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Effect of Soaking with Plant Growth Promoting Rhizobacteria on Rice Seed Germination

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Abstract. This study aims to determine the effect of immersing rice seeds in Plant Growth Promoting Rhizobacteria (PGPR) suspension. The study used a completely randomized design (CRD) with seed immersion treatment using PGPR bacterial suspension. The rice varieties used were Ciherang, Inpari 32, and IR64, where the seeds were soaked for 30, 45, 60, and 300 minutes, respectively. There were 12 treatment units plus one control by soaking the seeds in water for 60 minutes. All treatments and controls were repeated three times. The results showed that soaking rice seeds for 60 minutes gave longer root and sprout lengths of the three rice varieties. Soaking rice seeds for 30 and 45 minutes caused slower germination, and shorter root length and sprout length than the control. Soaking rice seedlings longer than 60 minutes did not alter germination speed than soaking for 60 minutes.

1 Introduction

Increasing food production, especially rice in Indonesia, is an effort to keep pace with population growth and meet the growing demand for commodities in line with an increase in population. The government needs to pay attention to rice production in Indonesia because any change in rice productivity can directly impact food security. Data shows that in 2020, the rice harvest area in Indonesia reached 10,786,814 hectares with a productivity of 55,160,548 tons of dry milled grain (GKG), an increase compared to 2019, which recorded a rice harvest area of 10,667,887 hectares with a productivity of 54,604,033 tons of GKG [1]. There was an increase of about 1.02% in rice harvested area and rice productivity.

The increase in rice production is of course greatly influenced by increasingly developing environmental conditions, so several factors can reduce production. The causes of low production yields can be caused by several factors, such as low quality and quantity of seedlings, use of local varieties that have low productivity, and lack of management intensity. Therefore, the role of providing superior seedlings is very important [2]. To increase the yield and productivity of rice, many tests are carried out to achieve the best results. This effort

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involves improving seed quality and developing technical culture systems that are continuously updated.

One alternative that can be used to increase rice production is to utilize rhizobacteria, a group of rhizosphere-inhabiting bacteria. This rhizobacteria isolate can stimulate plant growth, or what is known as Plant Growth Promoting Rhizobacteria (PGPR) [3]. These rhizobacteria can be found in the plant rhizosphere, which is the thin layer of soil that covers plant roots and has a positive impact on plant growth [4]. In general, the mechanism of PGPR in increasing plant growth involves the ability of PGPR to produce or change the concentration of plant hormones such as indoleacetic acid (IAA), gibberellic acid, cytokinins, and ethylene, as well as their precursors (1-aminocyclopropene-1-carboxylic deaminase) in plants. PGPR is also not symbiotic in N2 fixation and can dissolve mineral phosphate, affecting root formation [5].

The development of plant growth-promoting root bacteria (PGPR) is currently growing rapidly, especially in efforts to increase food production and improve environmental quality. Rhizobacteria has been applied to various types of plants because it can increase growth, seed germination in the field, and increase crop production. One factor that influences the successful application of this biological agent is the survival of rhizosphere bacteria and their ability to compete with other microorganisms in the field [6].

In previous research, many studies have shown the benefits of using rhizosphere bacteria to suppress the growth of pathogens, including research that used a suspension of *Pseudomonas alcaligenes* and *Bacillus* sp. bacteria to increase the growth and yield of bitter melon [7]. In other studies, it has been shown that the use of bacteria can increase the production and development of tomato plants [8], kale plants [9,10], and mustard plants [11]. other research shows that the utilization of bio-catharanthine did not reduce the germination rate of the Cempo Ireng seeds [12]. This research focused on the observation of the potential effect of PGPRs on seedlings, not throughout the rice growth phase (seedling-vegetative-mature stage).

2 Materials and Methods

2.1 Research Design

This study was designed using a Randomized Block Design (RBD) with seed immersion treatment using a suspension of bacterial isolates of PGPR., where each seed variety was soaked for 30, 45, 60, and 300 minutes respectively. The rice varieties used were Ciherang, Inpari 32, and IR64 varieties. There were 13 treatments including a control, and each treatment had three repetitions. Thus, there were 39 experimental units involved in this study.

2.2 Preparation of Bacterial Isolation of PGPR suspension

Bacteria PGPR Isolated in the Agrotechnology Laboratory, Faculty of Agriculture, Mahasaraswati University, Denpasar. PGPR isolates were grown on NB media and cultured for 24 hours in an Erlenmeyer with a capacity of 100 ml to obtain a colony density of 1.5 x 108 CFU/ml.

2.3 Observation Parameters and Data Analysis

Observation and measurement were conducted to germination time, plant height, and root length. The percentage of seed growth was calculated after the seventh day of observation.

Data were analyzed using SPSS v.17 for Windows. Different tests were performed on average using the Duncan Multiple Rings Test (DMRT) at the 5% level.

3 Results and Discussion

3.1 Germination Time, Seed Height, Root Length, and Seed Growing Percentage with PGPR.

The results of measurements of germination time, seedling height, root length, and percentage of seed growth by immersion treatment of PGPR suspension were presented in Figure 1, Table 1, Table 2, and Table 3

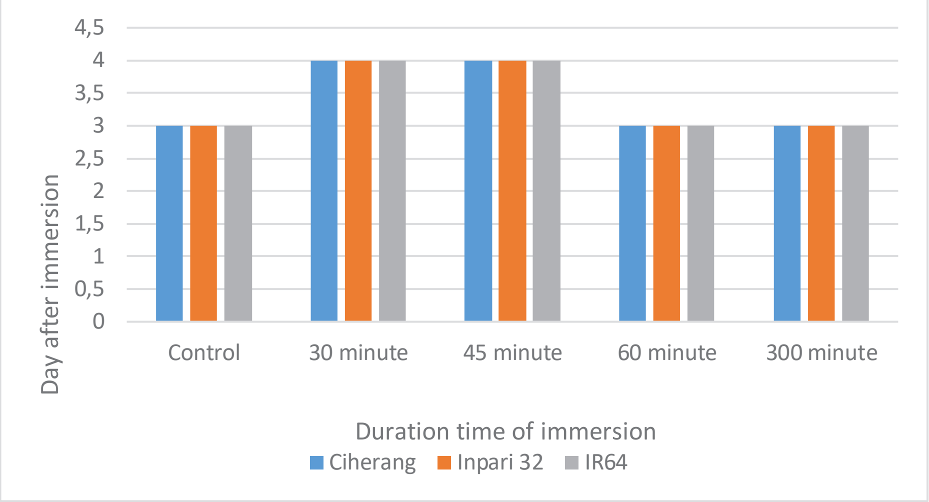

Fig 1. Seed Germination Time with PGPR suspension treatment immersion (day)

This research was conducted to determine the effect of soaking the seeds of several rice varieties with PGPR suspension against germination. In this study, the soaking treatment of several rice varieties was carried out using PGPR suspension with different seed soaking times. The results showed that the immersion treatment for 30 and 45 minutes required a longer growth time and imbibition process than the other treatments. This means that the seedlings experience delays in starting the process of germination and absorption of water.

So far, no research explains that bacteria can slow down the germination process in seedlings. However, several possible mechanisms can be considered in this study, including 1.) Bacteria produce inhibitory compounds that inhibit plant growth or development. These compounds can hinder the germination process or cause slower germination [13]. 2.) Certain bacteria may damage the nutrients contained in the seeds, such as sugar or vitamins, which are needed for plant growth. By destroying these nutrients, bacteria can slow down the germination process [14]. Some bacteria can become pathogens that cause disease in plants. These pathogenic infections can interfere with the development and growth of seedlings, thereby slowing down the germination process; and 4.) Bacteria cause disturbances in the hormonal system [15]. Bacteria can also interact with the plant's hormonal system and change the balance of hormones, which in turn affects the process of germinating. The following are just a few potential examples and need further research to understand how bacteria can affect plant germination. The results of observations on the average height of rice seedlings by immersion treatment of PGPR suspension can be seen in Table 1.

Table 1. Seedling height with PGPR suspension treatment immersion (cm)

Immersion treatment	Rice Varieties		
	Ciherang Variety	Inpari32 Variety	IR64 Variety
Control	6,53^{c}	7,79^{c}	6,30^{d}
30 minute	2,73^{a}	3,12^{a}	2,56^{a}
45 minute	3,98^{ab}	3,17^{ab}	2,88^{ab}
60 minute	6,93^{c}	8,20^{c}	6,55^{d}
300 minute	6,45^{c}	6,46^{c}	5,35^{c}

The results of observations on the average height of the seedlings of the Ciherang variety showed that the 30-minute and 45-minute immersion treatments gave very significantly different results from the control. Likewise, the observation of the average height of the seedlings of the Impari 32 variety showed that the 30-minute and 45-minute immersion treatments gave very significantly different results from the control. In contrast to the Ciherang and Inpari 32 varieties, the observation of the average seed height of the IR64 variety showed that each treatment gave different results to the control. The results of observations on the average root length of rice with the immersion treatment of PGPR suspension can be seen in Table 2.

Table 2. Root Length of Seedling with PGPR suspension treatment immersion (cm)

Immersion treatment	Rice Varieties		
	Ciherang Variety	Inpari32 Variety	IR64 Variety
Control	8,53^{bc}	12,04^{c}	7,64 ^{a}
30 minute	6,17^{a}	7,38^{a}	5,59 ^{a}
45 minute	7,78^{b}	8,22^{ab}	7,48 ^{a}
60 minute	9,02^{bc}	12,76^{c}	8,08 ^{a}
300 minute	8,19^{bc}	11,81^{c}	7,32 ^{a}

The results of observations on the average root length of several rice varieties with various immersion treatments with PGPR suspension showed different results. From the observation of the average root length of the rice seedlings in the Ciherang and Inpari 32 varieties, the results were significantly different from the control treatment, but the statements of the average root length of the rice seedlings in the IR64 variety gave results that were not substantially different from the control treatment. The results of observations on the percentage of rice seed growth by immersion treatment of PGPR suspension can be seen in Table 3.

Table 3. Percentage of Seed Growth by PGPR suspension treatment immersion (%)

Immersion treatment	Rice Varieties		
	Ciherang Variety	Inpari 32 Variety	IR64 Variety
Control	92	92	91
30 minute	87	82	85
45 minute	87	84	83
60 minute	87	82	88
300 minute	89	84	85

Based on the results of observations of the growth percentage of several varieties of rice seedlings treated with several soaking times, it showed that the control treatment gave a higher growth percentage compared to all other treatments. In this study, several varieties of rice seedlings were soaked with PGPR suspension. At different times as different treatments. The results showed that the group of grains soaked in water as a control showed a better percentage of growth than those soaked in the immersion treatment with PGPR suspension. This control treatment showed that immersion with PGPR suspension can reduce the growing percentage of all rice varieties.

So far, no research explains that bacteria can slow down the growth process of seed stems and roots. However, bacteria can interact with plants and influence their growth, for example by producing phytohormones and inhibitory compounds. Some bacteria produce compounds that function as phytohormones, such as auxins, cytokinins, or gibberellins. These phytohormones can affect plant growth and, in some cases, can shorten or stimulate plant growth, depending on the type and amount. Certain bacteria can also produce compounds that inhibit plant growth. These compounds can interfere with the process of cell division, cell elongation, or root development, which in turn can affect plant height [16].

The results showed that the soaking treatment of rice seeds with PGPR suspension had a significant effect on all treatments, especially in the treatment of immersion time of 30 minutes and 45 minutes. Soaking seeds with PGPR suspension significant effect on germination time, seed height, root length, and growing percentage of several rice varieties.

3.2. Growth Rate of Rice Seedlings

The daily growth rate of rice seedlings is an important parameter that describes how far the seedlings grow each day. This parameter was used to monitor and evaluate the growth rate of rice seedlings over a certain period. The daily growth rate of rice seedlings is measured by observing changes in size from day to day [17]. In this study, rice seedlings were measured by measuring the seedling epicotyl every day. The seed growth rate was measured by subtracting the seedling epicotyl today from the previous day and then dividing it by the period. The daily growth rate of rice seedlings can vary depending on various factors, including the rice variety used, environmental conditions, and treatments, such as fertilization, irrigation, and other protective treatments [18].

Figures 2, 3, and 4 show the average growth of rice seedlings from several varieties with different soaking times. The graph (Figure 2-4) shows that the 30-minute soaking time treatment had the lowest seed height, and the 60-minute treatment had the highest seed height in each rice variety. The daily growth rate of rice seedlings is presented in Table 4 below.

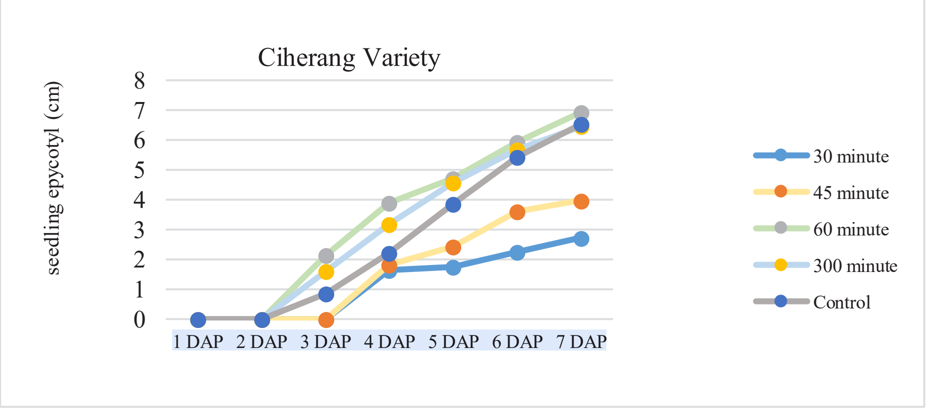

Fig 2. Graph of the growth rate of the Ciherang rice variety (DAP: day after planting)

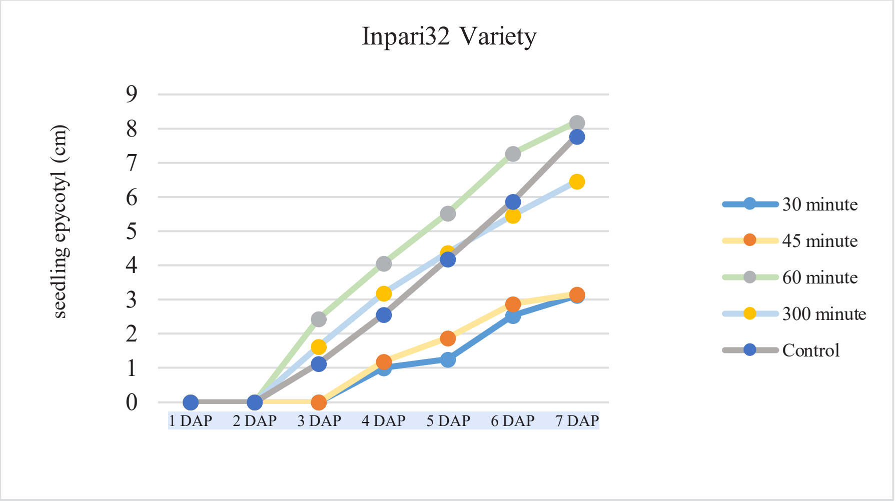

Fig 3. Graph of the growth rate of the Inpari 32 rice variety (DAP: day after planting)

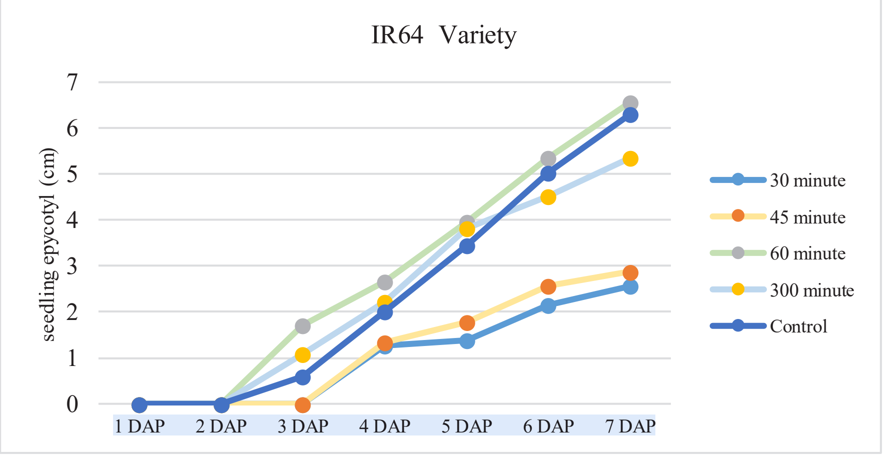

Fig 4. Graph of the growth rate of rice variety IR64 (DAP: day after planting)

Table 4. Average Daily Seed Growth Rate of Several Rice Varieties

Immersion treatment	Average Daily Growth Rate of Rice Seedlings (cm/day)		
	Ciherang Variety	Inpari 32 Variety	IR64 Variety
Control	0,93	1,11	0,90
30 minute	0,39	0,45	0,37
45 minute	0,57	0,45	0,41
60 minute	0,99	1,17	0,94
300 minute	0,92	0,92	0,76

From the results of the analysis of the daily growth rate of rice seedlings, it was shown that the 60-minute soaking treatment provided the highest growth rate for each rice variety. In the 30-minute soaking time treatment, the growth rate was slower than the control treatment. This shows that the immersion treatment with PGPR suspension gave different results in each treatment and variety. From the table above, it can be concluded that the immersion treatment with a time of 60 minutes is the best treatment in this study.

4 Conclusions

Germination of several varieties of rice plants was shown to be affected by immersion treatment with PGPR suspension. The immersion treatment for 30 and 45 minutes required a longer growth time and imbibition process than the other treatments. This means that the seedlings experienced delays in starting the process of germination and water absorption. The results showed that the treatment of soaking rice seedlings with PGPR suspension had a significant effect on all treatments, especially in the treatment of immersion time of 30 minutes and 45 minutes. Soaking seedlings with PGPR suspension also had a significant effect on seed height, root length, growth percentage, and growth rate of seedling**s**.

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