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Respiration Rate and Physiological Characters of Soybean (*Glycine max* (L.) Merr) Seeds After Invigoration Treatment

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Abstract: Degradation of seed quality occurs quickly, which makes it difficult to meet national soybean targets. This damage has the effect of making soybean seeds less viable. Invigoration is one of the methods used to improve seed viability. Two common methods of invigoration are *osmoconditioning* and *matriconditioning*. The primary objective of this research was to determine the most effective invigoration medium for enhancing the quality and viability of soybean seeds (*Glycine max* (L.) Merr) through invigoration techniques. Additionally, the study aimed to examine the correlation between the respiration rate and the physiological properties of the seeds. The research was conducted at PGRI University of Yogyakarta over a four-month period, from January to April 2022. The study utilized a completely randomized design (CRD) with five factors and three replications. Observed variables included water content (WC), seed germination (SG), vigor index (VI), and respiration rate (RR). The results of the study showed the low physiological quality of soybean seeds, especially in the germination indicator ranging from 56.67-68.67%, this indicates a decrease in the percentage of germination. Invigoration treatment using sawdust as a matriconditioning medium showed the best results when compared to other treatments. However, it was still unable to inhibit the rate of deterioration in the soybean seeds used.

Keywords: Invigoration, Priming, Seed Biochemistry, Seed Physiology, Seed Respiration

1. Introduction

Soybean (*Glycine max* (L.) Merr) is a high-protein crop, with its consumption projected to rise in tandem with population growth (Triyanti, 2020). According to data from the Central Statistics Agency (BPS) for 2020–2023, soybean productivity in Indonesia has shown a fluctuating trend. Productivity increased from 30.96 quintals per hectare in 2020 to 32.67 quintals per hectare in 2021. However, it declined to 30.29 quintals per hectare in 2022 and further decreased to 29.21 quintals per hectare in 2023 (Statistik, 2021; Statistik, 2022; Statistik, 2023; Statistik, 2024). These fluctuations highlight the need for improved agricultural practices to stabilize and enhance soybean production to meet future demands.

Despite efforts to enhance soybean production, storage conditions for soybean seeds in the field remain suboptimal, contributing to persistent production challenges. Many farmers continue to store seeds in environments that fail to meet ideal storage standards, particularly regarding humidity, temperature, and air circulation. These poorly controlled conditions often lead to seed damage, reducing their viability and overall quality. Addressing these storage issues is critical to ensuring seed performance and supporting sustainable increases in soybean production.

Seed deterioration is a common challenge during storage, resulting in a decline in biochemical and physiological quality (Rao et al., 2023). High-quality seeds are characterized by optimal genetic, physical, and physiological attributes (Jawak et al., 2016). However, improper storage conditions accelerate seed quality degradation over time. To mitigate this issue, strategic interventions are essential throughout the seed production and storage cycle to preserve seed viability and ensure sustained performance.

To enhance seed viability, various strategies have been developed, including seed invigoration techniques that improve physiological and biochemical seed quality prior to planting. These techniques include *hydropriming*, *matriconditioning*, and *osmoconditioning* (Fujikura et al., 1993; Garcia et al., 1995; Khan, 1992; Nejad, 2018; Thejeshwini et al., 2019). *Matriconditioning* involves using moist, solid materials such as sand, charcoal, vermiculite, ash, or sawdust to improve seed quality, while *osmoconditioning* employs osmotic solutions like PEG, KH_2PO_4, KNO_3, and NaCl to enhance seed performance during storage and germination (Thakur et al., 2019; Wahyuni, 2022). These methods have demonstrated significant potential in maintaining seed vigor and viability.

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This study aimed to identify the most effective invigoration media for enhancing the quality and viability of soybean seeds (*Glycine max* (L.) Merr) using invigoration techniques. Furthermore, it sought to deepen the understanding of the relationship between respiration rate and seed physiological characteristics, providing a foundation for developing improved seed management strategies to support soybean productivity.

2.0 Materials and Methods

2.1 Experimental Background

This study was conducted at Persatuan Guru Republik Indonesia (PGRI) University of Yogyakarta. The research utilized various tools, including plastic containers, oil paper, opaque paper, analytical scales, Erlenmeyer flasks, pipettes, stationery, and a camera for documentation. The materials used comprised soybean seeds of the Anjasmoro variety with a germination rate below 80%, distilled water, sulfuric acid (H_2SO_4), boric acid (H_3BO_3 4%), vermiculite and sawdust for *matriconditioning*, and 2% solutions of potassium nitrate (KNO_3) and sodium chloride (NaCl) for *osmoconditioning*.

2.2 Experimental Design and Treatments

The study employed a completely randomized design (CRD) with five treatments and three replications to evaluate the effects of different invigoration methods on soybean seeds. The treatments included: P0 (control), P1 (*matriconditioning* using vermiculite), P2 (*matriconditioning* using sawdust), P3 (*osmoconditioning* with 2% KNO_3), and P4 (*osmoconditioning* with 2% NaCl). This design allowed for a systematic comparison of the invigoration techniques to determine their impact on seed quality and viability.

Invigoration through *matriconditioning* was performed by combining 9 g of soybean seeds with 6 ml of water and 7 g of *matriconditioning* media, followed by incubation for 12 hours and subsequent drying. Similarly, *osmoconditioning* involved treating 50 g of seeds with 35 ml of an *osmoconditioning* solution for 12 hours, after which the seeds were dried to ensure appropriate moisture levels for storage and further testing (El-Abady, 2014). These procedures were designed to enhance seed viability by optimizing moisture absorption and biochemical activity during treatment.

After storage, several indicators are measured to assess seed quality. The moisture content is analyzed by taking a 10-gram seed sample and calculated using Equation (1). Germination capacity is tested using the rolled paper method, while vigor index is observed through germination in sand media, both calculated using the predetermined Equations (2) and (3). Additionally, soybean seed respiration is measured based on the amount of CO_2 produced, which is also calculated using Equation (4).

$$Water\ Content\ (\%) = \frac{Initial\ weight - Dry\ weight}{Initial\ weight}\ X\ 100\% \qquad \text{....... (1)}$$

$$Seed\ Germination\ (\%) = \frac{Number\ of\ germination\ seeds}{Total\ number\ of\ seeds\ tested} \times 100\% \qquad \text{....... (2)}$$

$$Vigor\ index = \frac{Normal\ number\ of\ sprout\ on\ the\ 4th}{Total\ number\ of\ seeds\ tested} \times 100\% \qquad \text{....... (3)}$$

$$Amount\ of\ CO_2\ produced = \frac{Molecular\ weight\ of\ CO_2}{Molecular\ weight\ of\ CaCO_3}\ x\ Weiht\ of\ CaCO_3\ precipitate \qquad \text{....... (4)}$$

2.3 Data Analysis

The final tests, conducted at the conclusion of the study, included assessments of water content, seed germination, vigor index, and respiration rate. Data obtained were analyzed using Analysis of Variance (ANOVA) at a 5% significance level. If significant differences were detected, post-hoc analysis was performed using Duncan's New Multiple Range Test (DMRT) at a 5% significance level to determine specific group differences.

3.0 Results

3.1 Physiological Characteristics of Soybean Seeds

The observed seed quality encompasses both biochemical and physiological aspects. Physiological quality is measured through key parameters: moisture content, germination rate, and vigor index. A comprehensive representation of the overall physiological quality of soybean seeds is shown in Figure 1 below.

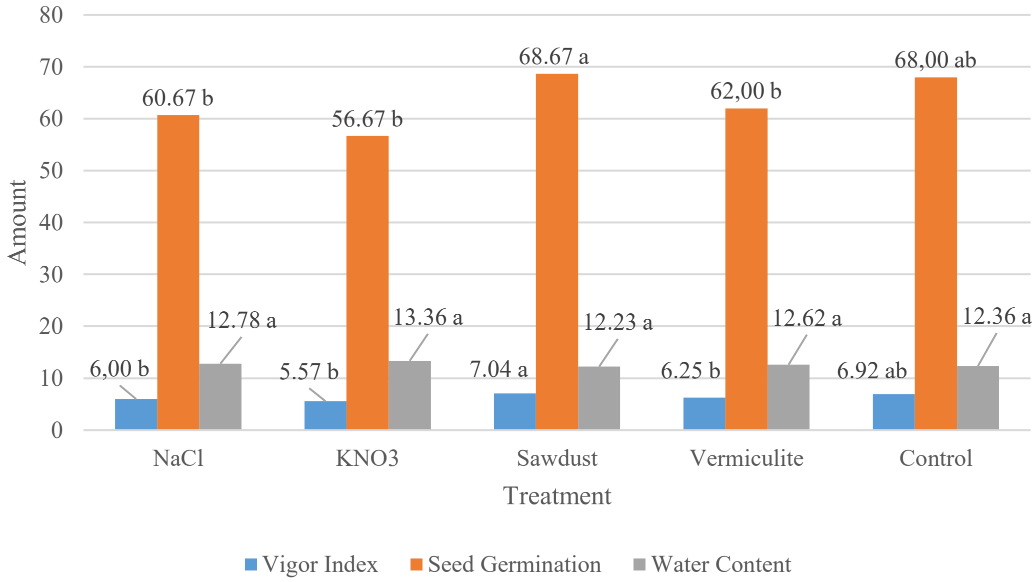

Fig. 1: Physiological quality of soybean seeds after treatment invigoration *Matriconditioning* and *Osmoconditioning*

3.1.1 Water Content

The invigoration treatments did not show significant differences in the water content variable. However, among the treatments, the sawdust media resulted in the lowest water content at 12.23%, outperforming other treatments, which ranged from 12.36% to 13.36%.

3.1.2 Seed Germination

In the germination variable, it can be seen that the invigoration carried out showed that there were significant differences between treatments. Based on the results of the study, the germination value obtained was relatively low, which was around 56.67-68.67%. This indicates a decrease in the percentage of germination when compared to the Anjasmoro seeds used, which have a germination percentage of around 70-80%. This indicates damage to the seeds which ultimately causes deterioration or decline in seed quality. Invigoration treatment using sawdust as a *matriconditioning* medium showed the best results when compared to other treatments. However, it was still unable to inhibit the rate of deterioration in the soybean seeds used.

3.1.3 Vigor Index

The vigor index showed significant differences between the *matriconditioning* and *osmoconditioning* invigoration treatments. This index is determined by the number of normal sprouts observed on the 4^{th} day after the germination test begins. The highest vigor index value was observed in the P2 treatment, specifically the sawdust *matriconditioning* treatment (Figure 1). However, the final vigor index remained low compared to the initial seed vigor index before treatment, indicating a decline in seed vigor as a result of the damage process.

3.2 Respiration Rate

The variation in respiration rates of soybean seeds after treatment is analyzed and shown in Table 1 below.

Table 1: Average of soybean seed respiration rate after given invigoration *matriconditioning* and *osmoconditioning*

Invigoration Treatment	Average[1]
--- Respiration rate (mg CO_2 kg^{-1} $hour^{-1}$) ---	
P0 (Without invigoration)	1.83 a
P1 (*Matriconditioning* vermikulit)	2.00 a
P2 (*Matriconditioning* serbuk gergaji)	1.86 a
P3 (*Osmoconditioning* KNO_3 2%)	2.05 a
P4 (*Osmoconditioning* NaCl 2%)	1.97 a

[1] Average values followed by the same letters show no differences according to DMRT 5%.

The variance analysis results indicated no significant differences in the respiration rates of soybean seeds treated with invigoration methods, either *matriconditioning* or *osmoconditioning* (Table 1). The respiration rate ranged from 1.83 to 2.05 mg CO_2 kg^{-1} $hour^{-1}$, exhibiting some fluctuation. This variation is likely linked to changes in seed water content, which can influence seed metabolism and, in turn, affect the biochemical quality of the seeds.

3.3 Relationship Between Respiration Rate and Seed Physiological Character

Regression and correlation analyses revealed a strong relationship between respiration rate and seed physiological factors. As shown in Figures 2 to 4, the regression analysis indicates a correlation between the respiration rate (RR) and several seed characteristics, including water content (WC), seed germination (SG), and vigor index (VI).

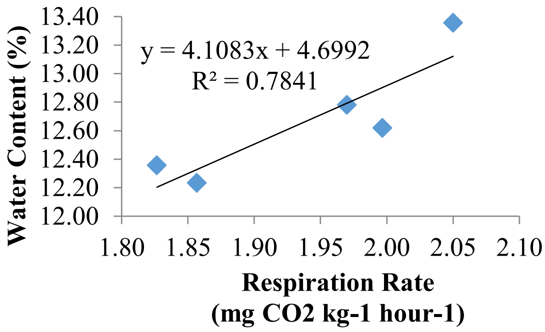

Fig. 2: Regression between respiration rate-water content

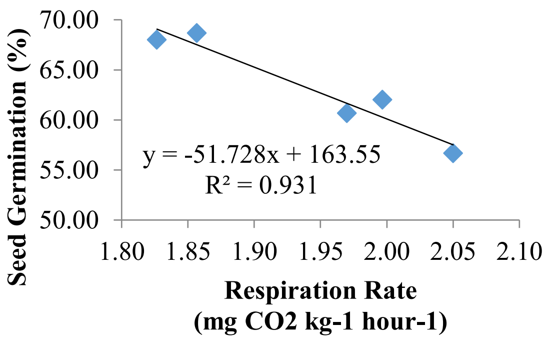

Fig. 3: Regression between respiration rate-seed germination

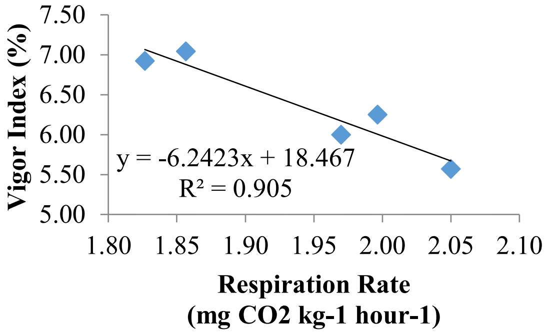

Fig. 4: Regression between respiration rate-vigor index

The relationship between respiration rate and water content (Figure 2) shows a positive correlation, indicating that as respiration rate increases, water content also increases. The correlation coefficient (r) in this experiment is 0.885, suggesting a strong relationship between the two variables. This implies that 78.3% of the variance in water content can be explained by the respiration rate, with the remaining variance attributed to other factors.

The relationships between respiration rate and germination (Figure 3) as well as between the respiration index and vigor (Figure 4) exhibit negative slopes, indicating that higher respiration rates are associated with lower germination rates and vigor indices. The correlation coefficients (r) in this experiment were -0.965 for germination and -0.951 for vigor, suggesting very strong negative relationships. The coefficient of determination (r^2) indicates that 93.2% of the variation in germination and 90.3% of the variation in the vigor index can be explained by the respiration rate, with the remaining variation attributed to other factors.

4.0 Discussion

The invigoration treatments (sawdust) had a distinct ability to retain less water compared to other media, as noted by (Day, 2022). Since higher water content increases the likelihood of soybean seed damage, maintaining lower water content is crucial for preserving seed quality. This statement is in line with research presented by Thirusendura & Saraswathy (2018), which stated that during the seed damage process, there is an increase in water content and electrical conductivity. However, at the same time, the carbohydrate and protein content in the seeds decreases.

Moreover, the invigoration treatment using sawdust as *matriconditioning* media demonstrated the best results in enhancing soybean seed germination. According to Day (2022), differences in the water retention ability of invigoration media contribute to these outcomes, with sawdust exhibiting superior water retention compared to other media. In contrast, the use of coarse vermiculite can damage the seed coat during the mixing process of media, water, and seeds. Therefore, sawdust is considered a more optimal choice for improving the germination of damaged soybean seeds.
Fluctuations in germination during the deterioration process are believed to result from genetic variations among different seed varieties. According to Ramdan et al. (2022), elevated storage temperatures and humidity significantly accelerate the deterioration of corn seeds during storage. Similarly, studies by Irma Noviana et al. (2017) and Nabila et al. (2016) found that the decline in germination rates over time is directly linked to a reduction in the physiological quality of soybean seeds.

According to Rosyad et al. (2016) and Irawan & Iwanudin (2019), internal factors influencing seed viability during storage include genetic traits, growth potential, vigor, and initial seed moisture content. Similarly, Hayati & Setiono (2021) highlighted several key factors affecting seed germination, namely: (1) initial germination rate, (2) initial moisture content, (3) storage humidity, (4) storage temperature, (5) presence of pests and diseases in the storage environment, and (6) duration of the storage period.

The vigor index showed significant differences between the *matriconditioning* and *osmoconditioning* invigoration treatments. According to Rosyad et al. (2016) and Irawan & Iwanudin (2019), a higher vigor index indicates faster germination, suggesting stronger seed vigor. In contrast, a lower index reflects weaker vigor. The vigor index is a key parameter in assessing seed quality, as seeds with higher vigor typically exhibit quicker and more robust germination, positively influencing their growth and establishment after planting. According to Sadjad (1989), rapidly germinating seeds are capable of overcoming various sub-optimal conditions. The decline in soybean seed germination capacity is accompanied by a decrease in vigor index values during storage, a consequence of the damage process that occurs within the seeds.

Furthermore, according to Nuraeni et al. (2019), respiration is a catabolic process in which organic compounds are broken down into inorganic compounds, such as glucose being metabolized to produce CO_2 and energy. The increase in respiration rate is likely associated with higher seed water content. As water content rises, physical changes occur in the seeds, including an increase in electrical conductivity. This suggests the release of compounds that may affect the integrity of the seed cell membrane. Consequently, both electrical conductivity and respiration rate are valuable indicators of seed damage, as they reflect physiological and biochemical changes during storage (Aruan et al., 2018). Monitoring these factors can provide valuable insights into seed condition and quality throughout storage.

Several studies have shown that respiration in seeds generates heat as energy is released. This energy, which should be stored for the germination process, results in a decrease in seed survival and strength (Fachruri et al., 2019; Kolo & Tefa, 2016; Pangastuti et al., 2019). Regression and correlation analyses revealed a strong relationship between respiration rate and seed physiological factors. As noted by previous studies (Singh et al., 2016; Singh et al., 2017), seed moisture content is a critical factor in seed deterioration. Elevated water content accelerates respiration, leading to an increased production of CO_2, water, and heat during storage. This heat can damage the protein structure of the seed cell membrane, contributing to seed deterioration. Over time, this damage results in a decline in both germination and seed viability (Purba et al., 2013). These findings indicate that high moisture content not only deteriorates the physiological condition of seeds but also makes them more prone to damage, ultimately reducing their shelf life.

Damage to the cell membrane leads to leakage, allowing essential compounds such as sugars, amino acids, and fats to escape. This increases the exudate content in the seed soaking water. Furthermore, the loss of energy required to support metabolic processes, such as membrane transport and maintaining cell integrity, disrupts cellular metabolism. Consequently, the breakdown of food reserves and synthesis of new compounds are hindered, ultimately diminishing seed viability. Studies by several researchers (Jasmi, 2018; Noviana et al., 2017) have demonstrated that membrane damage results in metabolic leakage.

5.0 Conclusion

This study showed a positive correlation between soybean seed respiration rate and water content, and a negative correlation with seed germination and seed vigor index. Respiration rate serves as an effective indicator of seed quality, reflecting biochemical changes that occur during storage. The results showed that sawdust, as an invigoration medium, outperformed other media in maintaining the physiological quality of seeds. Although this indicates a very low result, this is thought to be because soybean seeds have experienced deterioration, the seed deterioration process occurs gradually and cumulatively due to physiological and biochemical changes in seeds, so that the rate of deterioration or decline in seed quality cannot be inhibited. Further research is needed to investigate other factors that affect respiration and seed quality to achieve more optimal results

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Conflict of Interest

The authors declare no conflicts of interest.

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