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Effect of Seed Biopriming with *Trichoderma harzianum* for Increasing Seed Quality of Vegetable Soybean (Edamame)

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Abstract: A major constraint in edamame (*Glycine max* [L.] Merr.) cultivation is the rapid deterioration of seed viability, which limits crop productivity. Seed priming has been widely applied to improve the physiological quality of seeds by activating early metabolic processes prior to germination. This study aimed to determine the most effective seed priming treatment using *Trichoderma harzianum* and rice husk ash as matriconditioning media to stimulate edamame seed germination and vigor. The research was conducted from July to September 2025 at the Seed Technology Laboratory, Jember State Polytechnic, using a completely randomized design (CRD) with seven treatments: control (M1), matriconditioning with rice husk ash plus water for 6 hours (M2), matriconditioning with rice husk ash plus Trichoderma sp. isolate for 6 hours (M3), matriconditioning with rice husk ash plus Trichoderma sp. isolate for 12 hours (M4), matriconditioning with rice husk ash plus Trichoderma sp. isolate for 18 hours (M5), and matriconditioning with rice husk ash plus Trichoderma sp. isolate for 24 hours (M6). Observed parameters included germination percentage (GP), maximum germination potential (MGP), vigor index (VI), seedling uniformity, seedling biomass, and shoot and root length. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. The results suggested that *T. harzianum* seed treatments have not yet shown a significant effect on enhancing edamame seed viability, as indicated by the parameters of Germination Percentage, Maximum Germination Potential, and Vigor Index. These findings imply that further optimization of priming duration and inoculum concentration may be necessary to achieve more consistent improvements in germination performance and seed vigor.

Keywords: biopriming, edamame; seed quality

1. Introduction

Edamame (Glycine max L. Merril) is a type of soybean originating from Japan, characterized by its large pods and seeds. The pods are consumed fresh and regarded as a vegetable commodity that is widely cultivated and consumed across Asia. In Indonesia, edamame has been extensively developed and has become one of the leading agricultural commodities in Jember Regency. This plant produces pods with highly nutritious seeds, while its green stems can be used as animal feed or green manure to improve soil fertility for subsequent harvests within 65–75 days (Zhang et al., 2017). Edamame contains carbohydrates, sucrose, protein, and oil. The combination of sucrose, ascorbic acid, glutamic acid, and alanine provides a more pleasant taste compared to green peas. Moreover, edamame produces isoflavones with phenolic structures similar to estrogen, known as phytoestrogens, which serve as a natural alternative to postmenopausal hormone replacement therapy. Isoflavones and saponins are associated with potential health benefits related to age-related and hormone-dependent diseases. Its adaptability and pleasant flavor make edamame an ideal alternative to address malnutrition, particularly protein and iron deficiencies, in regions such as India.

Edamame differs from common soybeans primarily in its larger seed size and higher contents of sugar (3.0%), protein (11.4%), minerals (calcium 70 mg/100 g, potassium 140 mg/100 g, and phosphorus 140 mg/100 g), and vitamins (carotene 100 mg/100 g, ascorbic acid 27 mg/100 g, and alpha-tocopherol 12 μg/seed) (Khan et al., 2023). As a tropical country, Indonesia offers great potential for edamame development since its cultivation can be carried out year-round without seasonal constraints. In Jember, the productivity of edamame reaches approximately 6.6 tons/ha, with a relatively high selling price and well-established market access (Prasetyo, 2017). This condition has led to a shift in farmers' cultivation trends from tobacco to edamame due to better profitability, simpler cultivation and postharvest handling, and wider export market opportunities.

Edamame seed production is one of the activities carried out in the Project-Based Learning (PBL) program of students in the Seed Production Technology Study Program, specifically in the Seed Production of Legumes and Tuber Crops course. The main problem encountered in edamame cultivation during the PBL program is low seed quality, even when freshly harvested seeds are used. In general, dry soybean seeds contain around 40% protein and 20% oil (Sun & Yuan, 2022). As a soybean variant, edamame seeds have a similar composition and are prone to rapid seed deterioration. The relatively large size of edamame seeds makes them more sensitive to adverse environmental and edaphic conditions, including insufficient soil moisture, inappropriate temperature, and poor physical soil conditions. Large seeds are also more susceptible to reduced viability and vigor due to mechanical damage during harvesting and processing and are more likely to undergo aging during storage. Additionally, large seeds release more nutrients during imbibition, attracting soil-borne pathogens and increasing disease incidence. All these factors contribute to lower field emergence in edamame compared to common soybeans.

The problem of uneven germination affects the need for planting materials and can be overcome through seed priming, a pre-sowing treatment designed to enhance seed quality. Seed priming is performed to improve the physiological quality of edamame seeds by using germination-promoting agents. It is a controlled hydration process in which seeds are moistened to a level that allows pre-germinative metabolic activities to occur, while radicle emergence is still prevented (Raj & Raj, 2019). Priming improves the physiological condition of seeds, contributing to higher germination percentage, faster seedling establishment, and enhanced tolerance to various environmental stresses (Hussain et al., 2022)(Sakhno et al., 2025). It is often used to restore vigor in deteriorated seeds by enhancing both germination potential and stress tolerance (Paparella et al., 2015). Previous studies have shown that priming effectively improves germination in mung bean (AA et al., 2025), increases vigor index in yardlong bean seeds and maintains it for up to 15 weeks of storage (Utami et al., 2013), and strengthens root systems while enhancing nutrient uptake in soybean plants (Luta et al., 2024)

Matriconditioning is one of the seed priming techniques. It involves treating seeds with a moist solid medium characterized by low matric potential and negligible osmotic potential, aiming to improve seed physiology and biochemistry to prepare them for germination. This treatment stimulates metabolism and embryonic cell division within the seed, thereby enhancing germination rate and vigor. Matriconditioning has been reported to improve soybean seed viability in terms of germination percentage, growth rate, root length, and seedling height. Among various matriconditioning materials, finely ground bricks have been identified as the most effective, yielding 84% germination, 16.8% growth rate/day, 7.5 cm seedling height, and 6.8 cm root length (Colla & Rouphael, 2020). Moreover, combining matriconditioning with vermicompost extract solution (LEV) using rice husk charcoal as the medium in a seed:medium:LEV ratio of 9:6:7 (w/w/v) for 12 hours at 25 ± 2 °C significantly improved germination percentage (GP), vigor index (VI), and radicle emergence (RE), while reducing electrical conductivity (Permatasari, 2025).

Biopriming is another priming technique that incorporates beneficial microorganisms such as bacteria, fungi, or microalgae to control seed-borne pathogens and promote germination through the production of secondary metabolites, as well as to enhance resistance against biotic and abiotic stresses. Beneficial microbial agents, including biofertilizers and biopesticides, such as *Azotobacter*, *Rhizobium*, *Arthrobacter*, *Agrobacterium*, *Azospirillum*, *Enterobacter*, *Streptomyces*, *Bacillus*, *Burkholderia*, *Klebsiella*, *PSB*, *Pseudomonas fluorescens*, *Trichoderma viride*, *Trichoderma harzianum*, and Vesicular Arbuscular Mycorrhiza, can serve as biopriming agents. Biopriming applies to nearly all crops and provides an environmentally friendly alternative to chemical fungicides (Sarkar et al., 2021). Seed biopriming enhances early protein and DNA synthesis and facilitates more efficient mitochondrial development (Chakraborti et al., 2022). *Trichoderma* species are known to produce several plant hormones, such as cytokinin (zeatin-like) and gibberellins. These hormones can reduce the membrane stability index and malondialdehyde content, while *Trichoderma*-treated plants exhibit increased accumulation of proline, phenolics, and antioxidant enzyme activities, thereby improving overall physiological and morphological plant performance (Pehlivan et al., 2017). This study aimed to determine the most effective seed priming treatment using *Trichoderma harzianum* and rice husk ash as matriconditioning media to stimulate edamame seed germination and vigor.

2. Materials and Methods

The research conducted from July to September 2025 at the Seed Technology Laboratory, Jember State Polytechnic. The experiment will use a non-factorial completely randomized design (CRD). The treatments consist of matriconditioning using rice husk ash combined with Trichoderma sp. isolates. The treatment levels are: control (M1), matriconditioning with rice husk ash plus water for 6 hours (M2), matriconditioning with rice husk ash plus Trichoderma sp. isolate for 6 hours (M3), matriconditioning with rice husk ash plus Trichoderma sp. isolate for 12 hours (M4), matriconditioning with rice husk ash plus Trichoderma sp. isolate for 18 hours (M5), and matriconditioning with rice husk ash plus Trichoderma sp. isolate for 24 hours (M6).

The preparation of edamame seeds used in this study were obtained from a local seed production unit in Jember, East Java. The seeds used were seeds without any post-harvest treatment. The seeds were cleaned and sorted to ensure uniformity in size and color prior to the priming treatments.

Matriconditioning was carried out by mixing the seeds with a solid carrier material (this research using rice husk charcoal) that had been moistened with distilled water to achieve a water potential that allowed pre-germinative metabolic activities without radicle protrusion. The mixture ratio of seed:medium:water was maintained at 1:1:1 (w/w/w). Seeds were incubated for 12 hours at 25 ± 2 °C, then air-dried back to their initial moisture content.

Matriconditioning + Biopriming Seeds were soaked in a solid carrier material (this research using rice husk charcoal) and a suspension containing fungal bioagents (*Trichoderma harzianum*) at a concentration of 10^6 spores/mL. The mixture ratio of seed:medium:water was maintained at 1:1:1 (w/w/w). Seeds were incubated for 6 hours, 12 hours, 18 hours and 24 hours at room condition (25 ± 2 °C). the seed then air-drying at room temperature to reach the original seed moisture content.

Germination tests were conducted using the sand method following the ISTA (2023) rules. Sterilized river sand was placed in germination trays as the growth medium. A total of 50 seeds per replication were sown uniformly at a depth of approximately 1.5–2 cm. The sand was maintained at adequate moisture levels throughout the testing period.

Germination was carried out in a controlled environment at 25 ± 2 °C. Normal seedlings were counted on the 4th day (first count) and the 7th day (final count).

The following parameters were evaluated to assess seed physiological quality; Germination Percentage (GP): calculated as the proportion of normal seedlings after 8 days; Maximum Germination Potential: calculated as the proportion all germinate (normal and abnormal seedlings) at final count (8 days after planting); Vigor Index (VI): calculated from germination percentage at first count (4 days after planting). The data obtained will be analyzed using ANOVA and further tested with Duncan's Multiple Range Test at a 5% significance level.

3. Results and Discussion

The data analized showed non significant result of the seed treatment. The effect of different matriconditioning durations on the germination percentage of edamame seeds is presented in Figure 1. The results showed that the germination percentage varied among treatments, ranging from 32% to 48.5%. The highest germination percentage was observed in the control treatment (48.5%), which was statistically similar to 6-hour matriconditioning with rice husk ash (47.5%) and 12-hour matriconditioning with rice husk ash + *Trichoderma* (47%). These findings indicate that short-duration matriconditioning (6–12 hours) did not reduce the germination ability of seeds compared to the untreated control.

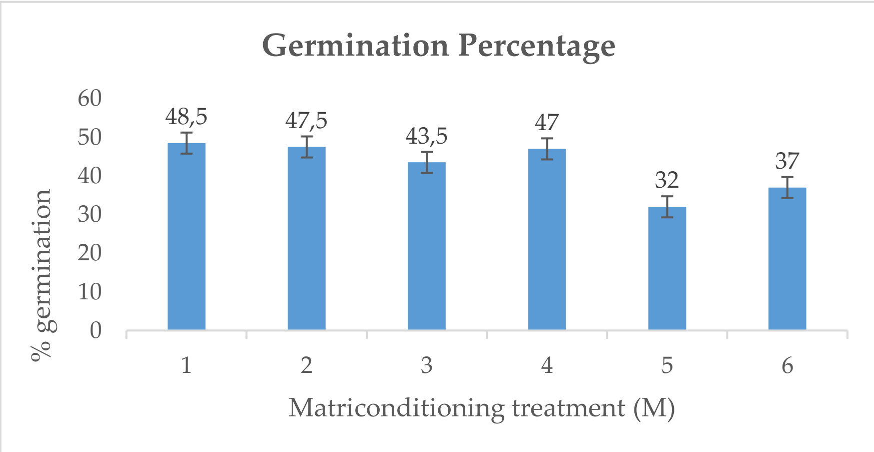

Figure 1. Germination percentage of matriconditioning edamame seed treatment.

Conversely, prolonged matriconditioning, especially 18 hours (32%) and 24 hours (37%), resulted in a marked decline in germination percentage. This suggests that extended exposure to high moisture content during maturation may have caused imbibitional injury or accelerated seed deterioration, leading to a loss of viability. Excess moisture during long priming periods can disrupt cell membrane integrity and increase metabolic imbalance, which reduces germination efficiency (Jayamohan et al., 2020). The inclusion of *Trichoderma* in matriconditioning treatments did not show a consistent improvement in germination compared to rice husk ash alone. This may be attributed to insufficient colonization time or microbial competition during extended incubation. *Trichoderma* spp. enhance seed germination and seedling vigor primarily through the production of growth-promoting substances and improvement of root system architecture. However, the beneficial effects of *Trichoderma* depend strongly on priming duration, moisture level, and compatibility between host seed and bioagent.

The relatively high germination in the control and short-duration matriconditioning treatments indicates that moderate hydration and limited exposure to priming media can maintain seed physiological quality. Optimal priming durations vary depending on seed size, seed coat permeability, and moisture absorption rate. In large-seeded legumes such as edamame, extended priming durations may lead to excessive water uptake, resulting in mechanical disruption of embryonic tissues (Farooq et al., 2010).

In this study, 6-hour matriconditioning using rice husk ash was identified as the most effective treatment for maintaining seed viability and achieving a comparable germination percentage to the control. This result highlights the potential of using organic carrier materials such as rice husk ash for short-duration priming, which provides sufficient hydration to trigger metabolic activation without causing cellular damage.

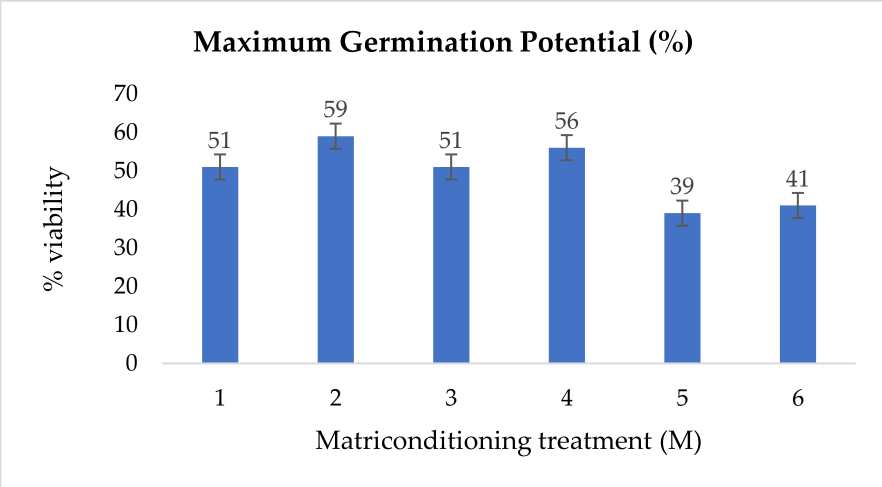

Figure 2. Maximum Germination Potential of matriconditioning edamame seed treatment

The effect of matriconditioning treatments on the maximum germination potential (MGP) of edamame seeds is presented in Figure 2. The results showed that different matriconditioning durations significantly influenced seed viability, ranging from 39% to 59%. The highest MGP was observed in Treatment 2 (59%), followed by Treatment 4 (56%), whereas Treatment 5 (39%) and Treatment 6 (41%) exhibited the lowest viability. The improvement in MGP under Treatment 2 indicates that moderate matriconditioning duration (6 hours) with rice husk ash provided an optimal balance of hydration, allowing activation of metabolic processes without inducing cellular stress. Controlled hydration during priming is known to facilitate membrane repair, mitochondrial activation, and antioxidant enzyme synthesis, all of which support early germination events and enhance viability(Kumar et al., 2022).

Conversely, the decline in seed viability observed in Treatments 5 and 6 suggests that prolonged matriconditioning (18–24 hours) may lead to overhydration and oxidative stress during subsequent drying. Extended exposure to high moisture content can trigger excessive respiration, reactive oxygen species (ROS) accumulation, and depletion of energy reserves, which compromise membrane integrity and reduce germination potential. The similar MGP values between Treatment 1 (Control, 51%) and Treatment 3 (51%) indicate that certain combinations of matriconditioning agents (rice husk ash + Trichoderma) did not significantly improve viability when compared with unprimed seeds. This outcome highlights that the efficacy of seed priming is strongly dependent on the interaction between hydration duration and microbial inoculation. Overall, the findings demonstrate that short-duration matriconditioning (6–12 hours) enhances MGP, while longer treatments may induce detrimental physiological responses. The use of rice husk ash as a solid-phase medium proved beneficial, likely due to its porous structure and moderate water retention capacity, which promotes uniform hydration. These results are consistent with previous reports that optimized priming conditions improve legume seed quality by balancing water uptake and oxidative metabolism (Nawaz et al., 2020).

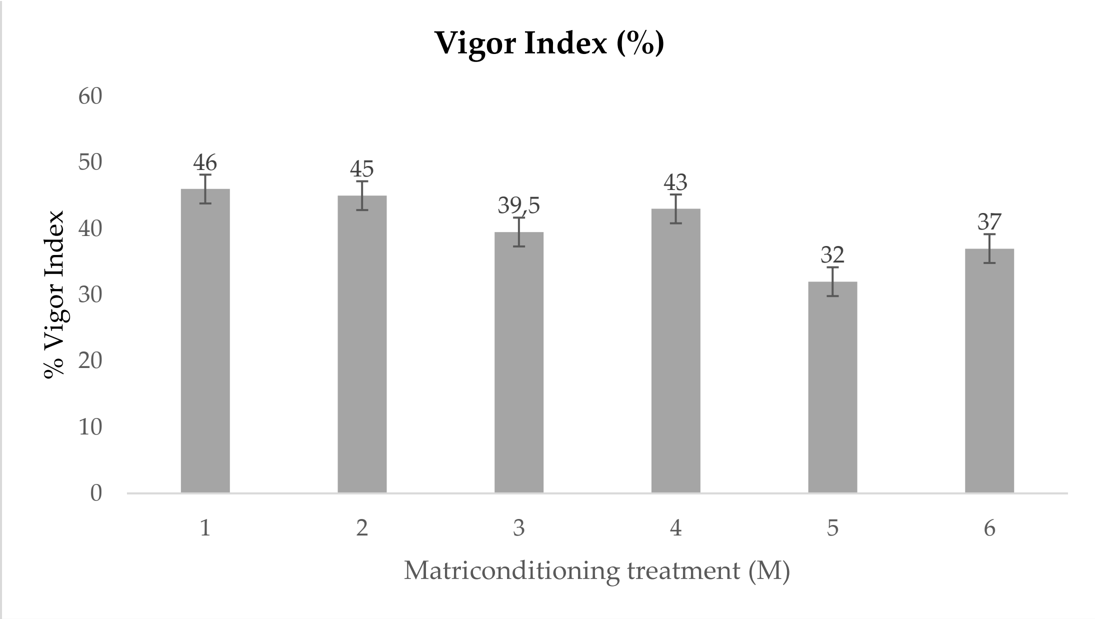

Figure 2. Vigor Index of matriconditioning edamame seed treatment

The effect of different matriconditioning treatments on the vigor index of edamame seeds is presented in Figure 3. The results show that matriconditioning influenced seed vigor, with values ranging from 32% to 46%. The highest vigor index was observed in the control treatment (46%), followed by Treatment 2 (45%) and Treatment 4 (43%), while the lowest vigor was recorded in Treatment 5 (32%) and Treatment 6 (37%).

Interestingly, seed vigor did not substantially improve after matriconditioning compared to the control, suggesting that prolonged or excessive hydration may have caused physiological stress or partial deterioration during the conditioning and drying phases. High vigor in the control treatment indicates that initial seed quality was relatively high, and excessive hydration during maturation conditioning may have negatively influenced membrane integrity, enzyme stability, and reserve mobilization, leading to reduced vigor(Ali et al., 2019). Moderate matriconditioning durations (6–12 hours) such as Treatment 2 and Treatment 4 maintained vigor index values close to the control, indicating that short-term conditioning can support limited metabolic activation without inducing desiccation injury. Controlled priming treatments that partially activate metabolic repair mechanisms can stabilize membranes and improve antioxidant defense, thereby maintaining seed vigor (Singh et al., 2020).

In contrast, the decline observed in Treatments 5 and 6 demonstrates that overhydration stress during 18–24 hours of matriconditioning likely led to ROS accumulation and ATP depletion, conditions detrimental to seed vigor(Huang et al., 2021). (Kumar et al., 2022)who noted that extended hydration periods in seed priming may disrupt normal mitochondrial activity, resulting in a loss of vigor during germination. Overall, these results highlight that the duration of matriconditioning plays a crucial role in determining seed vigor outcomes. While controlled hydration can be beneficial, excessive exposure time beyond the optimal threshold leads to physiological imbalances and reduced vigor index values. Therefore, short-duration matriconditioning (6–12 hours) using rice husk ash is more favorable for maintaining seed vigor compared to extended conditioning periods. These findings corroborate recent studies emphasizing that optimized priming regimes enhance vigor by promoting membrane reorganization, enzyme activation, and antioxidant response, whereas over-priming reduces seed quality and uniformity of germination

4. Conclusions

Matriconditioning seed treatments using *T. harzianum* have not yet shown a significant effect on enhancing edamame seed viability, as indicated by the parameters of Germination Percentage, Maximum Germination Potential, and Vigor Index. These findings imply that further optimization of priming duration and inoculum concentration may be necessary to achieve more consistent improvements in germination performance and seed vigor.

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