Rhizobacteria as Biological Agents in Enhancing the Viability and Vigor of Rice Seeds (*Oryza sativa* L.)

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Abstract.

This study aims to examine the effectiveness of rhizobacteria in stimulating plant growth and acting as a biofertilizer that can enhance the viability and vigor of rice (Oryza sativa L.*) seeds. The research was conducted at the Agronomy Unit Laboratory, Faculty of Agriculture, Halu Oleo University, from September to October 2024. The study was designed based on a randomized block design (RBD), consisting of 11 test isolates and 1 control. These isolates were tested for their effectiveness in improving the viability and vigor of rice (Oryza sativa* L.*). The test was carried out by soaking seeds in a rhizobacteria suspension, then germinating the seeds using a standard germination procedure. The observed variables included germination capacity, vigor index, seed uniformity, relative growth rate, and T50. The results showed that seed treatment with rhizobacteria significantly increased the viability and vigor of rice seeds (Oryza sativa* L.*), as seen in the variables of germination capacity, vigor index, T50, and relative growth rate. From this study, 2 isolates were selected that have the potential as growth promoters for rice seeds, namely DMW 4 and DSS 3. Further research is needed to test the effectiveness of these isolates in improving the growth and yield of rice (Oryza sativa* L.*) under field conditions.*

Keywords: *Rhizobacteria, seed viability, vigor index and biofertilizer.*

I. INTRODUCTION

Rice (*Oryza sativa* L.) is a major food commodity and a primary producer of rice, which is cultivated and developed in Indonesia. The demand for rice continues to increase in line with the progress of time, rapid population growth, and the shift in consumption patterns from non-rice to rice [14]. Southeast Sulawesi is one of the rice-producing regions in Indonesia. According to data from the Central Bureau of Statistics, rice production in Southeast Sulawesi in 2023 reached 482,371.05 tons, with a productivity of 4.15 tons per hectare. This productivity figure is still low compared to the national average of 5.26 tons per hectare [2].

The low rice production is influenced by both genetic and physiological quality. Genetic quality is determined by the level of genetic purity, while physiological quality is influenced by seed viability and vigor [18]. Seed quality significantly affects seed viability and vigor, where seeds are able to grow quickly and uniformly in environments with wide variability, resulting in better seedlings [27].

One environmentally friendly solution to improving physiological seed quality (viability and vigor) is by utilizing beneficial microorganisms. Rhizobacteria are microorganisms that naturally associate with host plants, living around the plant roots. Rhizobacteria can contribute directly or indirectly to promoting optimal plant growth through nitrogen fixation, inorganic phosphate solubilization, production of phytohormones, siderophores, and secondary metabolites with antimicrobial activity, which can protect crops from phytopathogens [3], [10]. The production of phytohormones (IAA) by microbes can improve root system performance by increasing the number, length, and surface area, thus helping the plant grow well by enhancing nutrient absorption [4], [9], [22].

The effectiveness of rhizobacteria as growth promoters varies among different isolates in their ability to stimulate plant growth and act as biofertilizers, biostimulants, and bioprotectants. This study aims to examine the effectiveness of rhizobacteria in improving the viability and vigor of rice seeds, such as germination capacity (GP), vigor index (VI), germination uniformity (UG), relative growth rate (RG-R), and time to 50% germination (T50).

II. METHODS

Research Location and Timeframe

This research was conducted at the Agrotechnology Laboratory, Agronomy Unit, Faculty of Agriculture, Halu Oleo University, and was carried out from September to October 2024.

Preparation of Rhizobacteria

The rhizobacteria isolates used were DMK 1, DMK 3, DMK 4, DMW 1, DMW 2, DMW 4, DMW 5, DMMti 1, DSS 3, DSS 7, and DST 7, which were obtained from laboratory selection/testing. These isolates were then cultured on TSA media and incubated for 48 hours in an incubator at room temperature. The colonies that grew were suspended in sterile distilled water until a concentration of 10^9 cfu/ml was achieved.

Seed Treatment with Rhizobacteria

The rice seeds were selected by placing them in distilled water. The seeds that floated were discarded, while the seeds that sank were collected and air-dried on tissue paper for 3 minutes [28]. A total of 10 g of rice seeds were soaked and shaken using a shaker for 12 hours in a suspension (concentration of 10^9 cfu/ml) of each bacterial isolate (20 ml) at 28°C. For the control, the seeds were soaked in sterile water under the same conditions and time. After the treatment, the seeds were air-dried for 30 minutes in a laminar air flow cabinet and were ready for use. The seeds were then planted in a germination tray with rice husk charcoal media, with 25 seeds per treatment unit.

Observation Variables

1. Germination percentage (GP), depicting seed potential viability, was measured based on the percentage of normal seedlings (NS) in the lates observation (7 dap) [17].
2. Vigor index (VI), depicting the growth rate vigor, was measured based on percentage of normal seedlings at the first observation [5].
3. Uniformity of Germination (UG) describes seed vigor [17] and is calculated based on the percentage of normal seedlings (NS) on the days between the first and second counts.
4. Relative Growth Rate (RG-R) is the ratio between the growth rate (RG) and the maximum growth rate (RG max). The maximum growth rate (RG max) is assumed to be 100% when the first count is made, and the normal seedlings (NS) have reached 100%. The relative growth rate is calculated based on the accumulated daily growth rates [17], [29].
5. T50, depicting seed vigor, is the time required to reach 50% of the total emergence of sprouts, observed by counting the number of seeds that germinate each day. T50 describes seed vigor, and is calculated using the formula [17], [23]:

$$T50 = ti + \frac{(n50\% - ni)}{nj - ni}(tj - ti)$$

Notes:

ti = time between, at or before the seeds germinate 50%

tj = time between, after the seeds germinate 50%

n50% = number of germinated seeds (50% of total germinated seeds)

ni = number of seeds germinated at time tj

nj = number of germinated seeds at time ti

Research Design

The research was conducted in the form of an experiment using a Completely Randomized Design (CRD) consisting of 12 treatments: R0 (control), R1 (Isolate DMK1), R2 (Isolate DMK3), R3 (Isolate DMK4), R4 (Isolate DMT4), R5 (Isolate DMW1), R6 (Isolate DMW2), R7 (Isolate DMW4), R8 (Isolate DMW5), R9 (Isolate DSS3), R10 (Isolate DSS7), and R11 (Isolate DST7). Each treatment was repeated 3 times, resulting in 36 experimental units.

IV. RESULT AND DISCUSSION

Germination Rate and Seed Vigor Index

The results of the viability and vigor tests on rice seeds inoculated with rhizobacteria showed a significant impact on two important parameters for seed growth, namely germination rate (GR) and seed vigor index (IV) (Fig. 1). The germination rate of rice seeds treated with rhizobacteria ranged from 86.67% to 93.33%, indicating that the inoculated seeds germinated quickly and uniformly. On the other hand, the seed vigor index, which measures the strength and ability of seeds to grow well after germination, also showed values ranging from 81.33% to 93.33%. The highest values for both parameters were found in the rhizobacterial isolates DMW4 and DSS3, which performed better than the treatments without rhizobacterial inoculation (control). This significant difference suggests that these two isolates have great potential in enhancing the growth capacity and quality of rice seeds. Therefore, the DMW4 and DSS3 isolates can be considered as biological agents that support rice plant growth, accelerate germination, and improve seed resilience to less favorable environmental conditions. The high percentage of germination rate and vigor index is related to the ability of the rhizobacterial isolates to produce growth hormones. The higher the vigor of a seed, the better its ability to germinate, which also positively impacts plant growth [20].

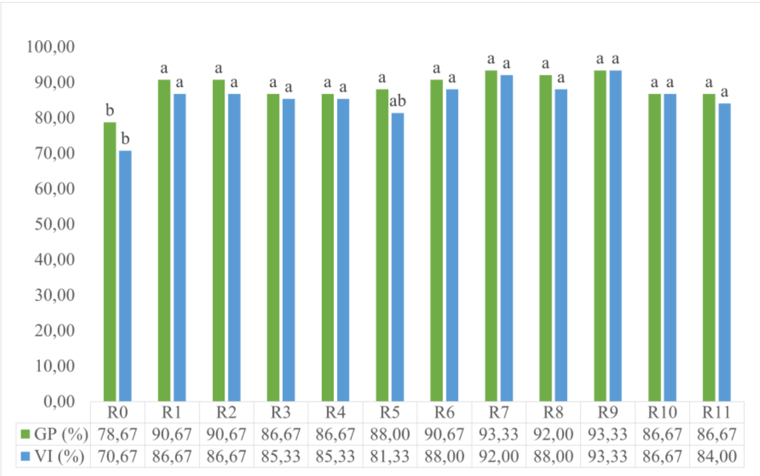

Fig. 1. Germination Rate and Seed Vigor Index of Rice Seeds Treated with Rhizobacteria

The increase in viability and vigor of rice seeds inoculated with rhizobacteria is related to their ability as plant growth promoters that play a role in providing nutrients (biofertilizers) to plants, such as the production of phytohormones, phosphate solubilization, and nitrogen fixation [3], [16]. The use of rhizobacteria as plant growth promoters is known to produce growth hormones such as Gibberellin and Indole-3-Acetic Acid (IAA). These growth hormones play a role in stimulating the formation of new roots, promoting plant growth, and influencing stem elongation [19]. Indole-3-Acetic Acid is the active form of the auxin hormone found in plants and plays a role in enhancing growth and increasing crop yield. The functions of Indole-3-Acetic Acid hormone for plants include promoting cell development, stimulating new root formation, accelerating growth, encouraging flowering, and increasing enzyme activity [11], [26].

Germination Uniformity (GU) and Relative Growth Rate (RGR)

Rhizobacterial treatments had a significant impact on seed germination uniformity, as evidenced by the clear differences in germination rates between the applied treatments. The highest uniformity value was recorded in the R9 treatment (DSS3), with a percentage of 93.33%, indicating that seeds inoculated with this isolate germinated more uniformly and faster. In contrast, the control treatment (R0), which did not receive rhizobacterial inoculation, showed a lower uniformity rate of only 76.00%. Although the rhizobacterial treatment did not significantly affect the relative growth rate of the seeds, the seeds that received rhizobacterial inoculation tended to exhibit a higher relative growth rate compared to those without inoculation. The highest relative growth rate was observed in the R7 treatment (DMW4), with a value of 91.70% per day, suggesting that inoculation with this isolate provided a greater boost to seed development compared to other treatments (Fig. 2). Rhizobacteria have an impact on both germination uniformity and relative growth rate in rice seeds. Through mechanisms such as the production of growth hormones, particularly IAA (Indole Acetic Acid) [8], [19]. Rhizobacteria can stimulate uniform seed germination, thus accelerating the germination process and enhancing germination uniformity.

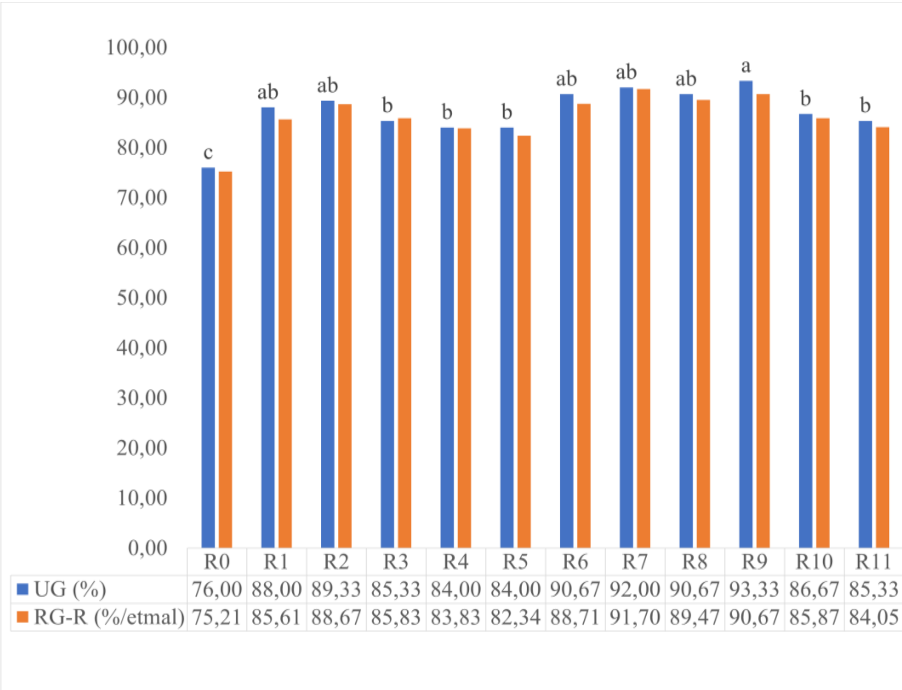

Fig. 2. Germination Uniformity (GU) and Relative Growth Rate (RGR) of Rice Seeds Inoculated with Rhizobacteria

Rhizobacteria have the ability to colonize roots and play an important role in plant growth, contributing 20% to 50% of nitrogen supply through nitrogen fixation (N) [1], [6]. The use of rhizobacteria as a biological agent can enhance plant growth because they act as growth stimulants (biostimulants) by synthesizing and regulating the concentration of various growth regulators. They also help facilitate the availability of essential nutrients and act as soil pathogen controllers (bioprotectants) [12].

Time to 50% Germination (T50)

Rhizobacterial treatment on rice seeds has proven to be effective in accelerating the time required to reach 50% germination (T50). The study showed that rice seeds inoculated with the rhizobacterial isolates DMW4 and DSS3 germinated faster compared to the control, which did not receive rhizobacterial treatment. The inoculated seeds required only about 2.43 days to germinate, which is 0.17 days faster than the control, which took 2.60 days (Fig.3). This increased germination speed is due to the ability of rhizobacteria to stimulate the seed germination process. As biological agents, these bacteria have the potential to influence factors that support germination, such as enhancing enzymatic activity, breaking seed dormancy, and increasing the availability of essential nutrients for early plant growth [13]. The use of rhizobacteria as a seed treatment can enhance or improve seed germination in plants [24], [25].

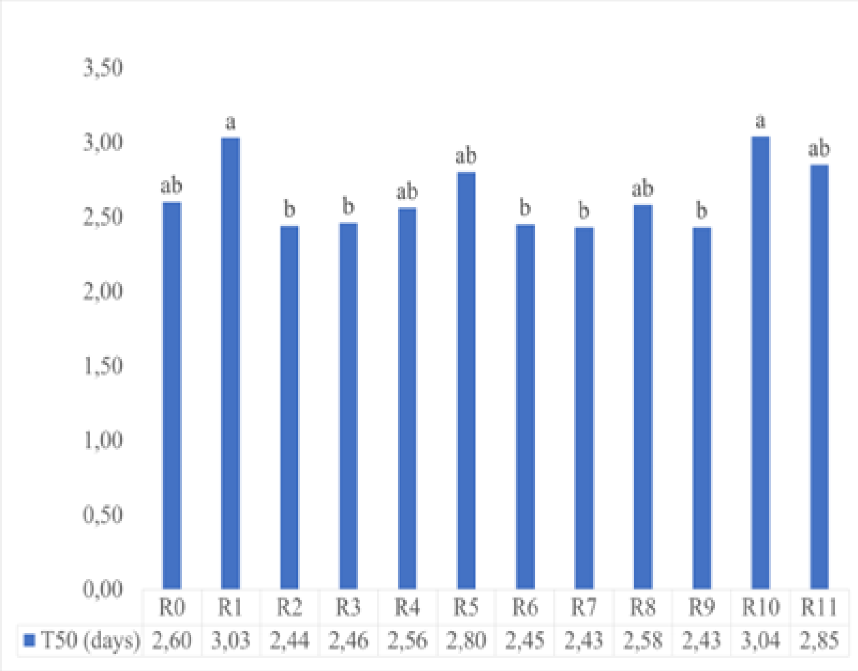

Fig. 3. Time to 50% germination (T50) of rice seeds inoculated with rhizobacteria.

Rhizobacterial activity plays a key role in supplying nutrients to plants, stimulating plant growth by producing Indole Acetic Acid (IAA), and improving soil health through nutrient solubilization and nitrogen fixation [21]. These rhizobacteria also possess genes involved in the biosynthesis of cytokinins. Cytokinins play a role in plant growth by shortening organ formation, aiding in cell division, and promoting cell enlargement [15]. Rhizobacteria can mitigate the detrimental effects of water deficiency on rice growth, leading to improvements in shoot length, the number of tillers, the number of panicles, and dry weight of shoots [7].

The performance of rhizobacterial isolates in synthesizing IAA hormone.

Rice seeds treated with the DMW4 and DSS3 isolates showed better results in terms of seed viability and vigor compared to other treatments. The research findings revealed that these two rhizobacterial isolates are capable of producing the auxin growth hormone in the form of Indole Acetic Acid (IAA).

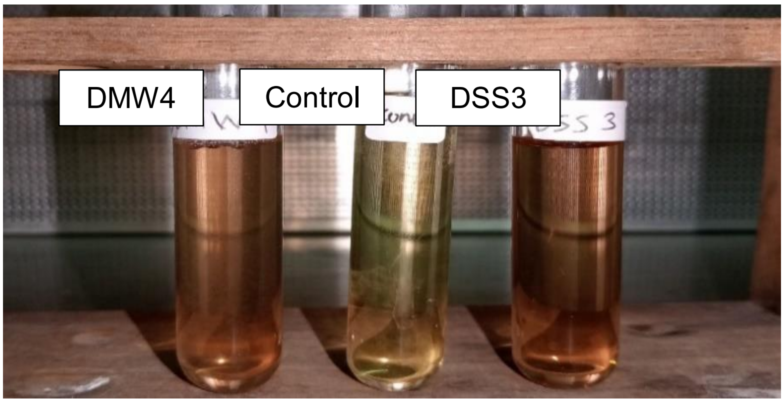

Fig. 4. Performance of rhizobacteria in synthesizing the growth hormone IAA.

The production of IAA is clearly indicated by the color change of the media to red, signifying the presence of this compound (Fig. 4). The presence of IAA has been proven to stimulate the germination process of rice seeds, thus enhancing the germination success rate and improving the growth quality of the treated rice seeds. Germination performance of rice seeds inoculated with rhizobacteria can be seen in Figure 5.

V. CONCLUSION

Seed treatment with rhizobacteria inoculation significantly increased the viability and vigor of rice seeds, as observed in the variables of germination rate, vigor index, relative growth rate, and time to 50% germination (T50). From this study, two isolates were selected with potential as growth promoters for rice seeds, namely DMW4 and DSS3. Further research is needed to test the effectiveness of these isolates in improving the growth and yield of rice (*Oryza sativa* L.) under field conditions.

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