes (Ahmad et al., 2021). Based on this evidence, we can conclude that gibberellin has the potential to mitigate plant stress and induce plant growth and seed germination under various stressful conditions.

## CONCLUSIONS AND RECOMMENDATIONS

Priming rice seeds with plant hormones, cytokinins, and gibberellin, could induce germination in salt stress conditions and promote growth in normal nursery conditions compared to those treated solely with distilled water, with gibberellin yielding higher results than cytokinins. Overall, utilizing gibberellin at concentrations of 50ppm and 100ppm appears to be the most effective in mitigating salt stress, inducing rice germination under such conditions, and promoting rice growth under normal conditions.

## ADVANCED RESEARCH

This study was conducted over a brief period. Subsequent experiments could explore the entire life cycle of rice to thoroughly assess the efficacy of hormonal priming with cytokinins and gibberellin.

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