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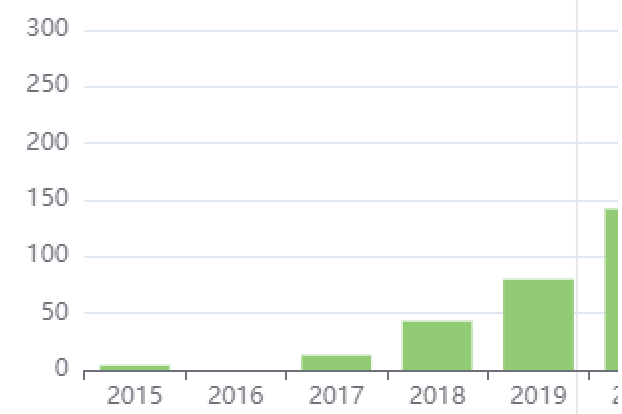

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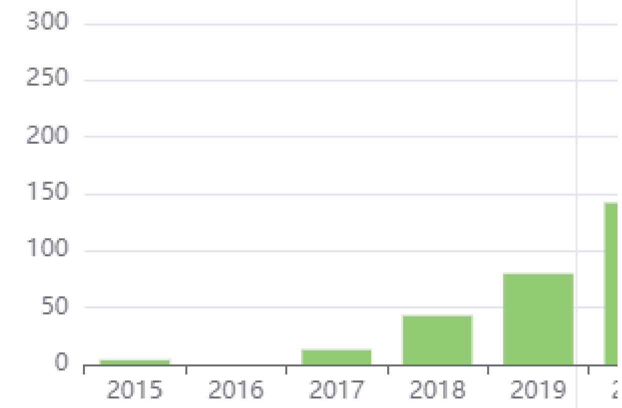

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Seed Bio-Priming to Enhance Seed Germination and Seed Vigor of Rice Using Rhizobacteria from The Northern Coast of Pemalang, Central Java, Indonesia

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ABSTRACT

The growth and yield of plants are strongly influenced by the early growth ability of the plants. Similar germination and good seed vigor will greatly support plant growth and increase production. Increasing the germination and vigor of seeds can be done through biopriming. The application of biopriming using rhizobacteria is developing environmentally friendly agricultural technology. This study aimed to determine the effect of inoculation of rhizobacteria from the north coast of Pemalang on rice plants' germination and vigor index. The study was arranged in a Randomized Block Design, consisting of 10 treatments with three replications. Ten rhizobacteria isolates were isolated from the North Coast of Pemalang, Central Java, consisting of Ju1, Jn3, Jn1, J, J12, J5, Kn1, A3, Jn, and K3. The biopriming with rhizobacteria isolated from the rice rhizosphere of the Northern Coast of Pemalang increased the seed germination rate, seed vigor index, and early vegetative growth of rice seedlings. Inoculation with isolate J12 produced the highest vigor index of 8280.01. The results of this study imply that the application of rhizobacteria from saline soil has the potential to increase the vigor of rice seedlings to impact better seedling growth in saline conditions.

Keywords: Biopriming, Germination, Rhizobacteria, Rice, Vigor

ABSTRAK

Pertumbuhan dan hasil tanaman sangat dipengaruhi oleh kemampuan tumbuh awal tanaman. Daya kecambah yang seragam dan vigor benih yang baik sangat mendukung untuk dapat tumbuh dengan baik dan mendukung peningkatan produksi. Upaya peningkatan daya kecambah dan vigor benih dapat dilakukan dengan perlakuan biopriming. Penerapan biopriming menggunakan rhizobakteri merupakan pengembangan teknologi pertanian yang ramah lingkungan. Penelitian ini bertujuan untuk menguji pengaruh inokulasi rhizobakteri dari tanah salin di pantai utara Pemalang terhadap daya berkecambah dan indeks vigor tanaman padi. Penelitian disusun menggunakan Rancangan Acak Kelompok, dengan tiga ulangan. Sebagai perlakuan, 10 isolat rhizobakteri diisolasi dari Pantai Utara Pemalang Jawa Tengah yakni Ju1, Jn3, Jn1, J, J12, J5, Kn1, A3, Jn, dan K3. Perlakuan biopriming dengan isolat rhizobakteri yang berasal dari rizosfer padi asal Pantai Utara Pemalang mampu meningkatkan kecepatan perkecambahan benih, indeks vigor benih dan pertumbuhan vegetatif awal benih padi. Inokulasi dengan isolat J12 mampu menghasilkan indeks vigor tertinggi sebesar 8280,01. Implikasi dari hasil penelitian ini adalah bahwa aplikasi rhizobcateri yang berasal dari lahan salin berpotensi untuk meningkatkan vigor bibit tanaman padi sehingga akan memberikan dampak terhadap pertumbuhan bibit yang lebih baik pada kondisi saline.

Kata kunci: *Biopriming, Perkecambahan, Rhizobakteria, Padi, Vigor*

INTRODUCTION

Rice is the staple food of the Indonesian people, and the consumption pattern of the people in urban areas is almost the same as that in rural areas (Saliem et al., 2019). On the other hand, Indonesian rice consumption has been quite high since 1996. However, there is a downward trend wherein 2020, and it has reached 78.42 kg per capita per year (Anggraeni, 2020). The trend of decreasing rice consumption is a positive thing. Nevertheless, national rice production must continue to be increased in terms of quality and food safety (Saliem et al., 2019; Anggraeni, 2020).

Increased agricultural production is strongly influenced by the interaction between environmental genetics and plant management. Good plant growth will start with good quality plant seeds in

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terms of seed germination and seed vigor (Ayalew et al., 2018). Seeds that can germinate quickly and have uniform seedling growth are essential in crop production (Hélnia et al., 2021). Seed vigor is a very important index of seed quality. It is a physiological marker of commercial seed lots mostly those with similar germination percentages, aiming to identify lots with a higher probability of performing well after sowing and/or during storage (Wen et al., 2018). Hao et al., (2020) stated that high seed vigor would determine the potential for rapid and uniform seed emergence and increase yields by up to 20 percent.

Various studies have reported that priming treatment was able to increase germination and seed vigor by using various materials, such as Polyethylene glycol, Calcium chloride, Calcium aluminum silicate, gibberellic acid (GA), salicylic acid, citric acid (CA), sodium chloride (NaCl), potassium chloride (KCl), zinc (Zn) and iron (Fe) (Nouri & Haddioui, 2021). The development of environmentally friendly priming technology is urgently needed. The use of beneficial microorganisms to increase seedling vigor is environmentally friendly, which positively affects plants and the soil environment. Beneficial microorganisms, such as Plant Growth Promotion Rhizobacteria (PGPR), have an important role in stimulating plant growth through N2 fixing mechanisms, suppressing ethylene levels, induction of resistance to pathogens, solubilizing nutrient, production of siderophores, and phytohormones (dos Santo et al., 2020). Bacterial inoculation methods to promote plant growth have been developed, among others, through seed coating, foliar application, direct application through the soil, and seed priming, by immersing the seeds in a bacterial suspension before the physiological process of the seed begins in the seed. At the same time, the radicle and plumule emergence is prevented (Mahmood et al., 2016). Madyasari et al., (2017) reported that seed priming using rhizobacteria increased the vigor of chili seeds after being stored for 24 weeks. Furthermore, Roslan et al., (2020) reported that *Enterobacter* spp. increased the vigor index 19.6% higher than without *Enterobacter* spp. inoculation promotes the initial vegetative growth of okra plants and increases leaf area and greenness.

Various researchers have reported that beneficial microorganisms can be utilized to increase the vigor index of seeds. *Pseudomonas fluorecence*to could increase the germination and vigor of the East Indian Sandalwood (*Santalum album* L) (Chitra & Jijeesh, 2021), Enterobacter spp. could increase the vigor of okra seeds (Roslan et al., 2020), and *Azospirillum, Azotobacter, and Bacillus* could increase the germination and vigor index of sorghum plants (Widawati & Suliasih, 2018).This condition opens up opportunities for using rhizobacteria originating from a saline environment to stimulate germination and early vegetative growth of rice plants. Saline soils in Indonesia are still very large, reaching 12,020 million ha or 6.20% of the total land area of Indonesia, and 9 million ha is potential for rice cultivation (Karolinoerita & Yusuf, 2020). Several PGPR isolates isolated from the rhizosphere of rice plants in saline rice fields can produce growth regulators of the auxin group and fix N. These isolates have the potential to stimulate growth, and in saline conditions, are expected to increase the vigor of rice seedlings. The effectiveness of Rhizobacteria derived from saline soils needs to be tested to determine their potential to improve the vigor index and early vegetative growth of rice plants. This study aimed to examine the effects of rhizobacteria inoculation from saline soils on the Northern Coast of Pemalang on the germination and vigor index of rice plants.

MATERIALS AND METHODS

The Seed Material

The rice seed used in this study was Inpari Unsoed 79 Agritan Rice Variety collection from the Laboratory of Plant Breeding and Biotechnology, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. The Inpari Unsoed 79 Agritan variety is a rice variety that is resistant to salinity stress.

Bacterial Culture Preparation

A total of 10 rhizobacteria isolates were prepared by cultivating them in a Nutrient Broth (Himedia) media. A total of 1 ose of bacterial colonies were inoculated on 250 ml of Nutrient Broth media, then incubated with a shaker at a speed of 120 rpm for 24 hours at room temperature to reach a population density of 10^7 CFU/mg.

Bacterial Inoculation

Each treatment consisted of 100 grains of rice seeds. Before being inoculated, the rice seeds were sterilized using sodium hypochlorite 0.02% for two minutes (Widawati & Suliasih, 2018) and washed with sterile distilled water three times. Sterile rice seeds were put in a petridish and then soaked in 20 ml of bacterial culture for 30 minutes. The inoculated rice seeds were then planted in a seed box with sterile sand media and maintained in a greenhouse until the age of 25 days after planting.

Experimental Design

The research was carried out in the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Jenderal Sudirman University, Purwokerto, Central Java, Indonesia. The study was conducted for two months, starting from September to October 2021. The study was arranged using a Randomized Block Design, consisting of 10 treatments with three replications. As treatments, 10 rhizobacteria were isolated from the North Coast of Pemalang, Central Java, consisting of Ju1, Jn3, Jn1, J, J12, J5, Kn1, A3, Jn, and K3.

Observed Variables

The seeds were planted in trays containing sterile sand, with each treatment comprising of 100 seeds. Germinated seeds were recorded every time they germinated from the total number of seeds sown. Based on the germination data, the percentage of germination was calculated according to the formula of Polaiah et al., (2020), and the germination rate was calculated by the formula of Chitra & Jijeesh, (2021) as follows :

Germination (%) =

$$\frac{\text{Number of seeds that germinated}}{\text{Total number of seeds}} x100\% \qquad (1)$$

Germination rate =

$$\frac{G1}{T1} + \frac{G2}{T2} + \frac{G3}{T3} + \ldots\ldots + \frac{Gn}{Tn} \qquad (2)$$

Remarks: G1, G2, G3, and Gn are % seeds germinate at T1, T2, T3, and Tn, respectively, and T1, T2, T3, and Tn are the first, second, third, and n day counting from sowing, respectively.

Variables of early vegetative growth of rice seedlings included plant height (cm), total root length measured by the intersection method (Bohm, 1979), leaf greenness measured by chlorophyll meter (Konica Minolta Chlorophyll Meter SPAD-502Plus), and biomass. The seed Vigor index was calculated based on the following formula:

Seed Vigor Index =

(shoot length + root length) x germination (%) (3)

Statistical analysis

The data obtained from this study were analyzed by ANOVA using SAS 9.1 software followed by DMRT at α=5%.

RESULTS AND DISCUSSION

Seed germination and germination rate

The observations found that the biopriming of rice seeds with various rhizobacteria isolates did not show any effect on rice seed germination. The percentage of rice seed germination was still high, ranging from 93.33% to 100.00 percent (Table 1). The high percentage of germination in all treatments was caused by the condition of the seeds where the seeds used were rice seeds that had just been harvested for about two months so that the seeds were still in good condition and had not deteriorated. The germination rate showed the impact of biopriming ($p<0.05$). The germination rate of rice seeds in different biopriming treatments varied between 32.89 – 24.99. The highest germination rate was obtained in the treatment of rhizobacteria of J5 isolate, while the lowest germination rate was obtained in isolate K3 (Table 1). The germination rate in treatment J5 isolate was not significantly different from that in control, J12, J, Ju1, and Jn (Table 1). Germination rate indicates the speed at which sprouts appear, and the ability of sprouts to emerge is strongly related to the energy for germination.

The results of this study indicated that biopriming with rhizobacteria could enhance seed vigor and early vegetative growth of rice seedlings. Biopriming treatment did not significantly affect the seed germination percentage, which was seen from the percentage of germination showing an insignificant difference between control and other treatments, ranging from 93.33 percent to 100 percent. This illustrates that the physiological quality of the seeds is still good. These results are in line with the results of Madyasari et al., (2017), where the seed biopriming treatment did not significantly affect seed germination because each seed had high vigor. The seed germination rate in this study showed a higher increase in the J5 isolate treatment of 32.89 seeds/day (Table 1). The increase in seed germination rate in the rhizobacteria inoculation treatment is closely related to the presence of plant growth substances that are capable of being synthesized by bacteria from the auxin, cytokinin and gibberellin groups, which trigger the activity of specific enzymes that promote faster germination, such as -amylase which helps starch assimilation (Nezarat & Gholami, 2009). Starch assimilation in the seed germination process will also increase the energy available for the germination process, which will cause an increase in the germination rate (Chitra & Jijeesh, 2021). According to Mitra et al., (2021), living microorganisms have different multifunctional capabilities, such as the production of plant growth regulators like auxins, cytokines, abscisic acid and gibberellins, which are produced as secretions of effector molecules and secondary metabolites through modulation of various pathways, which are the most suitable for the biopriming method. Murunde & Wainwright (2018) reported that biopriming treatment using *Bacillus subtilis* and *Serratia nematodiphila* increased the germination of onion seeds.

Seedling growth and biomass

Seed priming treatment in this study positively affected seedling growth and biomass. Seed biopriming with rhizobacteria had a significant effect on the variables of plant height ($p=0.0340$), root length ($p=0.0191$), leaf greenness ($p=0.0030$), and plant biomass variables. The treatment of rhizobacteria inoculation strongly influenced the root length of rice seedlings. Overall total root length increased by 83.41 percent compared to the control. The inoculation treatment of Kn1 isolate reached the highest plant height much higher than the control, although inoculation treatments of rhizobacteria isolates were not significantly different (Table 2). Biopriming treatment using rhizobacteria was able to increase plant height by 17.61 percent.

Table 1. The effect of rhizobacteria inoculation on seeds germination and germination rate

Treatments	Germination (%)	Germination Rate (germination/day)
Control	98.67 a	31.09 ab
Ju1	100.00 a	29.91 abc
Jn3	99.00 a	27.94 bcd
Jn1	98.67 a	28.67 bc
J	97.00 a	29.48 abc
J12	98.33 a	30.67 ab
J5	97.67 a	32.89 a
Kn1	98.33 a	28.78 bc
A3	97.67 a	26.48 cd
Jn	100.00 a	29.28abc
K3	93.33 a	24.99 d

Remarks: Means followed by same letters in the same column are not significantly different according to DMRT 5%.

Table 2. The effect of rhizobacteria inoculation on vegetative growth of rice seedling

Treatments	Plant Height (cm)	Roots Length (cm)	Leaf Greennes (SPAD unit)	Biomass (mg)
Control	24.72 b	26.66 b	17.84 c	32.67 c
Ju1	29.15 a	47.76 a	19.38 bc	44.67 a
Jn3	30.03 a	43.23 a	23.59 a	46.67 a
Jn1	28.85 a	42.72 a	19.27 bc	44.67 a
J	29.25 a	47.66 a	18.17 c	42.67 ab
J12	29.58 a	54.60 a	19.63 bc	42.67 ab
J5	29.24 a	51.67 a	19.73 bc	44.00 ab
Kn1	29.85 a	49.51 a	20.97 b	41.33 ab
A3	29.65 a	52.91 a	20.08 bc	36.67 bc
Jn	26.85 ab	51.46 a	20.34 bc	47.33 a
K3	28.28 a	47.45 a	19.55 bc	42.67 ab

Remarks: Means followed by same letters in the same column are not significantly different according to DMRT 5%.

The results indicated an increase in the greenness of the leaves. The greenness of the leaves reflects the total chlorophyll content in the plant leaves. The biopriming treatment with rhizobacteria isolates had a significant effect ($p=0.0030$) on increasing the greenness of the leaves, with an average value of 20.07 units.

The effect of biopriming treatment is clearly visible in the variable biomass of rice seedlings. Plant biomass in the biopriming treatment, on average, was able to produce biomass of 43.33 mg, which was greater than the control. The highest biomass of rice seedlings was achieved in the inoculation treatment of Jn isolate (Table 2). It can be seen that all rhizobacteria isolates were able to increase biomass production by 32.64 percent.

In general, biopriming treatment using rhizobacteria isolates increased the growth of rice seedlings. The application of rhizobacteria enhanced vegetative growth, which was triggered by the ability of rhizobacteria to produce auxins, especially indole acetic acid (IAA) (Chitra & Jijeesh, 2021; Chauhan et al., 2021). The ability of rhizobacteria to produce IAA will stimulate root elongation so that the

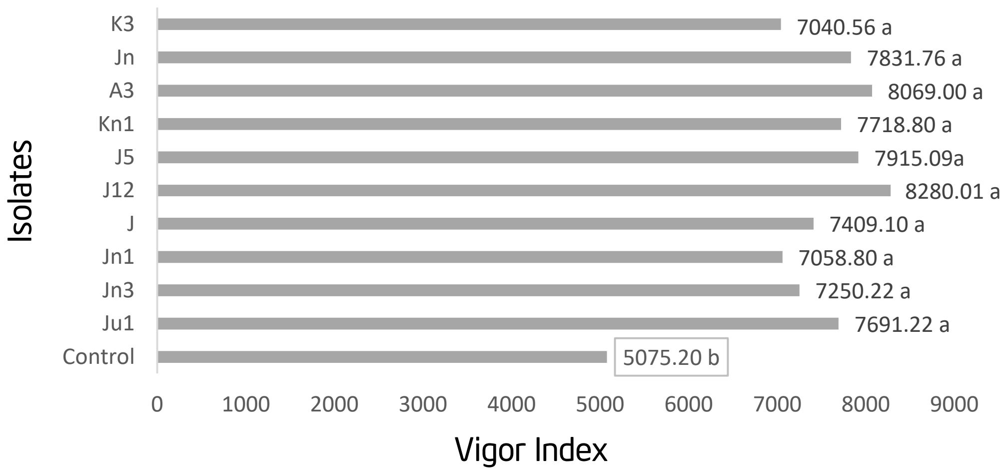

Figure 1. The effect of rhizobacteria isolates on seed vigor index

Table 3. Pearson Correlation Coefficient

	Plant Height	Germination	Root Length	Seed Vigor	Germination Rate	Leaf greenness	Biomass
Plant Height	1.00000						
Germination	-0.02904	1.00000					
Root Length	0.68563*	-0.17992	1.00000				
Seed Vigor	0.76862*	0.12582	0.94482*	1.0000			
Germination Rate	-0.18291	0.51039*	-0.20473	-0.06713	1.0000		
Leaf Greenness	0.22703	0.16298	0.00342	0.09224	-0.01063	1.0000	
Biomass	0.383882*	0.01838	0.43825*	0.45448*	-0.07033	0.29876	1.00000

root surface area that interacts with soil colloids increases and results in increased nutrient and water uptake (Purwanto et al., 2017; Purwanto et al., 2019; Rahma et al., 2019). Rahma et al., (2019) stated that the increase in root growth through the expansion of the root system was stimulated by hormones, thereby increasing nutrient uptake caused by the ability of rhizobacteria to dissolve nutrients such as P. Rhizobacteria can increase the availability of nutrients in the soil (N,P, K) so that nutrient uptake (N, P, K) increases, thereby increasing photosynthetic pigment and activity (Chauhan et al., 2021). Inoculation of rhizobacteria isolates can increase plant height and root length of rice seedlings through the ability to provide and mobilize the absorption of various nutrients in the soil through the ability to enhance capacity in synthesizing and modifying the concentration of numerous phytohormones, dissolving P elements, and producing the Indole Acetic Acid hormone (Rahma et al., 2019). The results of this study also showed that the biopriming treatment with rhizobacteria isolates was able to increase the biomass of rice seedlings. This result is in line with Moeinzadeh et al., (2010), stating that biopriming of sunflower seeds with *Pseudomonas fluorescens* significantly improved the growth of seedling height, root length, and biomass compared to control.

Seed vigor

The effect of biopriming rice seeds with rhizobacteria isolates was significant on the vigor of the seeds. The variance analysis showed that the rhizobacteria isolates' treatment significantly affected rice seedlings' vigor (p=0.0182). The observations found that the highest seed vigor was achieved in the J12 isolate treatment, and the lowest was in control (Figure 1).

Biopriming of rice seeds with rhizobacteria isolates significantly increased the vigor index. It can be seen that in all rhizobacteria isolate treatments, and the vigor index value increased compared to the treatment without biopriming (control). Seed biopriming increased rice seed vigor by 50.27 percent compared to control. The highest vigor index was achieved in biopriming with J12 isolate, where the vigor index value increased by 63.15 percent compared to the control. The germination percentage influences the increase in the vigor index. Still, it is also strongly influenced by the initial growth of rice seedlings, especially root growth and plant height. The results showed a significant correlation between plant height and vigor index (r=0.76862), as well as between root length and the vigor index variable (r=0.94482) (Table 3). The effect of biopriming on seed vigor index is induced by the ability of rhizobacteria to synthesize cytokines. This hormone stimulates cell division, and the effect of auxin as a hormone stimulates cell elongation (Agbodjato et al., 2016). Roslan et al., (2020) reported that inoculation of okra seeds with *Enterobacter* sp. increased the initial growth of okra seedlings compared to inoculation based on hypocotyl length, radicle, number of lateral roots and vigor index.

CONCLUSIONS

In general, it can be concluded that the biopriming treatment with rhizobacteria isolates derived from the rice rhizosphere from the Northern Coast of Pemalang increased the seed germination rate, seed vigor index, and early vegetative growth of rice seedlings. Inoculation with J12 isolates produced a higher vigor index than the control but was not significantly different from other isolates. The implication of the results of this study is that the application of rhizobacteria from saline soil has the potential to increase the vigor of rice seedlings so that it will have an impact on better seedling growth in saline conditions.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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