Bio-Invigoration of DETAP-1 Soybean (*Glycine max*) Seeds Through PGPR Application

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Abstract. Soybeans are a source of plant-based protein and a staple food ingredient, especially in Indonesia. When stored for extended periods, soybean seeds may experience quality deterioration, which can impact their productivity. An effort to improve the quality of deteriorated soybean seeds is through bio-invigoration treatment. The goal of bio-invigoration is to enhance seed metabolism through imbibition. One method that can be used as bio-invigoration treatment is the application of plant growth-promoting rhizobium (PGPR). This research was conducted from May to July 2024 at the Seed Analysis Laboratory, Politeknik Negeri Lampung. The research was arranged in a Completely Randomized Design (CRD) with two factors, namely concentration (K) and soaking duration (L). For the concentration factor, there were four levels: 0% (K0), 6% (K1), 12% (K2), and 24% (K3), and the soaking duration factor consisted of two levels: 3 hours (L1) and 6 hours (L2). The factors were combined and repeated four (4) times, resulting in 32 experimental units. Data were analyzed using variance analysis at 5% and 1% significance levels, and if significant differences were observed, further testing was conducted using the Least Significant Difference (LSD) test at the 5% level. The results showed that PGPR can improve the viability and vigor of Detap-1 soybean seeds that have experienced deterioration. Treatment with 12% PGPR with a 6-hour soaking duration can increase the seed's germination by approximately 11.33%, from the initial germination rate of 68% to 80%.

Keywords: soybeans, bio-invigoration, PGPR, viability, vigor.

INTRODUCTION

Soybeans are a primary source of plant-based protein and a fundamental ingredient in food, especially in Indonesia. Soybeans are categorized as secondary crops alongside potatoes, black-eyed peas, cassava, taro, mung beans, sorghum, and others [1]. Soybeans are known for their higher protein content compared to other legumes. They are highly versatile, contain protein, source of healthy fats, and can produce various processed products that serve as meat substitutes [2]. According to [3], soybeans function as a primary food source and as livestock feed and raw materials for both large and small-scale industries, including household use.

Soybeans were introduced to Indonesia in the 16th century, beginning in Java and eventually spreading to other islands. [4] stated that soybeans have a high protein content, along with water, fat, fiber, sugar, and other compounds. Despite the many benefits of soybeans, they are classified as orthodox seeds. One of the challenges with orthodox seed storage is that the seeds experience quality degradation if stored for an extended period. This is marked by a decrease in seed viability and vigor during storage [5]. The decline in seed quality is commonly referred to as deterioration, which cannot be avoided or stopped [6]. Soybeans deteriorate quickly when stored at sub-optimal temperatures because their high protein content accelerates moisture absorption.

[7] reported that soybean productivity increased by 15.78%, or 47.58 thousand tons, to 349.09 thousand tons, compared to 301.51 thousand tons in 2022. Soybean crop productivity can be enhanced by using high-quality seeds. High-quality seeds will also improve the effectiveness and efficiency of crop cultivation. Quality seeds have a clear identity, including labels and certificates, along with comprehensive information covering seed viability and purity [8]. The viability and vigor of the seeds can determine seed

quality. According to [9], invigoration treatment is one of the efforts to enhance seed viability and vigor. There are several common invigoration treatments, one of which is bio-invigoration. Bio-invigoration involves soaking seeds in an organic extract with a liquid texture and the addition of rhizobacteria [10]. One of the materials that can be used as a bio-invigoration medium is Plant Growth Promoting Rhizobacteria (PGPR). PGPR, which refers to rhizobacteria that promote plant growth, are microorganisms that have been researched for their ability to help plants obtain nutrients such as iron, nitrogen, and phosphorus. PGPR also prevents pathogen development, provides hormones like cytokinins and auxins, and supplies the enzyme ACC deaminase [11].

This research aimed to (1) determine the viability and vigor of deteriorated Detap-1 soybean seeds treated with PGPR at specific concentrations, (2) assess the viability and vigor of deteriorated Detap-1 soybean seeds about the soaking duration in the PGPR solution, (3) identify whether the response of soybean seed viability and vigor treated with PGPR at certain concentrations is influenced by the soaking duration, and (4) find the combination of treatments that can improve the viability and vigor of deteriorated soybean seeds.

METHODS

This research was conducted at the Seed Analysis Laboratory, Politeknik Negeri Lampung, from May to July 2024The materials used in this research include Detap-1 soybean seeds obtained from the Agricultural Instrument Standardization Agency (BSIP), Plant Growth Promoting Rhizobacteria (PGPR) containing *Bacillus subtilis* at 2.1 x 10^7 CFU/g, *Pseudomonas fluorescens* at 1.2 x 10^7 CFU/g, *Trichoderma harzianum* at 1.2 x 10^7 CFU/g, *Trichoderma sp*. at 1.3 x 10^7 CFU/g, and *Trichoderma viride* at 1.3 x 10^7 CFU/g as the concentration solution, rice straw paper, plastic, small tape, labels, tissue, and distilled water. The equipment used in this research includes scissors, tweezers, a ruler, writing tools, a germinator for seed germination, a grain moisture meter to measure initial moisture content, measuring cylinders, beakers, Petri dishes, trays, a hand sprayer, a stirrer, an analytical balance, and documentation tools.

The experimental design used in this study is a factorial Completely Randomized Design (CRD) consisting of two factors. The first factor is the concentration of the PGPR solution, represented by the letter (K), with four levels: 0% concentration (K0), 6% concentration (K1), 12% concentration (K2), and 24% concentration (K3). The second factor is the soaking duration, represented by the letter (L), with two levels: 3 hours (L1) and 6 hours (L2). Each treatment was replicated 4 times, resulting in a total of 32 experimental units. Each unit underwent a viability test consisting of 50 soybean seeds, requiring a total of 1,600 seeds. The data were then analyzed using Analysis of Variance (ANOVA) at the 5% and 1% significance levels, and if a significant difference was found, the analysis was followed by an LSD test at the 5% level.

This research was conducted in several stages, including seed preparation, seed testing in the laboratory (pre-research), preparation of bio-invigoration solution, and germination tests. The variables observed in this study related to seed viability and vigor improvement include seed germination (%), vigor index (%), abnormal seedlings (%), non-germinating seed (%), maximum growth potential (%), seed germination rate (%), seed germination uniformity (%).

RESULTS AND DISCUSSION

According to the analysis of variance summary in Table 1, the bio-invigoration concentration treatment showed a highly significant effect on the variables of seed germination, vigor index, seed germination rate, and seed germination uniformity. However, it only had a significant effect on the maximum growth potential, abnormal seedlings, and non-germinating seeds. The soaking duration treatment did not significantly affect any of the observed variables. As for the combination of treatments, the interaction between concentration and soaking duration had a significant effect on the seed germination, vigor index, maximum growth potential, and non-germinating seed. However, for the seed germination rate, seed germination uniformity, and abnormal seedlings variables, the combination of treatments did not show a significant effect.

Table 1. Analysis of variance recapitulation on observation variable.

Observation Variable	Concentration (K)	Soaking Duration (L)	Interaction (KxL)
Seed Germination (%)	**	ns	*

Vigor index (%)	**	ns	*
Seed Germination Rate (%/etmal)	**	ns	ns
Seed Germination Uniformity (%)	**	ns	ns
Maximum Growth Potential (%)	*	ns	*
Abnormal Seedling ( ) (%)	*	ns	ns
Non-germinating Seed ( ) (%)	*	ns	*

Note: ns = non-significantly different
* = significantly different (α = 5%)
** = significantly different (α = 1%)

Germination testing is a key variable in determining seed viability. Germination ability serves as a measure of each seed's potential to germinate normally under optimal environmental conditions [12]. Based on Table 2, the LSD test shows that the treatment combinations K0L2 and K0L1 had the lowest values in the seed germination variable. The K0L2 combination had a value of 54.5%, and the K0L1 combination had a value of 57.5%. This is likely because seeds not coated with PGPR (Plant Growth-Promoting Rhizobacteria) tend to deteriorate faster than seeds that are coated, resulting in lower germination in seeds with 0% PGPR concentration. This is consistent with the findings of [13], which showed that seed germinations were higher with PGPR soaking treatments than with 0% PGPR concentration soaking.[10] also reported that soaking without treatment resulted in the lowest seed germination, likely due to the absence of bio-invigoration to accelerate seed germination. [14] further explained that seeds soaked with 0% concentration for 6 hours had the lowest values due to rapid imbibition, which damaged the seed membrane.

Table 1. Effect of PGPR concentration and soaking duration on the seed germination, abnormal seedlings, and non-germinating seed.

No	Treatment Combinations (KxL)	Observation Variabel		
		Seed Germination (%)	Abnormal Seedlings (%)	Non-germinating Seed (%)
1	K0L1	57,50 d	26,00	16,50 bc
2	K0L2	54,50 d	16,00	29,50 a
3	K1L1	67,50 c	14,50	18,00 bc
4	K1L2	72,50 bc	6,00	21,50 ab
5	K2L1	71,50 bc	11,50	17,00 bc
6	K2L2	80,00 a	8,00	12,00 c
7	K3L1	75,00 ab	8,50	16,50 bc
8	K3L2	67,50 c	14,00	18,50 bc
	LSD 5%	7,46	-	7,3

Note: Different letters in the column indicate significant differences

The K2L2 treatment combination did not significantly differ from the K3L1 combination and had the highest seed germination. This is likely due to the increase in soybean seed viability and vigor influenced by soaking time. A concentration of 12% with a 6-hour soaking duration (K2L2) and a concentration of 24% with a 3-hour soaking duration (K3L1) indicate that higher concentrations are influenced by soaking time. Looking at the combination of concentration and soaking time, a 3-hour soaking time had a significant effect at 24% concentration, as this concentration represented the peak treatment in this study. Meanwhile, the 6-hour soaking time had a significant impact at 12% concentration, as this treatment's 6-hour duration was the peak. The seed germination in the K2L2 treatment combination reached 80%. This indicates that soybean seeds treated with a 12% concentration and soaked for 6 hours achieved seed germination that meets laboratory seed quality standards, as stated in the Indonesian Minister of Agriculture's Decree No. 966 of 2022. In this study, the treatment combination of 12% concentration (K2) and 6-hour soaking time (L2) increased seed germination rates by 12%, from an initial rate of 68% to 80%.

Non-germinating seeds are seeds that do not germinate by the end of the testing period. Non-germinating seeds can be classified as either fresh but ungerminated seeds or hard seeds [15]. The treatment combinations for the non-germinating seeds variable show that the K2L2 combination had the lowest value

at 12%, while the K0L2 combination had the highest value at 29.5%. This indicates that the K0L2 combination had a high percentage of dead seeds. This result aligns with the germination rate variable, where the K0L2 combination also had the lowest germination rate. According to [16], several factors that contribute to non-germinating seeds include environmental factors or growing media conditions, especially when the media is too moist. Seeds that do not imbibe water properly can quickly rot or die due to overly moist growing media, further exacerbated by prolonged soaking.

Table 2. Effect of PGPR concentration and soaking duration on the *vigor index and maximum growth potential.*

No	Treatment Combinations (KxL)	Observation Variabel	
		Vigor Index (%)	Maximum Growth Potential (%)
1	K0L1	57,50 c	83,50 ab
2	K0L2	51,00 c	70,50 c
3	K1L1	67,00 b	82,00 ab
4	K1L2	70,50 b	78,50 b
5	K2L1	71,50 b	83,00 ab
6	K2L2	80,00 a	88,00 a
7	K3L1	73,50 ab	83,50 ab
8	K3L2	66,50 b	81,50 ab
	LSD 5%	8,03	7,31

Note: Different letters in the column indicate significant differences

[12] stated that high vigor is characterized by rapid and uniform seed growth. The treatment combinations for the vigor index (VI) variable in Table 3 show that the K0L2 and K0L1 combinations did not significantly differ and had the lowest values, at 51% and 57.5%, respectively. This low vigor index is likely due to seeds not coated with PGPR (Plant Growth-Promoting Rhizobacteria), which could not germinate uniformly during the first count. According to [17], a low vigor index may result from non-uniform seedling growth. The vigor index represents the speed at which seeds grow and serves as an indicator of seed quality [18]. The highest vigor index was found in the K2L2 and K3L1 treatment combinations, with values of 80% and 73.5%. [19] suggested that the difference in vigor index values is due to the highest-performing treatments being more effective in the imbibition process, leading to more optimal seed growth compared to treatments with lower values. [17] noted that if soybean seeds grow uniformly in a short period, their vigor index is considered high. This finding is consistent with the germination rate variable, where the K2L2 treatment combination also showed the highest value compared to soaking without PGPR or soaking with 0% concentration.

According to the LSD test in Table 3, the highest value for the maximum growth potential variable was found in the K2L2 treatment combination, which significantly differed from the K0L2 and K1L2 combinations. Table 3 shows that the K2L2 treatment combination had the highest value at 88%, compared to K0L2, which had the lowest value at 70.5%. This is likely because seeds not coated with PGPR were not able to imbibe water efficiently. This aligns with the findings of [11], which showed that PGPR concentration effectively enhanced seed imbibition, resulting in rapid cell division and increased enzyme activity, leading to larger and more numerous cells. In this study, the maximum growth potential variable was also consistent with the germination rate variable, where the K2L2 combination showed the highest value compared to the K0L2 combination.

Table 3. Effect of PGPR concentration on the seed germination rate, seed germination uniformity, and abnormal seedlings.

No	PGPR Concentration	Seed Germination Rate (%)	Seed Germination Uniformity (%)	Abnormal Seedling (%)
1	K0	11,48 c	41,50 c	21,00 a
2	K1	16,96 ab	62,25 b	10,25 b
3	K2	18,71 a	71,00 a	9,75 b
4	K3	16,64 b	58,50 b	11,25 b
	LSD 5%	1,77	8,37	6,42

Note: Different letters in the column indicate significant differences.

The study by [20] demonstrated that PGPR can produce plant hormones such as auxins, gibberellins, and cytokinins, which enhance germination and speed of germination when compared to soaking without treatment or soaking in water. Based on the results of the LSD test in Table 4, the PGPR concentration for the seed germination rate variable at 12% (K2) did not significantly differ from the 6% (K1) concentration. However, the 12% (K2) concentration significantly differed from the 0% (K0) and 24% (K3) concentrations. The K1 concentration significantly differed from K0 but did not significantly differ from K3. Soaking seeds is aimed at enhancing seed germination rate through the imbibition process ([20]. According to [18], seed germination rate is an important indicator of seed viability and is closely related to vigor. Seeds with high germination rates are more resilient to sub-optimal environments. The 0% (K0) concentration showed the lowest value at 11.48%, likely because seeds without PGPR application did not absorb water optimally, causing slow seedling growth. This is in line with [21], who found that slow imbibition can delay seed germination, as water plays a crucial role in germination. The seed germination rate is influenced by specific treatments applied to seeds, ensuring proper growth. The study by [22] showed that the bacteria *Bacillus sp.* found in PGPR can dissolve phosphate, fix nitrogen, and increase the availability of P, which is essential for supporting seed germination and plant development.

In the variable of seed germination uniformity (Table 4), the 12% (K2) concentration, with a value of 71%, showed the highest value, while the lowest value was shown by the 0% (K0) concentration at 41.50%. The 12% (K2) concentration significantly differed from the 0% (K0), 6% (K1), and 24% (K3) concentrations. The K1 concentration did not significantly differ from the K3 concentration. This similarity between K1 and K3 is likely due to their equal impact on the seed germination uniformity, making them less effective for this variable. According to the study by [21], seed germination uniformity with a value of ≤70% indicates high vigor, suggesting that seeds treated with the 12% (K2) concentration had high vigor. This aligns with the vigor index variable, where seeds treated with the K2 concentration also had high values. Therefore, in this study, the 12% (K2) concentration was the most effective for the sed germination uniformity variable.

The abnormal seedling variable in Table 4 shows that the K0 concentration significantly differed from the K1, K2, and K3 concentrations. The K0 concentration had the highest abnormal seedling value at 21%, indicating that the 0% concentration had the highest number of abnormal seedlings. This study confirms that the application of PGPR to seeds can improve the viability and vigor of seeds that have undergone deterioration. Similar to previous studies on rice seeds, [23], demonstrated that the use of PGPR positively affects seed performance, consistently leading to improved growth results and better viability and vigor in seed invigoration treatments.

CONCLUSIONS

Based on the result of this research, it can be concluded that (1) the viability and vigor of Detap-1 soybean seeds that have deteriorated will increase with the application of PGPR concentrations up to 12%, (2) Soaking durations of 3 and 6 hours exhibit the same response in terms of improving the viability and vigor of Detap-1 soybean seeds that have deteriorated, (3) the improvement in the viability and vigor of deteriorated soybean seeds in response to PGPR concentration is influenced by the soaking duration, and (4) the best treatment combinations for increasing the viability and vigor of deteriorated soybean seeds are a 12% PGPR concentration with a 6-hour soaking duration (K2L2) and a 24% PGPR concentration with a 3-hour soaking duration (K3L1).

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