

# Germination Responses of Old Seeds from Lampung Local Rice to Magnetic Fields and Drought Stress

Submitted 16 April 2024 Revised 19 June 2024 Accepted 07 July 2024

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## Abstract

Germination is a critical phase in seed plants. Germination is vulnerable to drought stress. Apart from external factors, long seed storage can also affect seed viability. The magnetic field is known to positively impact the germination process because it can increase the activity of the  $\alpha$ -amylase enzyme. Lumbung Sewu Cantik is a local upland rice variety from Lampung, Indonesia. This study aims to determine the impact of exposure to a magnetic field of 0,2 mT for 11 minutes 44 seconds ( $M_1$ ) in improving the germination of LSC rice seeds given accelerated aging (So) and drought by 20% PEG 6000 ( $D_1$ ). The parameters observed were germination rate index (GRI), mean germination time (MGT), germination index (GI), final germination percentage (FGP), and percentage of abnormal germination (PAG). This research used a Completely Randomized Design (CRD). The experimental units consisted of Sn (normal seeds as control), So $D_1$ , So $M_1$ , and So $M_1D_1$ . Each treatment was repeated five times. The data obtained were tested for normality, analyzed of variance, and further analyzed with DMRT at the  $\alpha = 0,05$  level. The results showed that the magnetic field could accelerate germination in the So $M_1$  treatments based on the MGT parameters. The magnetic field also reduced the percentage of abnormal seedlings in the So $M_1D_1$  treatments compared to those without a magnetic field (So $D_1$  treatments).

Keywords: Aging of Rice Seeds, Drought Stress, Germination, Magnetic Field

## INTRODUCTION

Rice is a staple food in Asia, with more than 90% of world rice production and consumption contributed by the region. So rice (*Oryza sativa* L.) has become an important agricultural commodity for the people of Asia, one of which is Indonesia (FAO, 2014). The increase in demand for rice in Indonesia will be in line with the increase in population (Sawitri et al., 2018), so to comply with the domestic rice requirements, efforts must be made to increase rice production (Cahyadi et al., 2013). Rice production in Indonesia is still facing various obstacles, including drought due to climate change (Ma'sumah et al., 2016; Nasrudin & Firmansyah, 2020) and limited supplies of high-quality seeds due to processing and extended storage periods that cause damage or aging seeds (Dewi & Sumarjan, 2013; Navira et al., 2020).

Drought is one of the environmental factors that contribute most to limiting crop production (Bray, 2007). On the other hand, germination is the most critical stage in the development of seed plants because they are very susceptible to various abiotic stresses (Rajjou et al., 2012). Abiotic drought stress can inhibit germination and the growth process of seedlings (Zhu et al., 2006; Kizilgeci et al., 2017). In rice plants, drought conditions in the germination phase can reduce plumule length (Daksa et al., 2014; Chrisnawati et al., 2021), germination

speed, and percentage of normal germination (Cahyadi et al., 2013; Daksa et al., 2014), as well as chlorophyll levels (Putri et al., 2022).

Apart from external factors in the form of abiotic stress, internal factors such as seed quality also influence germination. The physiological quality of seeds is determined by age and storage process (Suparto & Nugraha, 2022). Initially, when the rice seeds have just been harvested, they cannot germinate even though they are planted under optimum conditions because the rice seeds are in the after-ripening period. The length of the after-ripening period varies depending on the rice variety, ranging from 0 to 12 weeks. New rice seeds can be planted over a specific interval after a dry storage process (Wahyuni et al., 2023). Rice seeds that have been stored for more than six months in inappropriate storage containers can experience a decrease in seed quality (deterioration), which is characterized by a decrease in vigor and viability (Suparto & Nugraha, 2022), as well as germination capacity (Rohandi & Widayani, 2016). Meanwhile, upland rice seeds used by farmers generally come from previous harvests with a storage period of 6-8 months (Kartika & Sari, 2015).

Using inappropriate storage containers during seed storage can worsen the decline in seed quality (Sari & Faisal, 2017) due to the increased water content of rice seeds (Tefa, 2017). The high water content of rice seeds during storage increases the seed respiration rate, increasing starch breakdown activity, increasing sugar levels, and reducing food reserves (Dewi & Sumarjan, 2013). Further depletion of seeds can cause cells to experience oxidative stress, which leads to damage to cell membranes and increased damage to genetic material (Ebony et al., 2019). Thus, if it is still to be used, it is necessary first to treat old rice seeds that have been stored for a long time (deteriorating) so that they can continue to germinate well, even if they are later planted in a location affected by drought.

Magnetic fields are known to influence the growth of obsolete seeds positively. The research results of Novitasari et al. (2019) showed that exposure to a magnetic field of 0,2 mT had the best effect in increasing the vigor of old tomato seeds (old stock) so that vegetative growth was the same as new stock seeds. The magnetic field also positively affected wheat embryo explants grown in a medium induced by drought stress (PEG-6000 60 g/L). Providing a magnetic field of 2,9-4,7 mT can increase levels of chlorophyll, carotenoids, and the activity of antioxidant enzymes in wheat plantlets (Sen & Alikamanoglu, 2014). Magnetic fields are known to increase antioxidants and reduce oxidative stress in plants when subjected to abiotic stress, such as drought, heavy metal contamination in the soil, and salt stress (Radhakrishnan, 2019). Additionally, exposure to a magnetic field can increase the activity of the  $\alpha$ -amylase enzyme, which plays a vital role in germination (Agustrina et al., 2013).

Based on the description above, this research aimed to determine the effect of exposure to magnetic fields in improving the germination process of old rice seeds germinated in drought conditions. The rice variety used in this research is the Lumbung Sewu Cantik (LSC) from Pringsewu Regency, Lampung, Indonesia. The LSC variety has the advantages of a fluffier rice texture, amylose content of 13,99%, resistance to lodging, and a very high yield of 3,8-4,0 tonnes per hectare without fertilizer (Adriyani et al., 2019). LSC varieties need to be researched further so that their existence is maintained. With this study, it is hoped that the use of magnetic fields can be an alternative solution to addressing the problem of drought stress and limited stocks of quality seeds to support national food security.

## **METHOD**

This research was conducted in October 2023 and is located at the Botany Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Lampung. This research used a Completely Randomized Design (CRD) which consisted of four treatments, namely Sn (normal rice seeds as a control), SoD<sub>1</sub> (rice seeds that were given accelerated aging and drought stress), SoM<sub>1</sub> (rice seeds that were given accelerated aging and exposed to a magnetic field of 0,2 mT), and SoM<sub>1</sub>D<sub>1</sub> (rice seeds that were given accelerated aging, exposed to a magnetic field of 0,2 mT, and drought stress). The magnetic field exposure duration was 11 minutes 44 seconds (Angraini et al., 2013). A simulation of drought stress treatment was carried out with PEG 6000 with a concentration of 20% (w/v) (Chrisnawati et al., 2021). Each treatment was repeated 5 times.

## **Materials and Tools**

The materials used in the research include local LSC variety rice seeds originating from Pringsewu Regency, straw paper, label paper, aluminum foil, 10% Bayclin solution (contains the active ingredient sodium hypochlorite/NaClO 5,25%), PEG 6000, distilled water, and 96% ethanol. The tools used in the research include measuring cups, beakers, plastic Petri dishes with a diameter of 9 cm, dropper pipettes, tweezers, analytical scales, stirring rods, solenoids as a source of magnetic fields, Gauss meters (measuring instruments for magnetic field strength), ruler, stopwatch, sprayer bottle, tray, and jerry cans.

## **Research Methods**

Rice seed selection is carried out manually by pressing lightly with fingers. If the rice seeds feel intact and not wrinkled, they are selected for use in research (Deanesia et al., 2014).

The selected seeds are also relatively uniform in size (Chrisnawati et al., 2021; Nurmalasari, 2018). The weight of LSC seeds used ranged from 0,027 to 0,031 gr.

The old rice seeds (So) were obtained by accelerated aging following the method from Belo & Suwarno (2012), with slight modifications. Rice seeds are soaked in 96% ethanol for 6 minutes and then air-dried for 30 minutes.

For normal seed treatment (Sn), the rice seed sterilization process involves soaking the seeds in a 10% Bayclin solution (v/v) for 15 minutes. Then, the rice seeds are rinsed three times with distilled water (Deanesia et al., 2014).

The prepared Sn and So rice seeds are soaked in distilled water for 24 hours (Chrisnawati et al., 2021). The magnetic field is applied during the soaked process (Agustrina et al., 2022) at 3 hours before the 24-hour soak ends. Exposure to a 0,2 mT magnetic field was given for 11 minutes 44 seconds using a solenoid. According to Angraini et al. (2013), prolonged exposure to a magnetic field of 11 minutes 44 seconds had the best impact on accelerating legume germination.

After soaking for 24 hours, the seeds were sowed in a 9 cm diameter petri dish lined with three layers of damp straw paper as a medium. PEG 6000 with a concentration of 20% (w/v) was given to the SoD<sub>1</sub> and SoM<sub>1</sub>D<sub>1</sub> treatments in the medium. Meanwhile, the control treatment (Sn) and SoM<sub>1</sub> were given distilled water. In each petri dish, 50 rice seeds were placed and germinated for about seven days. The number of rice seeds that germinate is observed every day to see the parameters GRI (germination rate index), MGT (mean germination time), and GI (germination index). The FGP (final germination percentage) and PAG (percentage of abnormal germination) parameters were calculated on the last day of germination (7th day). According to Kaur et al. (2023), seeds are germinated if the length of the emerging radicle is at least 2 mm. During the germination process, the condition of the seeds is monitored so that they remain moist. If it looks like it is starting to dry, spray it with a sprayer containing the solution according to the treatment.

### Calculation Formula

- a. GRI is calculated using equation (1) (Al-Mudaris, 1998):

$$GRI (\%/day) = G_1/1 + G_2/2 + \dots + G_x/x \quad (1)$$

with  $G_1$  = germination percentage on day one after sowing and  $G_2$  = percentage germination on the 2nd day after sowing.

- b. GMT is calculated using equation (2) (Al-Mudaris, 1998):

$$MGT (\text{days}) = \frac{\sum fx}{\sum f} \quad (2)$$

with  $f_x$  = number of germinated seeds on day  $x$ , and  $f$  = the number of seeds

germinate.

- c. GI is calculated using equation (3) (Kader, 2005):

$$GI = (7 \times n_1) + (6 \times n_2) + \dots + (1 \times n_7) \quad (3)$$

with  $n_1, n_2, \dots, n_7$  = number of germinated seeds on the first, second, and next until the 7th day.

- d. FGP is calculated using equation (4) (Liu et al., 2012):

$$FGP (\%) = n/N \times 100 \quad (4)$$

with  $n$  = number of germinated seeds on day 7 per petri dish, and  $N$  = total number seeds in each petri dish.

- e. PAG is calculated using equation (5) (Halindra et al., 2017):

$$PAG = \frac{\sum \text{Abnormal Sprouts}}{\sum \text{Germinated seeds}} \times 100\% \quad (5)$$

### Data Analysis

The data obtained was tested for normality and then analyzed for variance (ANOVA) with a confidence level of 5%. Suppose the results of the ANOVA show that there are significant differences. In that case, the parameter data is tested further using the DMRT (Duncan's Multiple Range Test) at a confidence level of 5%. ANOVA and DMRT were carried out using IBM SPSS Statistics application version 25.

### RESULTS AND DISCUSSION

The results of ANOVA and further DMRT tests showed that the treatments provided significantly different GRI, MGT, GI, and PAG values, but not significantly different for FGP (Table 1).

Table 1. DMRT Test Results

Treatment	Parameters (Mean ± Std. Dev)				
	GRI (%/day)	MGT (days)	GI	FGP (%)	PAG (%)
Sn (normal rice seeds as a control)	30,30 ± 1,64 <sup>a</sup>	3,07 ± 0,10 <sup>b</sup>	223,80 ± 13,19 <sup>a</sup>	90,80 ± 3,29 <sup>a</sup>	3,20 ± 2,68 <sup>d</sup>
S <sub>0</sub> D <sub>1</sub>	26,40 ± 1,52 <sup>b</sup>	3,58 ± 0,12 <sup>a</sup>	199,00 ± 9,65 <sup>b</sup>	90,00 ± 3,74 <sup>a</sup>	58,80 ± 11,37 <sup>a</sup>
S <sub>0</sub> M <sub>1</sub>	27,69 ± 1,09 <sup>b</sup>	3,23 ± 0,13 <sup>b</sup>	208,60 ± 7,40 <sup>b</sup>	87,60 ± 3,35 <sup>a</sup>	14,00 ± 4,15 <sup>c</sup>
SoM <sub>1</sub> D <sub>1</sub>	26,51 ± 1,64 <sup>b</sup>	3,64 ± 0,16 <sup>a</sup>	199,00 ± 13,15 <sup>b</sup>	91,20 ± 3,63 <sup>a</sup>	45,20 ± 4,00 <sup>b</sup>

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the DMRT test at the  $\alpha = 0,05$  level.

The DMRT test results in Table 1 show that the Germination Rate Index (GRI) resulting from the control treatment was significantly higher (30,3%/day) compared to the other three

treatments (Table 1). GRI shows the percentage value of seeds germinating per day in each petri dish during the germination period (Al-Mudaris, 1998). So, based on the GRI value, the application of a magnetic field has not been able to improve the germination metabolism of old LSC seeds or those subjected to drought stress.

Furthermore, in MGT value, the results of the SoM<sub>1</sub> treatment (3,23 days) showed that they were not significantly different from the results of the control treatment (3,07 days). These two treatments produced the smallest MGT values compared to those given drought stress (SoD<sub>1</sub> and SoM<sub>1</sub>D<sub>1</sub>) (Table 1). Al-Mudaris (1998) states that the lower the MGT value produced, the faster a seed population will germinate. So, based on SoD<sub>1</sub> and SoM<sub>1</sub>D<sub>1</sub> treatment data, drought treatment significantly slowed down germination, and this condition could not be improved by magnetic field treatment. However, the SoM<sub>1</sub> treatment shows that magnetic field treatment can increase the germination speed of old LSC rice seeds.

The GI parameter shows similar results to the GRI; the control treatment has the highest GI value (223,8) compared to the other three treatments (Table 1). Thus, based on GI parameters, exposure to a magnetic field has not been able to restore the germination level in old LSC rice seeds or those subjected to drought stress.

FGP is a parameter that estimates seed viability (Aliu et al., 2015). In FGP parameters, the four treatments gave results that were not significantly different. So, based on the FGP parameters, the drought and accelerated aging treatment given to LSC rice seeds did not significantly reduce the number of rice seeds that managed to germinate compared to the control.

Finally, the PAG parameters. Abnormal germination is sprouts that have damaged or incomplete conditions, such as no cotyledons, broken embryos, short primary roots, twisted plumules, and swollen hypocotyl, epicotyl, and cotyledons. Coleoptiles that are broken or have no leaves also have abnormal germination, as are stunted or soft sprouts (Nurrachmamilia & Saputro, 2017). In the results of this study, the differences between normal and abnormal sprouts in each treatment are presented in Figure 1.

The analysis results showed that the treatments produced significantly different PAG values. Treatments given aging and grown in drought conditions produced the most significant PAG values, while the control treatment produced the smallest PAG values (Table 1).



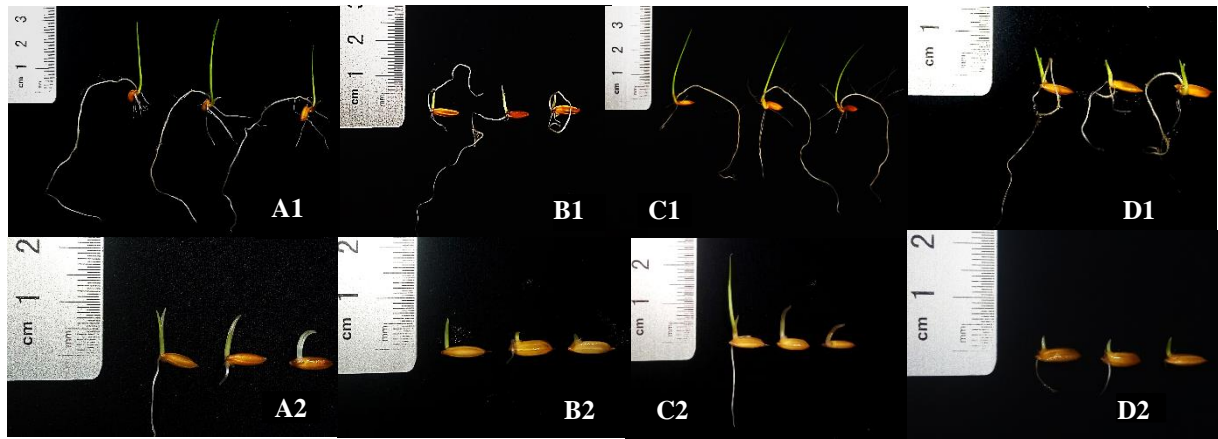


Figure 1. Comparison of normal sprouts in treated Sn as control (A1), SoD<sub>1</sub> (B1), SoM<sub>1</sub> (C1), and SoM<sub>1</sub>D<sub>1</sub> (D1) with abnormal sprouts in treated Sn as control (A2), SoD<sub>1</sub> (B2), SoM<sub>1</sub> (C2) and SoM<sub>1</sub>D<sub>1</sub> (D2)

The measurement results on PAG show apparent differences from the measurement results on FGP. Not all treatments caused significant differences in FGP, but the treatments caused significant differences in PAG. Thus, the treatment of desolation and drought stress did not affect the quantity of LSC rice seeds that germinated on FGP but impacted the quality of the sprouts produced based on PAG. The results of this research align with the research of Mulyanti et al. (2013), which states that accelerated aging treatment with ethanol can effectively increase the percentage of abnormal germination. Apart from being caused by depletion treatment, the increase in PAG was also caused by drought stress. According to Daksa et al. (2014), normal upland rice sprouts decreased due to drought stress given by PEG 6000.

In this research, applying a magnetic field was proven to reduce the PAG value in old LSC rice seeds. The treatment that was subjected to drought stress without a magnetic field (SoD<sub>1</sub>) produced the highest PAG value; however, in the treatment that was subjected to drought stress and was subjected to a magnetic field (SoM<sub>1</sub>D<sub>1</sub>) there was a decrease in the PAG value with a ratio of 58,80%: 45,20% respectively. These results are in line with the research results of Lette et al. (2019), which prove that magnetic field induction can increase normal germination in rice plants.

Based on the results of the research above, it is known that accelerated aging treatment significantly reduces various germination parameters. This is likely because the ethanol used in the seed aging process can cause damage to the seed membrane. Apart from dissolving phospholipids in membranes, ethanol also allows the denaturation of some membrane proteins (Priestley & Leopold, 1980). Damage to the membrane causes the membrane to pass through water more efficiently, and the function of the cell membrane as a selector for various molecules decreases (Pujiastuti & Sudrajat, 2017). Magnets can help improve the germination of old seeds.

Old seeds may experience cell membrane damage (Ebony et al., 2019). However, according to Reina & Pascual (2001), magnetic fields can interact with ionic currents that pass through cell membranes so that magnetic fields can change the ionic conductivity of membranes. As a result, there is a change in osmotic pressure, which impacts the seed imbibition mechanism.

Drought stress also dramatically affects the germination of LSC rice seeds. Giving PEG 6000 can reduce the water potential in the seed-growing medium, making it difficult for rice seeds to absorb water (Nurmalasari, 2018). However, based on this research, magnetic fields have been proven to improve the quality of sprouts produced from old rice seeds affected by drought based on the PAG value. According to Radhakrishnan (2019), magnetic fields can increase antioxidants and reduce oxidative stress in plants when subjected to abiotic stress such as drought.

## CONCLUSION

Based on the results of this research, the application of a magnetic field shows a positive impact in improving germination metabolism, as indicated by an increase in the germination rate of old LSC rice seeds (SoM<sub>1</sub> treatment) based on the MGT value. In addition, the magnetic field can improve the quality of the sprouts produced based on the PAG value, as evidenced by the decrease in PAG in the treatment of old seeds subjected to drought stress (SoM<sub>1</sub>D<sub>1</sub>) when compared to the treatment of old seeds without a magnetic field and subjected to drought stress (SoD<sub>1</sub>). However, the magnetic field treatment has not shown a positive effect on the GI and GRI values, and all treatments have no impact on the FGP value.

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