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Recovery of deteriorated upland rice seeds through ultra-low magnetic field exposure and its effects on germination and enzyme activity

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Abstract. *Saputri DA, Agustrina R, Irawan B, Yusnita. 2026. Recovery of deteriorated upland rice seeds through ultra-low magnetic field exposure and its effects on germination and enzyme activity. Asian J Agric 10 (1): g100138. https://doi.org/10.13057/asianjagric/g100138.* Aged upland rice (*Oryza sativa* L. var. Lumbung Sewu Cantik, LSC) seeds often exhibit reduced germination capacity and metabolic activity due to prolonged storage. Pretreatment with a low-intensity static magnetic field (magnetopriming) demonstrated the potential to revitalize and improve the quality of aged seeds. This study examined the effect of a low-intensity static magnetic field (magnetopriming) at 0.2 mT on the germination performance and enzyme activity of 10-month-old LSC seeds. Seeds were exposed to the magnetic field at different exposure durations, with untreated seeds as controls. Several germination indices and enzyme activities were evaluated. The results showed significant improvement in Final Germination Percentage (FGP) at optimal exposure durations, with no substantial changes were observed in Mean Germination Time (MGT), First Day Germination (FDG), Last Day of Germination (LDG), Coefficient of Velocity of Germination (CVG), Germination Index (GI), Germination Rate Index (GRI), and Time Spread of Germination (TSG). Enzyme assays revealed that α-amylase and protease activities were significantly increased following magnetic field exposure, whereas dehydrogenase activity showed only slight, non-significant enhancement. The study demonstrates that low-intensity magnetic field exposure can serve as an eco-friendly, non-chemical priming method that partially enhances the performance of aged seeds and improves early seedling vigor. Within the scope of this study, the observed improvements in germination and enzyme activity suggest that this non-chemical approach may support more efficient seed utilisation under sub-optimal storage conditions.

Keywords: Enzyme activity, magnetic field, magnetopriming, seed priming, seed viability

INTRODUCTION

Seed deterioration is a complex process involving morphological, physiological, and biochemical changes that progressively reduce seed viability. It is characterized by seed coat discoloration, cellular damage, loss of membrane integrity, decreased enzymatic activity, and impaired mitochondrial function, resulting in reduced germination and energy production. The accumulation of Reactive Oxygen Species (ROS) during storage further accelerates lipid, protein, and DNA damage, ultimately reducing seed vigor and germination capacity (Sano et al. 2016; Ebone et al. 2019; Ratajczak et al. 2019; Li et al. 2022).

To mitigate these effects, seed priming is widely recognized as an effective approach. One promising method is magnetopriming, which involves exposing seeds to a Static Magnetic Field (SMF) of specific intensity and duration before sowing. This technique is considered eco-friendly and non-chemical. Previous studies have shown that magnetic field exposure can enhance germination (Lette et al. 2019), promote root and shoot growth (Afzal et al. 2021), and increase hydrolytic enzyme activity such as α-amylase (Luo et al. 2022) and protease (Sharma et al. 2021). It has also been associated with improved photosynthetic efficiency and biomass accumulation (Kataria et al. 2021), contributing to higher yield and stress tolerance (Vashisth and Joshi 2017). However, the underlying mechanisms remain unclear.

Lumbung Sewu Cantik (LSC) is an important upland rice variety in Indonesia, registered in 2018 by the Center for Plant Variety Protection and Agricultural Licensing (PPVTPP), Ministry of Agriculture (Putri et al. 2023). It is widely cultivated in Pringsewu Regency, Lampung Province, Indonesia, due to its favorable traits, including drought tolerance, the ability to grow without chemical fertilizers, and yields of 3.8-4.0 t/ha. Morphologically, LSC produces an average of 10.3 tillers per clump and is resistant to lodging. Its grains are white-husked, and the cooked rice is soft and aromatic, with an amylose content of 13.99%. However, its relatively long growth cycle (≈5 months) limits planting to one season per year (Adriyani et al. 2018), requiring prolonged storage and increasing susceptibility to deterioration.

Magnetic field exposure in aged or aged seeds has shown promising results. Magnetic fields are thought to influence biological systems by modulating ion fluxes, redox balance, and electron transfer processes, thereby affecting cellular metabolism. In deteriorated seeds, this may help reactivate metabolic pathways required for germination. Improved germination has been reported in aged rice seeds of the Ciherang variety (Putra et al. 2015) and Lembata Putih Besi seeds stored for 36 months (Lette et al. 2019), as well as in aged radish and green pea seeds (Bhardwaj et al. 2016). Magnetopriming has also been shown to enhance enzymatic activity, including increased α-amylase in brown rice (Han et al. 2024), protease in soybean (Asghar et al. 2016) and Cucumis melo (Iqbal et al. 2016), and dehydrogenase in coffee (Braga Júnior et al. 2020). Under ambient conditions, rice seeds generally retain high viability for only 6-9 months, after which germination declines significantly (Afzal 2023; Paul and Sanjoy 2025). In tropical environments, germination may drop below 70% within six months under uncontrolled humidity (Prasad et al. 2023). However, most studies have focused on fresh or artificially aged seeds using moderate to high magnetic field intensities (≥10 mT). Few studies have investigated low-intensity magnetic fields (≤1 mT), particularly in naturally aged seeds under tropical storage conditions. Addressing this gap is important for developing cost-effective and environmentally friendly strategies to restore seed vigor. In this study, 10-month-old LSC seeds were categorized as aged.

The novelty of this study lies in evaluating the effects of an extremely low-intensity magnetic field (0.2 mT) on germination performance and key enzymatic activities (α-amylase, protease, and dehydrogenase) in naturally aged 10-month-old LSC upland rice seeds. Unlike previous studies focusing on fresh or artificially aged seeds with higher magnetic intensities, this research specifically targets deteriorated seeds under tropical storage conditions. Although no specific shelf-life standard exists for LSC, previous studies indicate that rice seeds stored for longer than nine months under ambient conditions undergo significant deterioration; therefore, the seeds used in this study were considered aged. This study provides insight into the potential of low-intensity magnetic fields as an eco-friendly approach to improving seed viability and germination performance.

MATERIALS AND METHODS

Experimental design

The research was conducted at the Botany Laboratory, Universitas Lampung, Lampung, Indonesia, in January and February 2025. A Completely Randomized Design (CRD) was used in this study with four levels of magnetic field exposure duration at 0.2 mT, with a total of 24 experimental units. The treatments included: control (no magnetic field exposure), 3 min. 54 sec, 7 min. 48 sec, and 11 min. 42 sec, with six replications for each treatment. These exposure times were determined based on calculations of cumulative magnetic field energy received by the seeds, representing low, moderate, and high exposure doses under a constant magnetic field intensity (0.2 mT). The exposure durations were determined based on calculations of the cumulative magnetic energy received by the seeds during treatment. The total magnetic energy stored in the solenoid was calculated using the following equation:

$$\mathbf{UB} = \frac{1}{2}\mathbf{LI}^2$$

Where, UB is the total magnetic energy (J), L is the inductance of the solenoid (H), and I is the electric current (A). The total magnetic energy within the solenoid volume was also estimated using:

$$\text{UB} = \mu\text{B} \times \text{Volume}$$

Where, μB is the magnetic energy density (J m^{-3}), and V is the effective volume of the solenoid (m^3). The Exposure power was calculated as:

$$P = W/t$$

Where, P is the power (W), W is the total energy (J), and t is the exposure time (sec).

Based on these calculations, three exposure durations (3 min 54 sec, 7 min 48 sec, and 11 min 42 sec) were selected to represent low, moderate, and high cumulative magnetic energy doses under a constant magnetic field intensity of 0.2 mT. A priori power analysis was performed using G*Power 3.1 (Faul et al. 2009) for one-way ANOVA (fixed effects). Assuming a medium effect size (f=0.25), α=0.05, and power (1-β) = 0.80, the required total sample size was 24. Six replications per treatment were appropriate to detect moderate to strong effects. Randomization was carried out by assigning serial numbers (1-24) to all Petri dishes, and treatment allocation was determined using random numbers generated by the RAND () function in Microsoft Excel. The random numbers were sorted in ascending order, and treatments were assigned sequentially according to the randomized list (Festing 2020). The activity of germination-related enzymes was measured using a UV-Vis spectrophotometer (PG Instruments T-70 UV-VIS) with germinated seeds collected at 72 hours after imbibition (HAI).

Exposure time point

LSC upland rice seeds were collected from farmers in Pringsewu Regency, Lampung Province, Indonesia. Seeds used in this study were 10 months old and derived from a single lot. The seeds were stored under ambient laboratory conditions, which are commonly applied in seed aging and magnetopriming studies. Seed screening was performed by soaking them in distilled water. Seeds settled at the bottom were classified as good quality and employed for further treatment. The selected seeds were sterilized with 0.5% NaOCl for 10 min, followed by three rinses with distilled water. Fifty-five sterilized aged upland rice seeds were soaked in 20 mL of distilled water in 1-inch plastic vials (seed-to-water ratio 1:3-4). After 24 h, the water level was adjusted to the seed height for magnetic field exposure. Subsequently, 50 seeds were transferred to Petri dishes for germination. Rice seeds were exposed to a Static Magnetic Field (SMF) generated by a solenoid connected to a DC power supply, 12 V, 0.05 A. To ensure accurate measurement, the magnetic field strength produced by the solenoid was measured with a TD 8620 gauss meter. The solenoid was made by winding 320 turns of 0.15 mm enamel wire over a 5 cm length of a 3/4-inch PVC pipe. It was connected in series with a 220 Ω 2 W resistor and a 12 V power supply to generate a 0.05 A current and a 0.2 mT magnetic field. The solenoid was placed vertically, and the sample vial was positioned at its end. Seeds were placed along the central axis of the solenoid, where the magnetic field is most uniform, and the setup was calibrated before each experimental run to ensure a stable field.

Rice seeds exposed to a magnetic field were germinated in 9-cm Petri dishes containing moist germination paper at a density of 50 seeds per dish. Petri dishes were maintained at room temperature for 7 days for germination assessment (Lette et al. 2019), while moisture was preserved by spraying distilled water. All Petri dishes were incubated in the same room under dark and stable ambient conditions to ensure consistent environmental exposure across treatments. The ambient conditions remained stable during the germination period. Germination observations were carried out daily. Seeds were considered germinated when the radicle or the plumule had reached a length of 2 mm (Li and Yang 2020). Germination parameters measured included Final Germination Percentage (FGP), Mean Germination Time (MGT), Germination Index (GI), Coefficient of Velocity of Germination (CVG), Germination Rate Index (GRI), and Time Spread of Germination (TSG), calculated according to Makhaye et al. (2021), as well as First Day of Germination (FDG) and Last Day of Germination (LDG) according to Saeed et al. (2022).

Enzyme assay overview

Crude α-Amylase and Protease extracts were prepared from 0.5 g of 72-HAI germinated seeds ground in 2 mL of 0.9% NaCl and 2 mL of phosphate buffer (pH 7) at 4°C, followed by centrifugation at 10,000 rpm for 3 min for amylase and 10 min for Protease extract (Anwar et al. 2021). This procedure uses a strictly standardized tissue-to-buffer ratio (0.5 g tissue per mL buffer) for all treatment groups. This standardization serves as the primary control, ensuring valid relative comparisons of enzyme activity per unit of initial fresh tissue mass, a widely applied methodology in seed germination and developmental studies (Binodh et al. 2022). Amylase activity was determined by the DNS method (Posoongnoen and Thummavongsa 2020), using a glucose standard curve, and expressed as μmol glucose min^{-1} mL^{-1} ($U.mL^{-1}$) (Nisa et al. 2021). Protease activity was determined using a modified Anson method (Iqbal et al. 2016) with L-tyrosine as the standard. Protease activity expressed as units (U) per mL of enzyme extract ($U.mL^{-1}$) (Sulistiono et al. 2022). Similar to alpha-amylase, the $U.mL^{-1}$ unit is utilized for relative comparison across treatments, reflecting the enzyme activity extracted from a standardized amount of tissue.

The activity of the dehydrogenase enzyme was quantitatively determined using the TTC method with 2,3,5-triphenyl tetrazolium chloride (TTC), as described by Xie et al. (2022), with modification. Enzyme activity is expressed in $mg.g^{-1}$ FW of formazan released from TTC under the specified conditions (Wang et al. 2023). A standard or calibration curve was prepared using TCC solution with $Na_2S_2O_4$ added to reduce TTC to 1,3,5-triphenyl formazan (TTF). The resulting reaction mixtures were evaporated to dryness (Wang et al. 2023). This unit of expression $mg \cdot g^{-1}$ FW directly normalizes the result to the initial fresh weight, thereby providing a robust parameter for comparative analysis. All enzyme assays were conducted with six replicates, and absorbance values were corrected using blanks; tissue mass and extraction volumes were kept uniform to ensure comparability across treatments.

Statistical method

Germination data and enzyme activity are presented as mean ± standard deviation. Data normality and homogeneity of variance were verified before by one-way ANOVA with IBM Statistical Product and Service Solutions (SPSS) version 27.0.0, followed by DMRT at $\alpha=0.05$. The correlation between α-amylase and protease activities was analyzed using Pearson's correlation in Microsoft Excel 2021 to evaluate the direction and strength of their relationship. This correlation analysis was performed to explore potential metabolic interactions between enzymes during germination. Figures were prepared and refined using Adobe Illustrator 2020 for improved graphical presentation.

RESULTS AND DISCUSSION

Germination metric

The germination parameters of aged upland LSC rice seeds exposed to a 0.2 mT magnetic field are summarized in Table 1. Final Germination Percentage (FGP) was significantly higher in the 7 min 48 sec exposure compared to the control and other treatments ($p<0.05$). Other germination parameters showed no statistically significant differences among treatments ($p>0.05$). Although not statistically significant, numerical trends suggest that shorter exposures tended to increase FGP, whereas the longest exposure tended to decrease it. These observations are reported descriptively and do not imply statistical significance.

Enzyme activity

Amylase activity

The α-amylase activity of germinated aged LSC rice seeds after 72 hours of imbibition is shown in Figure 1. Activities at 3 min 54 sec and 7 min 48 sec exposure were comparable to the control, whereas a significant increase was observed at 11 min 42 sec.

Protease activity

Protease activity is presented in Figure 2. Activities at 3 min 54 sec and 7 min 48 sec exposure were not significantly different from control, while a higher value was observed at 11 min 42 sec, although the difference was not statistically significant (p>0.05).

Dehydrogenase activity

Dehydrogenase activity at 72 hours after imbibition is presented in Figure 3. Activity slightly increased with longer exposure; however, no statistically significant differences were observed among treatments (p>0.05).

Correlation between α-amylase and dehydrogenase activity

Figure 4 illustrates the relationship between α-amylase and dehydrogenase activities. Pearson's correlation revealed a weak, non-significant association ($R^2 = 0.2231$, $p = 0.5725$), indicating no statistically supported relationship.

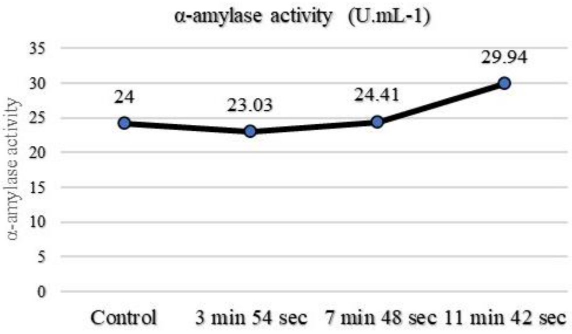

Figure 1. α-Amylase activity in germinated aged upland LSC rice seeds at 72 HAI treated with different durations of 0.2 mT magnetic field exposure

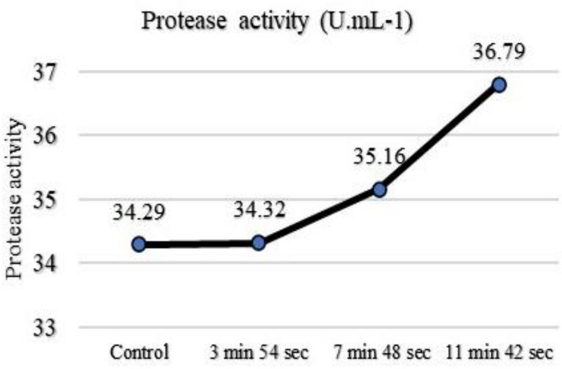

Figure 2. Protease activity in germinated aged upland LSC rice seeds at 72 HAI treated with different durations of 0.2 mT magnetic field exposure

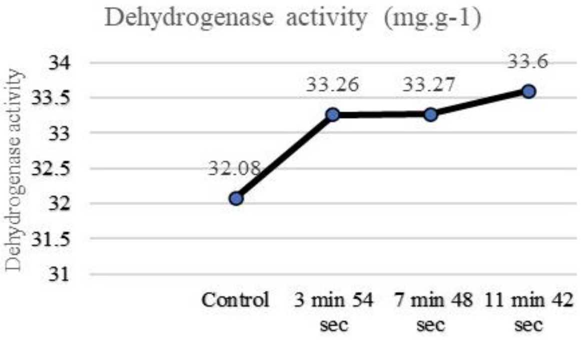

Figure 3. Dehydrogenase activity in germinated aged upland LSC rice seeds at 72 HAI treated with different durations of 0.2 mT magnetic field exposure

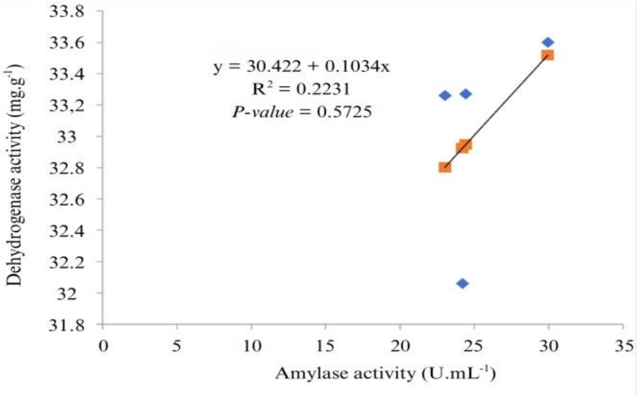

Figure 4. Correlation between α-amylase and dehydrogenase activities in germinated aged seeds of upland rice LSC rice. Blue diamond symbols represent the actual observed data, while orange squares represent the estimated values derived from the regression equation y = 30.422 + 0.1034x

Table 1. Germination parameters of aged seeds of upland LSC rice treated with a 0.2 mT magnetic field

Exposure duration	FGP	MGT	FDG	LDG	CVG	GI	GRI	TSG
	%	day	day	day	-	-	% /day	Day
Control	86.92±5.04 a	4.69±0.102 a	3±0.00 a	6±0.409 a	21.09±0.45 a	141.63±10.18 a	25.19±2.53 a	3.83±0.41 a
3 min. 54 sec	88.08±2.28 a	4.74±0.145 a	3±0.00 a	7±0.00 a	20.83±0.36 a	142.08±4.25 a	25.70±0.94 a	4.00±0.00 a
7 min. 48 sec	92.50±1.41 b	4.81±0.057 a	3±0.00 a	7±0.00 a	20.94±0.14 a	145.58±2.18 a	26.53±0.71 a	4.00±0.00 a
11 min. 42 sec	88.16±2.18 a	4.69±0.076 a	3±0.00 a	7±0.00 a	21.32±0.34 a	147.33±3.75 a	26.49±0.49 a	4.00±0.00 a

Note: Numbers followed by the same letter in the column indicate no significant difference based on the DMRT test (α = 0.05)

Discussion

Germination test

Exposure to a 0.2 mT magnetic field showed a *slight increase* in FGP of aged upland LSC rice seeds. However, most germination variables, including Mean Germination Time (MGT), Coefficient of Velocity of Germination (CVG), Germination Index (GI), Germination Rate Index (GRI), and Time Spread Germination (TSG), did not differ significantly among treatments. These results suggest that ultra-low magnetic fields had a limited effect on overall germination performance under the conditions examined in this study.

Although numerical trends indicated that moderate exposure (7 min 48 sec) tended to improve FGP, the tendencies were not statistically supported and were therefore reported descriptively. This observation is supported by Tirono (2022), who reported that exposure to magnetic fields ranging from 0.1-0.5 mT accelerated germination in soybean seeds, with 0.2 mT reducing mean germination time from 2.4 days to 1.2 days. Although conducted in a different species, this finding suggests that moderate-intensity magnetic fields may enhance early germination dynamics. A similar non-significant trend has been reported in previous low-intensity studies of magnetic fields (Agustrina et al. 2018). The delayed germination observed in some treatments is consistent with the natural aging effects in seeds (Amirmoradi and Feizi 2017). Furthermore, Erez and Özbek (2024) emphasized that magnetic field responses are highly dependent on exposure duration and plant species, which may explain the variability observed in this study.

Several physiological mechanisms have been proposed in previous studies, including stabilization of the cell membrane, promotion of ion transport, and facilitation of water uptake (Hu et al. 2024). However, these mechanisms remain hypothetical in the present study, as no direct measurement of membrane integrity, ion flux, or aquaporin activity was conducted. Therefore, interpretations on membrane permeability and hydration processes should be treated as literature-based assumptions rather than experimental evidence. Despite the limited effect on germination parameters, the observed enzymatic responses suggest that metabolic activation may precede visible improvement in germination. This supports the hypothesis that physiological recovery in aged seeds may begin internally before being expressed at the whole-seed level.

Enzyme enhancement mechanisms

In this study, exposure of aged upland rice (LSC) seeds to a 0.2 mT magnetic field significantly increased α-amylase activity by 23.72% and protease activity by 7.29% after 11 min 42 sec exposure compared to the control. These results indicate enhanced mobilization of starch and protein under longer exposure duration. Similar responses have been reported in brown rice for amylase activity (Luo et al. 2022), and in aged *Cicer arietinum* (chickpea) seeds for protease activity following magnetic treatment (Sharma et al. 2021)

Importantly, this finding demonstrates the novelty of the present study, showing that an ultra-low 0.2 mT field can induce a significant 23.72% metabolic boost in truly aged LSC rice seeds, in contrast to previous studies that required fields of 10 mT.

Dehydrogenase enzymes play an important role during germination as catalysts in redox reactions essential for producing ATP, the energy used in various germination processes. In contrast, dehydrogenase activity shows only a weak and non-significant increase, suggesting limited stimulation of respiratory metabolism. Comparable trends have been reported in wheat and rice seeds (Verma et al. 2017; Senapati et al. 2019). Although not statistically significant, this positive trend indicates an early recovery of energetic metabolism. Mitochondrial enzymes may require higher SMF intensity, stronger magnetic field strength, or longer exposure duration to exhibit significant adaptations. Moreover, the biological response to magnetic fields is influenced by field strength, plant species, and physiological conditions. Therefore, the ultra-low 0.2 mT SMF may act as a priming stimulus that supports the enhancement of α-amylase and protease activities, without yet inducing full mitochondrial enzymatic remodeling.

The weak correlation between amylase and dehydrogenase activity observed in this study further indicates that enhanced reserve mobilization was not consistently accompanied by increased respiratory activity. This may be because soluble carbohydrates released by α-amylase are not used exclusively for respiration but are also allocated to other metabolic processes, including cell wall synthesis, osmotic regulation, and synthesis of structural and regulatory compounds during early germination. In cereal seeds, soluble sugars have been shown to regulate metabolic activity beyond their role as respiratory substrates (Yu et al. 1996).

The biophysical mechanism underlying enzyme enhancement was not directly investigated in this study. Based on previous reports, the magnetic field may hypothetically influence enzyme conformation and activity through interactions with paramagnetic ions, changes in molecular energy states, and electron spin dynamics (Wang et al. 2022; Kashtoh and Baek 2023). The explanations, including those related to the Radical Pair Mechanism, remain speculative and require further experimental validation.

Recent studies suggest that low-intensity Static Magnetic Fields (SMFs) regulate Ca^{2+} ion-channel fluxes and Reactive Oxygen Species (ROS) signaling, thereby supporting redox balance and protein stability (Čėsnienė et al. 2023). However, since ROS levels and antioxidant activity were not evaluated in the present study, the interpretation is indirect and based on the literature. Compared with studies applying high-intensity magnetic fields, the physiological responses observed here were more moderate. High-field treatments have often produced greater improvements in germination and respiratory activity (Hozayn et al. 2015; Verma et al. 2017; Afzal et al. 2021), whereas the ultra-low 0.2 mT field used in this study mainly affected hydrolytic enzyme activity. Therefore, direct comparisons between low- and high-intensity magnetic treatments should be interpreted with caution, given differences in exposure intensity, duration, and experimental conditions.

Overall, the findings indicate that ultra-low magnetic field exposure can partially enhance metabolic activity in aged rice seeds, particularly through increased α-amylase and protease activity. However, its influence on germination performance and mitochondrial metabolism remains limited under the tested conditions. Further studies involving direct assessments of membrane integrity, ROS production, antioxidant enzyme activity, and mitochondrial function are recommended to clarify the mechanisms involved and to optimize magnetopriming protocols for practical application.

Magnetic field application at very low intensity has been reported to enhance germination performance and enzyme activities in various plant species, particularly in aged and stressed seeds. Exposure of wheat seeds to a weak magnetic field (10 Hz, 7.5 mT) below 1 mT improved germination rate and early seedling growth by stimulating enzymatic activation and protein content (Urnukhsaikhan et al. 2025). Similarly, pea seeds treated with a low-intensity magnetic field (0.1-0.5 mT) exhibit improved germination percentage, reducing MGT and amylase activities, indicating improved reserve utilization (Carbonell et al. 2000). In sweet corn, exposure to a weak magnetic field of 0.1-0.3 mT accelerates the germination process (Sapitri et al. 2024)

From an agronomic perspective, such ultra-low magnetopriming approaches represent an eco-friendly and scalable strategy for rejuvenating deteriorated seeds without the risk associated with high-intensity magnetic exposure (Flórez et al. 2007). Notably, most previous studies employed magnetic fields at or above 0.5 mT, whereas the present study demonstrates that even a 0.2 mT magnetic field is sufficient to induce a measurable physiological response in aged seed.

In conclusion, exposure of long-stored (10-month) upland LSC rice seeds to a low intensity (0.2 mT) magnetic field resulted in partial biological enhancement. The Final Germination Percentage (FGP) was significantly higher at 7 min 48 sec exposure, while other germination parameters showed no significant differences, where significant trends were described descriptively. Amylase, protease, and dehydrogenase activities tended to increase, with a significant rise in amylase at 11 min 42 sec. These findings suggest that low-intensity, short-duration magnetic fields can enhance certain germination-related physiological processes in aged rice seeds. However, this study was limited to a single seed lot under controlled laboratory conditions, and the molecular mechanisms were not assessed. Further research is needed under field conditions with multiple varieties and storage histories, along with molecular analyses of germination-related enzymes and stress-response pathways, to confirm practical applicability and elucidate underlying mechanisms.

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