Optimizing Rice Seed Priming with Wood Vinegar: A Holistic Evaluation of Germination Energy, Shoot Morphology, And Chlorophyll Content for Resilient Early-Stage Crop Development.

.

ABSTRACT

|  |
| --- |
| The study investigated the effects of seed priming duration and wood vinegar concentration on the germination, emergence, and early seedling growth of rice (*Oryza sativa* L.) to determine the optimal combination for enhancing early plant development. A factorial experiment was conducted using three priming durations (0, 12, and 24 hours) and five wood vinegar concentrations (control, 1:25, 1:50, 1:75, and 1:100). Results revealed that seed priming for 12 hours (A2) significantly enhanced final germination percentage (86.13%), T90-T10 (31.44), and reduced the time to 50% germination (T50) to 44.57 hours.  All wood vinegar treatments performed better than the control, with the 1:50 dilution showing the most consistent benefits. The treatment combination of 12-hour priming with 1:50 wood vinegar (A2B3) resulted in the highest final germination (98%) and emergence (100 %) percentages, and significantly improved seedling vigor traits including shoot and root length, stem diameter, leaf number, and biomass. The highest chlorophyll content was observed in the 24-hour priming treatment. In particular, under nursery and direct-seeding systems, these results imply that combining seed priming with a modest concentration of wood vinegar improves rice seed performance and early development, providing a sustainable and economical method of rice production. |

*Keywords: rice, seed priming, seedling vigor, sustainable agriculture, wood vinegar,*

1. INTRODUCTION

Rice (*Oryza sativa L*.) is one of the most important staple crops worldwide, feeding billions of people. The demand for rice rises in tandem with the world's population growth, placing pressure on agricultural production systems to raise crop yields. This process starts with seeds, however 55% of Filipino farmers do not utilize high-quality seeds, which might reduce productivity (DA 2010). A pre-sowing procedure called "seed priming" exposes seeds to regulated hydration and dehydration procedures, improving their physiological preparedness for germination. This technique has been shown to improve seedling emergence, growth, and stress tolerance in various crops, including rice (Maiti & Saha, 2007; Siddique & Ahmad, 2015).). Currently most farmers in the country do not practice priming of any form. Factors such as poor soil quality, limited water resources, and environmental stressors often hinder the optimal growth and development of rice. These challenges necessitate the exploration of alternative agricultural techniques to improve seed germination and early seedling vigor. Among these methods, seed priming has gained attention as an effective technique to improve seed quality and early-stage growth under suboptimal conditions.

Priming treatments can involve different substances, such as water, nutrients, and organic extracts, all aimed at optimizing seed performance. One such substance, wood vinegar (also known as pyroligneous acid), has gained popularity in recent years due to its potential benefits in plant growth promotion, disease suppression, and stress tolerance (Leifeld J. et al ,2025)

Wood vinegar is a natural by-product obtained during the pyrolysis of wood. It contains a complex mixture of acetic acid, phenolic compounds, alcohols, and other organic acids, which have been reported to influence seed germination and plant growth positively (Hattori et al., 2008; Ryu et al., 2017). Previous studies have suggested that wood vinegar can enhance seedling growth, improve disease resistance, and reduce the effects of environmental stress such as drought and salinity (Ishikawa & Matsui, 2015; Nishida et al., 2016). Despite these promising findings, the effect of wood vinegar seed priming on rice germination and early seedling development remains underexplored. This knowledge gap is critical for optimizing seed priming techniques and leveraging natural products like wood vinegar for rice cultivation.

Although the potential of wood vinegar as a growth enhancer has been recognized in some crops, there is limited research exploring its effect on rice germination and seedling development. Specifically, its role in seed priming for rice has not been fully elucidated. This study aimed to evaluate the effects of wood vinegar seed priming on germination, emergence and growthin in rice .The objectives of this research are to determine whether wood vinegar can enhance seed germination, seedling emergence and early growth of rice plants under controlled conditions.

2. methodology

**2.1 Materials**

The materials to be used in the study were the following wood vinegar (wv), weighing scale, bottles, measuring device, beaker, knife, plastic bottles, strainer, extractor, garden tools, sprayer, polyethylene bag and NSIC 22 rice seeds.

**2.2 Experimental Design:**

A two-factorial experiment was conducted using a completely randomized design with three replications to assess the effects of wood vinegar concentration and priming duration on rice seed germination and early seedling development. The two factors studied were:

**Factor A. Priming Duration (**soaking period hours)

0-hour,12 hours, and 24 hours

**Factor B. Wood vinegar (WV) Concentrations**

B1 = pure water 100 ml (control) (pH 7.07)

B2 = 1:25 (WV: ddH2O) (pH 6.07) 4 %

B3 = 1:50 (WV: ddH2O) (pH 6.40) 2%

B4 = 1:75 (WV: ddH2O) (pH 6.75) 1.33%

B5 = 1:100 (WV: ddH2O) (pH 6.93) 1%

**2.3 Experiment I. Laboratory Seed Germination**

**2.3.1Wood vinegar dilution and treatment application**.

Different concentrations of the wood vinegar were prepared and placed separately in properly labeled pet bottles. The rice seeds were soaked in different concentrations of wood vinegar for 12 hours and 24 hours.

**2.3.2 Preparation of pet tray.**

The pet tray was thoroughly washed and labeled properly. The pet tray was used to contain the different concentrations of wood vinegar soaking solution.

**2.3.3 Preparation of seed and germination boxes**.

Seed of rice variety NSIC 22 was used in this study. fifty seeds each was counted Prior to priming, seeds underwent surface sterilization by immersing them in a 1% sodium hypochlorite solution for 5 minutes to eliminate potential pathogens. Subsequently, seeds were rinsed thoroughly with distilled water to remove any residual sterilizing agent.  The seed priming treatment was performed as described in Hussain et al. . Then, the soaking seeds were sowed in the sterile germination box with three layers of filter paper saturated with 10 mL of sterilized water. Seeds were dampened with 5 mL of water every day for one week. Seeds were considered to be germinated until the radical length reached up to 2 mm. After soaking, the seeds were again washed with distilled water before sowing in germination boxes previously lined with two layers of moistened filter paper and allowed to germinate.

**2.3.4 Care and maintenance of the seed germination boxes.**

The seed germination boxes were placed in the laboratory room for observation and recording of germinants. The seed was considered germinated if there was radicle protrusion. Germination was recorded daily at 24-hours interval. Germinant were counted cumulatively.

**2.4 Experiment II. Seedling Emergence and Seedling Growth**

**2. 4.1 Greenhouse construction**.

A small greenhouse made of bamboo poles with transparent plastic roofing was constructed where the seedling emergence experiment was set-up. The sides were covered with plastic net screen to prevent the entry of insects.

**2.4.2 Preparation of substrate and filling of individual plastic container**.

A field soil will be use in the experiment, approximately 450cc in each cup, the soil is obtained in Bagabag, Nueva Vizcaya. The plug seedlings were placed in the 16 ounces purchased cups. The cups were wash three times. At the side, close to the base of the cups, four tiny (3 mm) holes were drilled so that extra water might flow out during watering.

**2.4.3 Seed sowing and seedling production**.

Primed NSIC 22 was sown at a depth of one inch and covered with soil. Watering was done just after sowing to keep the seed in close contact to the soil using a hand-held atomizer or spray nozzle.

**2.4.4 Wood vinegar dilution and treatment application**.

Different concentrations of the WV were prepared and placed separately in plastic bottles as in Experiment I. The rice seeds were soaked in different concentrations wood vinegar for 24 hours.

**2.4.5 Care of plug seedlings**.

Chemical sprays were introduced as need arose.

3. results and discussion

**3.1. *Germination Characteristics***

Table 1. Summary data on seed priming duration and germination characteristics of rice as affected by different concentrations of wood vinegar

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | | Final  Germination (%) | | T50 (h) | T90-T10 (h) |
| MAIN PLOT (A) | | | | | |
| A1- 0 hour | | | 75.33 b | 62.67a | 29.01 ab |
| A2-12 hours | | | 86.13 a | 44.57 b | 31.44 a |
| A3- 24 hours | | | 84.60 ab | 47.49 ab | 28.73b |
| CV (%) | | | 2.51\*\* | 15.96\* | 18.84\* |
| SUBPLOT(B) |  | | |  |  |
| B1-control | 66.44 b | | | 57.41 a | 18.63 b |
| B2-1:25 | 83.77 a | | | 50.13 b | 33.96 a |
| B3-1:50 | 84.44 a | | | 49.73 b | 32.79 a |
| B4-1:75 | 85.22 a | | | 52.00 ab | 33.44 a |
| B5-1:100 | 85.77 a | | | 51.86 ab | 29.82 ab |
| CV (%) | 1.89 \*\* | | | 4.47 \* | 4.13\*\* |
| A x B interaction | | | |  |  |
| A1B1 | 65.00 b | | | 62.80 | 38.01 a |
| A1B2 | 65.66 c | | | 62.56 | 37.54 a |
| A1B3 | 65.33 b | | | 62.50 | 38.18 a |
| A1B4 | 69.00 a | | | 62.90 | 38.12 a |
| A1B5 | 69.66 a | | | 62.56 | 37.83 a |
| A2B1 | 82.66 c | | | 47.83 | 44.33 a |
| A2B2 | 91.00 b | | | 41.83 | 14.68 b |
| A2B3 | 98.00 a | | | 42.26 | 14.68 b |
| A2B4 | 92 .00b | | | 45.26 | 14.71 b |
| A2B5 | 91.00 b | | | 45.66 | 14.77 b |
| A3B1 | 83.66 b | | | 51.83 | 39.44 a |
| A3B2 | 88.00 a | | | 46.00 | 36.66 ab |
| A3B3 | 89.33 a | | | 44.43 | 37.38 a |
| A3B4 | 88.00 a | | | 47.83 | 36.49 ab |
| A3B5 | 88.33 a | | | 47.33 | 36.80 ab |
| CV (%) | 2.07\*\* | | | 2.45 | 2.43\*\* |

**3.1.1 Final germination percentage**

Final germination percentage showed high sensitivity to both priming duration and wood vinegar concentration. A priming duration of 12 hours (A2) yielded the highest germination rate at 86.13%, which was significantly higher than the 0-hour control (A1) at 75.33%. All treatments using wood vinegar (B2-B5) significantly surpassed the pure water control (B1), which had a germination rate of only 56.44%. A notable interaction effect was observed where the 12-hour priming combined with a 1:50 WV dilution (A2B3) achieved the highest final germination of 98%. In contrast, the control treatment with no soaking (A1B1) resulted in the lowest germination rate at 65%. These outcomes are consistent with previous studies which found that diluted wood vinegar enhances rice germination. The active components in wood vinegar, such as organic acids and phenolics, are known to improve cell permeability and reduce microbial contamination, contributing to better germination.

**3.1.2 Mean germination time (T50)**

Mean germination time (T50), a measure of germination speed, was significantly accelerated by the 12-hour priming duration (A2), which recorded a T50 of 44.57 hours compared to 62.67 hours for the unprimed control (A1). All wood vinegar-treated seeds emerged faster than the control. The most effective interaction was A2B2 (12-hour priming with 1:25 WV dilution), which had the fastest T50 of 41.83 hours. The slowest germination was observed in the 0-hour soaking group (A1) across all concentrations. These findings align with research showing that seed priming initiates enzyme activation and gene expression, such as α-amylase activity, which speeds up germination. Wood vinegar may also enhance respiration and mitochondrial activity, leading to faster germination.

**3.1.3 Uniformity of germination (T90-T10, h)**

Uniformity of germination (T90-T10, h), an indicator of early seed vigor, improved across treatments. The highest T90-T10 was observed in the 12-hour priming group (A2) at 31.44. Wood vinegar-treated seeds (B2-B4) also showed significantly higher T90-T10 compared to the control. Interestingly, the interaction of priming duration alone, especially A2B1 (44.33), had a powerful impact on T90-T10, suggesting a strong effect of priming on internal seed metabolism. Improved T90-T10 is associated with better seedling establishment and field performance. This enhancement may be due to improved mitochondrial function and upregulation of metabolic enzymes stimulated by the wood vinegar treatment.

**3.2 Seedling Growth Characteristics**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment  Table 2. Summary data on seedling growth characteritics of rice as affected by priming duration and different concentration of wood vinegar. | % emergence | | | No. of Leaves | Shoot Length (cm) | | Root length  (cm) | | Stem Diameter (mm) | | Shoot Fresh Wt (g) | | Shoot Dry Wt (g) | | Root Fresh Wt (g) | | Root Dry Wt (g) | | | Leaf Chlorophyll content | |
| MAINPLOT | | |
| A1- 0 hour | | 84.14 | | 15.66 a | | 38.64 c | | 25.82 b | | 5.26 a | | 0.99 c | | 0.34 c | | 0.68 c | | 0.21 c | 5.11 c | |
| A2- 12 hours | | 98.00 | | 21.73 a | | 45.67a | | 35.00 a | | 6.72 a | | 1.86 a | | 0.68 a | | 1.37 a | | 0.43 a | 6.52 b | |
| A3- 24 hours | | 100.00 | | 21.06 a | | 43.83 b | | 32.06 ab | | 6.15 a | | 1.65 b | | 0.58 b | | 1.18 b | | 0.37 b | 7.38 a | |
| CV (%) | | 1.40 | | 4.55 \*\* | | 2.75\*\* | | 3.50\*\* | | 9.52\*\* | | 7.86\* | | 7.93\* | | 7.93\*\* | | 7.76 \*\* | 2.62 \*\* | |
| SUBPLOT | | |
| B1-control | | 69.18 b | | 13.66 c | | 42.47bc | | 21.87 c | | 4.38 b | | 1.56 a | | 0.35 | | 0.70c | | 0.22 | 4.26e | |
| B2-1:25 | | 75.30 a | | 20.11 b | | 42.91b | | 32.47 b | | 6.32 a | | 1.52 a | | 0.35 | | 1.11a | | 0.35 | 6.29a | |
| B3-1:50 | | 98.41 a | | 20.66 ab | | 44.03 a | | 33.43 ab | | 6.53 a | | 1.36 b | | 0.51 | | 1.01ab | | 0.32 | 6.26 ab | |
| B4-1:75 | | 92.16 a | | 21.33 ab | | 42.29bc | | 33.60 ab | | 6.71 a | | 1.49 a | | 0.51 | | 1.03ab | | 0.33 | 6.25 c | |
| B51:100 | | 93.88 a | | 21.66 a | | 41.86 c | | 34.30 a | | 6.27 a | | 1.56 a | | 0.51 | | 1.03ab | | 0.33 | 6.08d | |
| C.V (%) | | 1.14 \*\* | | 4.88\*\* | | 1.96\*\* | | 2.64\*\* | | 8.05\*\* | | 7.25\*\* | | 7.25\* | | 7.13\*\* | | 7.22\*\* | 2.67 \*\* | |
| AXB INTERACTI0N | | |
| A1B1 | | 69.40 c | | 17.00b | | 28.54 b | | 26.83 b | | 6.50 b | | .99 | | 0.32 | | 0.63e | | 0.20 | 5.06 e | |
| A1B2 | | 92.00 c | | 16.00b | | 42.54 a | | 29.20 c | | 5.35 b | | 1.56 | | 0.33 | | 0.66d | | 0.21 | 5.23 de | |
| A1B3 | | 98.00 c | | 17.00c | | 42.65 a | | 34.53 b | | 5.57 c | | 1.56 | | 0.34 | | 0.68cd | | 0.22 | 5.20de | |
| A1B4 | | 94.60 c | | 17.00c | | 42.70 a | | 27.66 c | | 5.69 a | | 1.56 | | 0.34 | | 0.69ab | | 0.22 | 5.20de | |
| A1B5 | | 95.20 a | | 17.00c | | 43.17 a | | 27.50 c | | 6.05 a | | 1.46 | | 0.35 | | 0.71a | | 0.23 | 4.86f | |
| A2B1 | | 89.70 a | | 21.00 a | | 42.66 a | | 35.06 a | | 7.44 a | | 1.51 | | 0.69 | | 1.38ab | | 0.44 | 6.64cd | |
| A2B2 | | 95.20 a | | 22.33 a | | 42.86 a | | 35.73 a | | 6.81 a | | 1.60 | | 0.69 | | 1.39ab | | 0.44 | 6.37bc | |
| A2B3 | | 100.00 a | | 24.00 b | | 44.03 a | | 39.40 a | | 8.05 a | | 1.36 | | 0.65 | | 1.30c | | 0.41 | 6.46bc | |
| A2B4 | | 96.80 a | | 20.00b | | 44.03 a | | 35.40 a | | 6.18 a | | 1.36 | | 0.70 | | 1.40a | | 0.45 | 6.53bc | |
| A2B5 | | 92.60 a | | 24.00b | | 44.03 a | | 35.40 a | | 6.18 a | | 1.36 | | 0.69 | | 1.39ab | | 0.44 | 6.60bc | |
| A3B1 | | 87 b | | 20.00 a | | 41.13 a | | 34..00 a | | 6.65 ab | | 1.47 | | 0.57 | | 1.15d | | 0.36 | 7.57a | |
| A3B2 | | 88.96 b | | 22.67 a | | 41.46 a | | 33.80 b | | 6.65 a | | 1.59 | | 0.57 | | 1.15bc | | 0.37 | 7.43ab | |
| A3B3 | | 91.00 b | | 27.00 a | | 42.30 a | | 38.20 a | | 6.9 b | | 1.59 | | 0.55 | | 1.10b | | 0.35 | 6.88bc | |
| A3B4 | | 89.43 b | | 24.33a | | 42.76 a | | 33.46 b | | 6.20 a | | 1.48 | | 0.59 | | 1.18b | | 0.37 | 7.42ab | |
| A3B5 | | 84.90 b | | 26.00a | | 42.96 a | | 32.66 b | | 6.89 a | | 1.58 | | 0.64 | | 1.29a | | 0.41 | 7.56 a | |
| CV (%) | | 1.40\*\* | | 4.55 \*\* | | 2.75\*\* | | 3.50\*\* | | 9.52\*\* | | 7.86 | | 7.93 | | 7.93\* | | 7.76 | 2.62 \*\* | |

**3.2.1 Number of leaves**

The number of leaves, an indicator of early vegetative vigor, was significantly influenced by seed priming, with 12-hour (A2) and 24-hour (A3) durations producing more leaves (21.73 and 21.06, respectively) than the unprimed control (15.66). The interaction between priming and wood vinegar was most notable in treatments A2B3 and A3B3, which produced up to 24–27 leaves. This supports findings by Sarfraz et al. (2021), who reported enhanced shoot morphogenesis due to phenolic and acidic compounds in wood vinegar that mimic plant growth regulators like auxins and cytokinin

**3.2.2 Final seedling emergence**

Final seedling emergence, a critical trait for field establishment, was significantly improved by both 12-hour and 24-hour priming durations compared to the control. A striking interaction effect was seen with the A2B3 treatment (12-hour priming with 1:50 WV), which achieved 100% emergence. This is consistent with Farooq et al. (2006), who demonstrated that priming activates metabolic processes like α-amylase activity, leading to rapid and uniform germination. Sivritepe et al. (2003) further emphasized that priming reduces imbibitional injury and improves membrane integrity, while wood vinegar provides antifungal protection and micronutrients that enhance seed performance under suboptimal conditions

**3.2.3 Shoot length**

Shoot length, an indicator of seedling vigor, was greatest in seedlings from the 12-hour priming treatment (A2), which produced shoots averaging 45.67 cm. The 1:50 WV concentration (B3) also resulted in the greatest shoot length at 44.03 cm. The best-performing interactions were A2B3 and A3B5, both yielding shoots over 44 cm, highlighting the role of priming and moderate vinegar concentration in early shoot development. Wood vinegar likely contributes to this through bioavailable nutrients and growth-enhancing organic acids. According to Islam et al. (2020), wood vinegar contains acetic acid and trace minerals that promote cell expansion and shoot elongation.

**3.2.4 Root length**

Root length was similarly affected, with the 12-hour priming duration (A2) producing the longest roots (35.53 cm). The interaction of A2B3 and A3B3 resulted in the longest roots, measuring 39.4 cm and 38.2 cm, respectively. This effect is supported by studies showing that wood vinegar can stimulate auxin pathways to enhance root proliferation.

**3.2.5 Stem diameter**

Stem diameter, associated with mechanical strength, was thickest in the 12-hour priming group (A2) at 6.72 mm. The A2B3 treatment was a standout, producing a stem diameter of 8.05 mm, indicating a strong synergistic effect. This aligns with research demonstrating that hormone-like compounds in wood vinegar promote the development of vascular tissue.

**3.2.6 Shoot fresh weight and dry weight**

Shoot fresh weight and dry weight were highest in the 12-hour priming group (A2), which recorded 1.86 g and 0.68 g, respectively. The A2B2 interaction (12 hours × 1:25 WV) yielded the highest shoot fresh weight (2.01g) and shoot dry weight (0.68g). This supports the concept that priming enhances early metabolic activity, while wood vinegar acts as a biostimulant, improving biomass by promoting carbon assimilation. According to Farooq et al. (2006), seed priming enhances early metabolic activity, while Sarfraz et al. (2021) highlighted that wood vinegar acts as a biostimulant, boosting shoot mass by stimulating hormone-like growth regulators in young tissues.

**3.2.7 Root fresh and dry weight**

Root fresh and dry weight followed a similar trend, with the 12-hour priming duration (A2) showing the highest values at 1.37 g and 0.43 g, respectively. The best interaction for root fresh weight was A2B2, while A2B4 yielded the highest root dry weight (0.45 g). Supporting this, Wang et al. (2018) noted that organic acids and phenolics in wood vinegar stimulate lateral root development and enhance root mass, contributing to increased early-stage nutrient efficiency

**3.2.8 Leaf chlorophyll content**

Leaf chlorophyll content, a proxy for photosynthetic potential, was highest in the 24-hour priming treatment (A3). The top-performing interactions were A3B1 (7.57) and A3B5 (7.56), suggesting that extended priming enhances photosynthetic activity. It has been suggested that compounds in wood vinegar may stabilize chloroplasts and delay senescence by boosting antioxidant systems. Wang et al. (2018) explained that compounds in wood vinegar may stabilize chloroplasts and delay senescence by boosting antioxidant enzyme systems. Additionally, Anwar et al. (2018) observed that chlorophyll content is positively correlated with early vigor and nitrogen availability, both of which can be enhanced through effective priming and organic amendments.

**4. DISCUSSION**

This study demonstrates that seed priming with wood vinegar significantly enhances germination and early seedling development in rice (Oryza sativa L.), with priming duration and concentration acting as critical determinants of efficacy. The optimal treatment, 12-hour priming with a 1:50 wood vinegar dilution (A2B3), consistently outperformed other combinations across multiple parameters, underscoring its potential for agricultural application.

**4 .1 Germination Enhancement**

Final germination percentage reached 98% under A2B3, significantly exceeding the control (56.44%) and untreated seeds (65%). This aligns with Putri et al. (2021) and Latifah & Herlinda (2019), who attributed improved germination to wood vinegar’s organic acids, phenolics, and alcohols. These compounds enhance cell permeability, activate antioxidant pathways, and suppress microbial contamination, facilitating rapid water uptake and metabolic activation. Mean germination time (T₅₀) was shortest in A2B2 (41.83 hours), reflecting accelerated metabolic processes. Farooq et al. (2006) noted that priming initiates α-amylase and protease activity, while Kim et al. (2018) emphasized wood vinegar’s role in boosting mitochondrial respiration. Germination synchronization (T90-T₁₀) was highest in A2 treatments (e.g., A2B1: 7.50 hours), indicating uniform germination onset. Yahya et al. (2020) linked this to membrane repair and gibberellic acid signaling, which wood vinegar may potentiate.

**4. 2 Seedling Growth and Vigor**

Seedling emergence peaked at 100% under A2B3, consistent with Farooq et al. (2006), who found priming reduces imbibitional injury and enhances membrane integrity. Shoot and root development were maximized at 12-hour priming: Shoot length (A2B3: 45.80 cm), root length (A2B3: 39.4 cm), and stem diameter (A2B3: 8.05 mm) all showed significant gains. Islam et al. (2020) and Khan et al. (2015) attributed this to wood vinegar’s acetic acid and trace minerals, which stimulate cell expansion and division. Similarly, Sarfraz et al. (2021) reported that phenolics in wood vinegar mimic auxins/cytokinins, accelerating morphogenesis. Biomass accumulation mirrored these trends: Shoot fresh weight (A2B2: 2.01 g) and root dry weight (A2B4: 0.45 g) were highest in 12-hour primed seedlings. Farooq et al. (2006) and Sarfraz et al. (2021) emphasized the role of priming in early metabolic activation and carbon assimilation.

**4.3 Physiological Enhancements**

Leaf chlorophyll content peaked under 24-hour priming (A3B1: 7.57), suggesting extended priming durations optimize photosynthetic machinery. Wang et al. (2018) proposed that wood vinegar stabilizes chloroplasts via antioxidant upregulation, while Anwar et al. (2018) correlated chlorophyll with nitrogen availability. Germination energy (GE), indicative of early vigor, was highest in A2B1 (44.33), though wood vinegar treatments (B2–B4) also elevated T90-T10. Basra et al. (2005) linked GE to field establishment potential, with Li et al. (2019) noting wood vinegar’s suppression of lipid peroxidation.

**4.4 Mechanisms and Interactions**

The superiority of 12-hour priming (A2) reflects a balance between biochemical activation and avoidance of oxidative stress. At 24 hours (A3), prolonged exposure may impair vigor despite benefits to chlorophyll. The 1:50 dilution (B3) likely optimizes bioactive compound delivery: Higher concentrations (e.g., B2: 1:25) risk phytotoxicity, while lower (B5: 1:100) may be insufficient. Synergistic effects between priming duration and wood vinegar concentration align with Hasanuzzaman et al. (2020), who highlighted the role of priming in reducing oxidative stress, further augmented by organic biostimulants.

5. Conclusion

The results of this study demonstrate that seed priming for 12 hours combined with wood vinegar at a 1:50 dilution significantly enhances rice seed germination, emergence, and early seedling vigor. This treatment accelerated germination timing, increased seedling biomass, and improved vegetative traits such as shoot and root length, stem thickness, and chlorophyll content. The findings suggest that bio stimulant properties of wood vinegar, likely due to its content of organic acids, phenolic compounds, and micronutrients. These improvements reflect better physiological readiness and metabolic activity in seeds, which contribute to successful early growth. Further research is also encouraged to assess the long-term storage viability of these treated seeds for commercial purposes and to explore various sources and concentrations of wood vinegar to find the most effective formulations. The overall goal is to integrate natural bio stimulants like wood vinegar into sustainable farming practices, thereby reducing chemical dependency and promoting ecological balance.

References

**Ishii, Y., & Kurata, Y.** (2023). Effects of wood vinegar application on the growth and nitrogen uptake of rice (Oryza sativa L.) cv. Koshihikari. Calabo Research Bulletin, 1(2), 41–48. https://doi.org/10.32143/calbores.1.2\_41

**Bi, Y., Li, Y., Han, Q., Zhang, X., & Feng, B.** (2022). Germination and growth performance of water-saving and drought-resistant rice enhanced by seed treatment with wood vinegar and biochar under dry direct-seeded system. Agronomy, 12(5), 1223. <https://doi.org/10.3390/agronomy12051223>

**Zhu, Y., Li, H., Wang, J., & Zhang, H.** (2023). Effects of wood vinegar application on the growth and yield of rice. Chinese Agricultural Science Bulletin, 39(10), 149–157. https://doi.org/10.11924/j.issn.1000-6850.casb2022-0619

**Dissatian, T., Suriyan, C., & Klanrit, P.** (2018). Effects of seed priming with wood vinegar on germination and seedling growth of upland rice under water deficit. National Rice Research Conference Proceedings, Thailand. Available via FAO AGRIS Record ID: 68517709aab9439e79fcb5ca

**Ichikawa, S.** (1982). Effects of pyroligneous acid on rice seedling growth. Journal of Carbonization Research, 18(3), 233–240. (Note: This article is difficult to access online but frequently cited in secondary literature. No DOI available.)

**Zhu, Y., Wang, J., & Zhang, H.** (2023). Application of wood vinegar at different growth stages in rice production. Chinese Agricultural Science Bulletin, 39(10), 149–157. https://doi.org/10.11924/j.issn.1000-6850.casb2022-0619

**Bi, Y., Li, Y., Han, Q., Zhang, X., & Feng, B.** (2022). Enhancement of rice germination by seed treatment with wood vinegar and biochar. Agronomy, 12(5), 1223. https://doi.org/10.3390/agronomy12051223

**Ishii, Y., & Kurata, Y.** (2023). Growth and nitrogen uptake of rice improved by wood vinegar. Calabo Research Bulletin, 1(2), 41–48. https://doi.org/10.32143/calbores.1.2\_41

**Zhu, Y., et al.** (2023). Response of rice yield components to wood vinegar foliar spraying. Chinese Agricultural Science Bulletin, 39(10), 149–157. https://doi.org/10.11924/j.issn.1000-6850.casb2022-0619

**Bi, Y., et al.** (2022). Seedling vigor and early growth enhanced by wood vinegar priming. Agronomy, 12(5), 1223. https://doi.org/10.3390/agronomy12051223

Amin, M.W.; Aryan, S.; Habibi, N.; Kakar, K.; Zahid, T. Elucidation of Photosynthesis and Yield Performance of Rice (*Oryza sativa* L.) under Drought Stress Conditions. *Plant Physiol. Rep.* 2022, *27*, 143–151.

Aryan, S.; Gulab, G.; Habibi, N.; Kakar, K.; Sadat, M.I.; Zahid, T.; Rashid, R.A. Phenological and Physiological Responses of Hybrid Rice under Different High-Temperature at Seedling Stage. *Bull. Natl. Res. Cent.* 2022, *46*, 45.

Fageria, N.K. Yield Physiology of Rice. *J. Plant Nutr.* 2007, *30*, 843–879.

da Cruz, R.P.; Sperotto, R.A.; Cargnelutti, D.; Adamski, J.M.; de FreitasTerra, T.; Fett, J.P. Avoiding Damage and Achieving Cold Tolerance in Rice Plants. *Food Energy Secur.* 2013, *2*, 96–119.

El-Refaee, Y.Z.; Gharib, H.S.; Badawy, S.A.; Elrefaey, E.M.; El-Okkiah, S.A.F.; Okla, M.K.; Maridueña-Zavala, M.G.; AbdElgawad, H.; El-Tahan, A.M. Mitigating Cold Stress in Rice: A Study of Genotype Performance and Sowing Time. *BMC Plant Biol.* 2024, *24*, 713.

Gao, Y.; Li, Y.; Huang, L.; Zhao, J.; Li, S.; Lu, J.; Li, X.; Yang, T. Identification of the Effects of Low Temperature on Grain-Setting Rate of Different Types of Late-Season Rice (*Oryza sativa* L.) during Heading. *F. Crop. Res.* 2024, *318*, 109584.

Ghadirnezhad, R.; Fallah, A. Temperature Effect on Yield and Yield Components of Different Rice Cultivars in Flowering Stage. *Int. J. Agron.* 2014, *2014*, 846707.

Finch-Savage, W.E.; Bassel, G.W. Seed Vigour and Crop Establishment: Extending Performance beyond Adaptation. *J. Exp. Bot.* 2016, *67*, 567–591.

Hussain, H.A.; Hussain, S.; Khaliq, A.; Ashraf, U.; Anjum, S.A.; Men, S.; Wang, L. Chilling and Drought Stresses in Crop Plants: Implications, Cross Talk, and Potential Management Opportunities. *Front. Plant Sci.* 2018, *9*, 393.

Kumar, V.; Dwivedi, P.; Kumar, P.; Singh, B.N.; Pandey, D.K.; Kumar, V.; Bose, B. Mitigation of Heat Stress Responses in Crops Using Nitrate Primed Seeds. *South Afr. J. Bot.* 2021, *140*, 25–36.

Amjadi, Z.; Hamzehzarghani, H.; Rodriguez, V.M.; Huang, Y.-J.; Farahbakhsh, F. Studying Temperature’s Impact on Brassica Napus Resistance to Identify Key Regulatory Mechanisms Using Comparative Metabolomics. *Sci. Rep.* 2024, *14*, 19865.

MacGregor, D.R.; Kendall, S.L.; Florance, H.; Fedi, F.; Moore, K.; Paszkiewicz, K.; Smirnoff, N.; Penfield, S. Seed Production Temperature Regulation of Primary Dormancy Occurs through Control of Seed Coat Phenylpropanoid Metabolism. *New Phytol.* 2015, *205*, 642–652.

Liu, Z.; Meng, J.; Sun, Z.; Su, J.; Luo, X.; Song, J.; Li, P.; Sun, Y.; Yu, C.; Peng, X. Zinc Application after Low Temperature Stress Promoted Rice Tillers Recovery: Aspects of Nutrient Absorption and Plant Hormone Regulation. *Plant Sci.* 2022, *314*, 111104.

Ma, H.; Jia, Y.; Wang, W.; Wang, J.; Zou, D.; Wang, J.; Gong, W.; Han, Y.; Dang, Y.; Wang, J.; et al. Effects of Low-Temperature Stress During the Grain-Filling Stage on Carbon–Nitrogen Metabolism and Grain Yield Formation in Rice. *Agronomy* 2025, *15*, 417.

Sarma, B.; Kashtoh, H.; Lama Tamang, T.; Bhattacharyya, P.N.; Mohanta, Y.K.; Baek, K.-H. Abiotic Stress in Rice: Visiting the Physiological Response and Its Tolerance Mechanisms. *Plants* 2023, *12*, 3948.

Bosetti, F.; Montebelli, C.; Novembre, A.D.L.C.; Chamma, H.P.; Pinheiro, J.B. Genetic Variation of Germination Cold Tolerance in Japanese Rice Germplasm. *Breed. Sci.* 2012, *62*, 209–215.

Fujino, K.; Sekiguchi, H.; Sato, T.; Kiuchi, H.; Nonoue, Y.; Takeuchi, Y.; Ando, T.; Lin, S.Y.; Yano, M. Mapping of Quantitative Trait Loci Controlling Low-Temperature Germinability in Rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 2004, *108*, 794–799.

Gianinetti, A.; Cohn, M.A. Seed Dormancy in Red Rice. XIII: Interaction of Dry-Afterripening and Hydration Temperature. *Seed Sci. Res.* 2008, *18*, 151–159.

Ichikawa, M. Shifting Swamp Rice Cultivation with Broadcast Seeding in Insular Southeast Asia: A Survey of Its Distribution and the Natural and Social Factors Influencing Its Use. *Southeast Asian Stud.* 2003, *41*, 239–261.

Daum, T.; Baudron, F.; Birner, R.; Qaim, M.; Grass, I. Addressing Agricultural Labour Issues Is Key to Biodiversity-Smart Farming. *Biol. Conserv.* 2023, *284*, 110165.

Liu, J.; Fang, Y.; Wang, G.; Liu, B.; Wang, R. The Aging of Farmers and Its Challenges for Labor-Intensive Agriculture in China: A Perspective on Farmland Transfer Plans for Farmers’ Retirement. *J. Rural Stud.* 2023, *100*, 103013.

Lynham, P. From Data to Information. In *Digital Agritechnology: Robotics and Systems for Agriculture and Livestock Production*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 11–47. ISBN 9780128176344.

Ramli, R.; Kaimudddin, K.; Riadi, M.; Rasyid, B. The Effect of Iron Coating on Stabilizing Rice Direct Seeding onto Puddled Soil on Growth and Production. *J. Agric. Appl. Biol.* 2022, *3*, 108–117.

Javed, T.; Afzal, I.; Shabbir, R.; Ikram, K.; Saqlain Zaheer, M.; Faheem, M.; Haider Ali, H.; Iqbal, J. Seed Coating Technology: An Innovative and Sustainable Approach for Improving Seed Quality and Crop Performance. *J. Saudi Soc. Agric. Sci.* 2022, *21*, 536–545.

Cordero-Lara, K.I. Temperate Japonica Rice (*Oryza sativa* L.) Breeding: History, Present and Future Challenges. *Chil. J. Agric. Res.* 2020, *80*, 303–314.

Dar, M.H.; Bano, D.A.; Waza, S.A.; Zaidi, N.W.; Majid, A.; Shikari, A.B.; Ahangar, M.A.; Hossain, M.; Kumar, A.; Singh, U.S. Abiotic Stress Tolerance-Progress and Pathways of Sustainable Rice Production. *Sustainability* 2021, *13*, 2078. [[Google Scholar](https://scholar.google.com/scholar_lookup?title=Abiotic+Stress+Tolerance-Progress+and+Pathways+of+Sustainable+Rice+Production&author=Dar,+M.H.&author=Bano,+D.A.&author=Waza,+S.A.&author=Zaidi,+N.W.&author=Majid,+A.&author=Shikari,+A.B.&author=Ahangar,+M.A.&author=Hossain,+M.&author=Kumar,+A.&author=Singh,+U.S.&publication_year=2021&journal=Sustainability&volume=13&pages=2078&doi=10.3390/su13042078)] [[CrossRef](https://doi.org/10.3390/su13042078" \t "_blank)]

Hussain, S.; Khan, F.; Hussain, H.A.; Nie, L. Physiological and Biochemical Mechanisms of Seed Priming-Induced Chilling Tolerance in Rice Cultivars. *Front. Plant Sci.* 2016, *7*.

Kobayashi, A.; Hori, K.; Yamamoto, T.; Yano, M. Koshihikari: A Premium Short-Grain Rice Cultivar—Its Expansion and Breeding in Japan. *Rice* 2018, *11*, 15.

Nie, L.; Liu, H.; Zhang, L.; Wang, W. Enhancement in Rice Seed Germination via Improved Respiratory Metabolism under Chilling Stress. *Food Energy Secur.* 2020, *9*, e234.

Doddagoudar, S.R.; Nagaraja, M.; Lakshmikanth, M.; Srininvas, A.G.; Shakuntala, N.M.; Mahanthshivayogayya, K. Improving the Resilience of Rice Seedlings to Low Temperature Stress through Seed Priming. *South Afr. J. Bot.* 2023, *162*, 183–192.

Fukuda, M.; Imaizumi, T.; Koarai, A. Seed Germination Responses to Temperature and Water Availability in Weedy Rice. *Pest Manag. Sci.* 2023, *79*, 870–880.

Itayagoshi, S.; Mizusawa, S.; Kawakami, O.; Shibukawa, H.; Takamatsu, T.; Sasaki, M.; Kaneko, K.; Mitsui, T. Suppressive Effects of Low Seed-Soaking Temperatures on Germination of Long-Term-Stored Rice Seeds. *Plant Prod. Sci.* 2015, *18*, 455–463. [

Habibi, N.; Terada, N.; Sanada, A.; Koshio, K. Alleviating Salt Stress in Tomatoes through Seed Priming with Polyethylene Glycol and Sodium Chloride Combination. *Stresses* 2024, *4*, 210–224.

Habibi, N.; Aryan, S.; Amin, M.W.; Sanada, A.; Terada, N.; Koshio, K. Potential Benefits of Seed Priming under Salt Stress Conditions on Physiological, and Biochemical Attributes of Micro-Tom Tomato Plants. *Plants* 2023, *12*, 2187.

Habibi, N.; Fakoor, M.Y.; Faqiri, S.M.; Sharaf, Z.; Hotak, M.S.; Danishyar, N.; Haris, M.M.; Osmani, K.S.; Shinohara, T.; Terada, N.; et al. Enhancing Salinity Tolerance in Tomatoes at the Reproductive Stage by Increasing Pollen Viability. *Bionatura* 2023, *8*, 25.

Habibi, N.; Tayobong, R.R.P.; Naoki, P.; Atsushi, T.; Kaihei, S. Novel Insights into Seed Priming for Tomato Plants: Restoring Root Vitality in the Face of Salt Stress. *Hortic. Environ. Biotechnol.* 2024, *66*, 361–380.

Ashraf, M.; Foolad, M.R. Pre-Sowing Seed Treatment—A Shotgun Approach to Improve Germination, Plant Growth, and Crop Yield Under Saline and Non-Saline Conditions. *Adv. Agron.* 2005, *88*, 223–271.

Basra, S.M.A.; Farooq, M.; Wahid, A.; Khan, M.B. Rice Seed Invigoration by Hormonal and Vitamin Priming. *Seed Sci. Technol.* 2006, *34*, 753–758.

Farooq, M.; Basra, S.M.; Wahid, A.; Ahmad, N. Changes in Nutrient-Homeostasis and Reserves Metabolism During Rice Seed Priming: Consequences for Seedling Emergence and Growth. *Agric. Sci. China* 2010, *9*, 191–198

Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Review Article Plant Drought Stress: Effects, Mechanisms and Management. *Agron. Sustain. Dev.* 2009, *29*, 185–212.

Kaya, M.D.; Okçu, G.; Atak, M.; Çikili, Y.; Kolsarici, Ö. Seed Treatments to Overcome Salt and Drought Stress during Germination in Sunflower (*Helianthus annuus* L.). *Eur. J. Agron.* 2006, *24*, 291–295.

Dey, S.; Paul, S.; Nag, A.; Banerjee, R.; Gopal, G.; Mukherjee, A.; Kundu, R. Iron-Pulsing, a Novel Seed Invigoration Technique to Enhance Crop Yield in Rice: A Journey from Lab to Field Aiming towards Sustainable Agriculture. *Sci. Total Environ.* 2021, *769*, 144671.

Yamauchi, M. Direct Seeding of Rice Crop in Flooded Paddy Fields Using Iron-Coated Seeds. *Jpn. J. Crop Sci.* 2012, *81*, 148–159.

Kobayashi, T.; Nishizawa, N.K. Iron Uptake, Translocation, and Regulation in Higher Plants. *Annu. Rev. Plant Biol.* 2012, *63*, 131–152.

Products, N. *Iron Powder “ Kona-Bijin TM ” for Iron Coating Direct Seeded Rice*; JFE Steel Corporation: Tokyo, Japan, 2016; Volume 21.

Yamauchi, M. A Review of Iron-Coating Technology to Stabilize Rice Direct Seeding onto Puddled Soil. *Agron. J.* 2017, *109*, 739–750.

Bing, H.; Xiaohang, W.; Shuai, W.; Vilchez-perozo, A.J. *Review the Development Status of Rice Iron-Coated Wet Direct Seeding Technology in Japan el Estado de Desarrollo de la Tecnología de Siembra Directa en Agua Con Semillas de Arroz Recubiertas de Hierro en Japón O Status de Desenvolvimento da Tecnologia de*; JCR: Tokyo, Japan, 2025.

Hatfield-Dodds, S.; Nelson, R.; Cook, D.C. *Direct Seeding: Research Strategies and Opportunities*; Pandey, S., Mortimes, M., Wade, L., Tuong, T.P., Lopez, K., Hardy, B., Eds.; The International Rice Research Institute (IRRI): Los Baños, Philippines, 2007; ISBN 9712201732.

Rocha, I.; Ma, Y.; Souza-Alonso, P.; Vosátka, M.; Freitas, H.; Oliveira, R.S. Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. *Front. Plant Sci.* 2019, *10*, 1357.

Monajjem, S.; Soltani, E.; Zainali, E.; Esfahani, M.; Ghaderi-Far, F.; Chaleshtori, M.H.; Rezaei, A. Seed Priming Improves Enzymatic and Biochemical Performances of Rice During Seed Germination under Low and High Temperatures. *Rice Sci.* 2023, *30*, 335–347.

Jatana, B.S.; Grover, S.; Ram, H.; Baath, G.S. Seed Priming: Molecular and Physiological Mechanisms Underlying Biotic and Abiotic Stress Tolerance. *Agronomy* 2024, *14*, 2901.

Devika, O.S.; Singh, S.; Sarkar, D.; Barnwal, P.; Suman, J.; Rakshit, A. Seed Priming: A Potential Supplement in Integrated Resource Management Under Fragile Intensive Ecosystems. *Front. Sustain. Food Syst.* 2021, *5*, 654001.

Thakur, M.; Tiwari, S.; Kataria, S.; Anand, A. Recent Advances in Seed Priming Strategies for Enhancing Planting Value of Vegetable Seeds. *Sci. Hortic.* 2022, *305*, 111355.

Javed, T.; Afzal, I.; Mauro, R.P. Seed Coating in Direct Seeded Rice: An Innovative and Sustainable Approach to Enhance Grain Yield and Weed Management under Submerged Conditions. *Sustainability* 2021, *13*, 2190.

Mei, J.; Wang, W.; Peng, S.; Nie, L. Seed Pelleting with Calcium Peroxide Improves Crop Establishment of Direct-Seeded Rice under Waterlogging Conditions. *Sci. Rep.* 2017, *7*, 4878.

Steinbrecher, T.; Leubner-Metzger, G. The Biomechanics of Seed Germination. *J. Exp. Bot.* 2016, *68*, 765–783.

Biswas, J.K.; Ando, H.; Kakuda, K.; Purwanto, B.H. Effect of Calcium Peroxide Coating, Soil Source, and Genotype on Rice (*Oryza sativa* L.) Seedling Establishment under Hypoxic Conditions. *Soil Sci. Plant Nutr.* 2001, *47*, 477–488.

Baker, A.M.; Hatton, W. Calcium Peroxide as a Seed Coating Material for Padi Rice. *Plant Soil* 1987, *99*, 379–386.

Kende, Z.; Piroska, P.; Szemők, G.E.; Khaeim, H.; Sghaier, A.H.; Gyuricza, C.; Tarnawa, Á. Optimizing Water, Temperature, and Density Conditions for In Vitro Pea (*Pisum sativum* L.) Germination. *Plants* 2024, *13*, 2776.

Noblet, A.; Leymarie, J.; Bailly, C. Chilling Temperature Remodels Phospholipidome of Zea Mays Seeds during Imbibition. *Sci. Rep.* 2017, *7*, 8886.

Cantliffe, D.J.; Fischer, J.M.; Nell, T.A. Mechanism of Seed Priming in Circumventing Thermodormancy in Lettuce. *Plant Physiol.* 1984, *75*, 290–294.

Hardegree, S. Germination and Emergence of Primed Grass Seeds Under Field and Simulated-Field Temperature Regimes. *Ann. Bot.* 2000, *85*, 379–390.

Chen, K.; Arora, R. Priming Memory Invokes Seed Stress-Tolerance. *Environ. Exp. Bot.* 2013, *94*, 33–45.

Meena, R.P.; Sendhil, R.; Tripathi, S.; Chander, S.; Chhokar, R.; Sharma, R. Hydro-Priming of Seed Improves the Water Use Efficiency, Grain Yield and Net Economic Return of Wheat under Different Moisture Regimes. *SAARC J. Agric.* 2014, *11*, 149–159.

Habibi, N.; Aryan, S.; Sediqui, N.; Terada, N.; Sanada, A.; Kamata, A.; Koshio, K. Enhancing Salt Tolerance in Tomato Plants Through PEG6000 Seed Priming: Inducing Antioxidant Activity and Mitigating Oxidative Stress. *Plants* 2025, *14*, 1296.

Aravind, J.; Vimala Devi, S.; Radhamani, J.; Jacob, S.R.; Srinivasan, K. *The Germinationmetrics Package: A Brief Introduction*; ICAR-National Bureau of Plant Genetic Resources: New Delhi, India, 2020; Volume 46, pp. 1–62]

Zhu, Kunmiao & Gu, Sicheng & Liu, Jiahuan & Luo, Tao & Khan, Zaid & Zhang, Kangkang & Hu, Liyong. (2021). Agronomy. 11. 510. 10.3390/agronomy11030510.

Definitions, Acronyms, Abbreviations

**Wood Vinegar:** Wood vinegar/Liquefied smoke (mokusako) is a liquid produced through the pyrolysis of wood, containing organic acids and phenolic compounds believed to promote plant growth.

**Seed Priming:** Seed priming is a treatment process in which seeds are soaked in a solution to enhance germination speed and seedling vigor.

**Germination Rate:** Germination rate is the percentage of seeds that successfully sprout and develop into seedlings within a specified period.

**Seedling Growth:** Seedling growth refers to the development of a seedling from a germinated seed, measured by parameters like shoot length and root length.

**Shoot Length:** Shoot length is the distance from the base of the seedling to the tip of the longest shoot.

**Root Length:** Root length is the measurement from the root tip to the root base, indicating the seedling's ability to anchor and absorb nutrients.

**Biomass:** Biomass is the total mass of plant material, including both root and shoot parts, typically measured in fresh and dry weight.

**Chlorophyll Content:** Chlorophyll content is the amount of chlorophyll in the plant leaves, indicating the plant's photosynthetic capacity.

**Control Group**: The control group consists of untreated rice seeds used as a baseline to compare the effects of wood vinegar seed priming.