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Seed Bio-Priming to Enhance Seed Germination and Seed Vigor of Rice ~~by~~ Using Rhizobacteria Origin 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 ability to grow early plants. Uniform germination and good seed vigor are very supportive to be able to grow well and support the increase in production. Efforts to increase seed germination and seed vigor can be done with biopriming treatment. The application of biopriming using rhizobacteria is the development of environmentally friendly agricultural technology. This study to examine the effect of rhizobacteria inoculation from saline soils on the North coast of Pemalang on germination and vigor index of rice plants. The study was arranged using a Randomized Block Design, with three replications. As treatment, 10 isolates of rhizobacteria were isolated from the North Coast of Pemalang, Central Java, which consisted of 10 isolates namely Ju1, Jn3, Jn1, J, J12, J5, Kn1, A3, Jn, and K3. The biopriming treatment with rhizobacteria isolates derived from the rice rhizosphere origin from The Northern Coast of Pemalang was able to increase the rate of seed germination, seed vigor index and early vegetative growth of rice seedlings. Inoculation with isolate J12 was able to produce the highest vigor index of 8280.01.

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Key words : biopriming, rhizobacteria, germination, vigor, rice

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.

Kata kunci : biopriming, rhizobakteria, perkecambahan, vigor, padi

INTRODUCTION

Rice is the staple food of the Indonesian people and the consumption pattern of the people is almost the same between urban and rural areas (Saliem et al., 2019). But on the other hand, the Indonesian population's per capita consumption of rice has been quite high since 1996; however, there is a downward trend wherein 2020 it has reached 78.42 kg per capita per year (Anggraeni, 2020). The trend of decreasing rice consumption is a positive thing; however, national rice production must continue to be increased, both 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 terms of seed germination and seed vigor (Ayalew, 2018). Seeds that are able to germinate quickly and have uniform seedling growth are very essential attributes in crop production (Hélnia et al., 2021). Seed vigor is a very important index of seed quality and is a physiological marker of commercial seed lots mostly those with similar germination percentages, aiming to identify lots with higher probability to perform 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 would increase yields higher 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 a technology that is environmentally friendly and has a positive effect on plants and the soil environment. Beneficial microorganisms such as Plant Growth Promotion Rhizobacteria (PGPR) have an important role in stimulating plant growth through N_2 fixing mechanisms, suppressing ethylene levels, induction of resistance to pathogens, solubilizing nutrient, production of siderophores, and phytohormones (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 while the radicle and plumule emergence is prevented (Mahmood et al., 2016). Madyasari, et al., (2017) reported that seed priming using rhizobacteria was able to increase the vigor of chili seeds after being stored for 24 weeks. Furthermore, Roslan et al., (2020) reported that *Enterobacter* spp. able to increase the vigor index 19.6% higher than without *Enterobacter* spp. inoculation and the initial vegetative growth of okra plants were faster and increased leaf area and greenness.

Various researchers have reported that beneficial microorganisms can be utilized to increase the vigor index of seeds including *Pseudomonas fluorecence*to increase the germination and vigor of The East Indian Sandalwood (*Santalum album* L) (Chitra & Jijeesh, 2021), *Enterobacter* spp. to increase vigor of okra seeds (Roslan et al., 2020), *Azospirillum, Azotobacter, Bacillus* to increase germination and vigor index of sorghum plants (Widawati & Suliasih, 2018). This condition opens up opportunities for the use of 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 of potential for rice cultivation (Karolinoerita & Yusuf, 2020). Several PGPR isolates that have been isolated from the rhizosphere of rice plants in saline rice fields have the ability to produce growth regulators of the auxin group and are able to 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 increase the vigor index and early vegetative growth of rice plants. This study aimed to examine the effect 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 seeds used in this study were 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 Culturre 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 Innocultaion

Each treatment consisted of 100 grains of rice seeds, before being inoculated the rice seeds were sterilized using sodium hypochlorite 0.02% for 2 minutes (Widawati & Suliasih, 2018), then washed with sterile aquades 3 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 seedbox with sterile sand media and maintained in a green house 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 2 months starting from September to October 2021. The study was arranged using a Randomized Block Design, with three replications. As a treatment, 10 isolates of rhizobacteria were isolated from the North Coast of Pemalang, Central Java, which consisted of 10 isolates namely 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 measured by the formula of Chitra & Jijeesh, (2021) as follows :

Germination (%)= $\frac{Number\ of\ seeds\ that\ germinated}{Total\ number\ of\ seeds}\ x100\%$ ……………………………………….(1)

Germination rate = $\frac{G1}{T1}+\frac{G2}{T2}+\frac{G3}{T3}+\ldots\ldots+\frac{Gn}{Tn}$ ……………………………………………………………..(2)

Here, G1-% seeds germinate at T1, G2-% seeds germinate at T2, G3-% seeds germinate at T3, Gn-% seeds germinate at Tn, and T1 day is the first from sowing until the count, T2 day is the second count from sowing, T3 day from sowing to the third count, and Tn day from sowing to day count.

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

$seed\ Vigor\ Index = (\text{shoot length} + \text{root length})$ x germination (%) ....……………………(3)

Statistical analysis

The data obtained from this study were analyzed by ANOVA using SAS 9.1 software, and if the results of the ANOVA were significantly different, it was continued with DMRT at α=5%.

RESULTS AND DISCUSSION

Seed germination and germination rate

Based on observations, it was found that biopriming of rice seeds with various rhizobacteria isolates did not shown any effect on rice seed germination. The percentage of rice seed germination is 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 2 months so that the seeds were still in good condition and had not deteriorated. The germination rate variable showed the impact of biopriming on the of germination rate of rice seeds ($p<0.05$). The germination rate of rice seeds in different biopriming treatments varried between 22.89 – 24.99. The highest germination rate was obtained in the treatment of isolate rhizobacteria J5, while the lowest germination rate was obtained in the treatment of isolate K3) (Table 1). Germination rate in treatment J5 was not significantly different from control, J12, J, Ju1, and Jn

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(Table 1). Germination rate indicated the speed at which sprouts appeared, and the ability of sprouts to emerge was strongly related to the energy possessed for germination.

The results of this study indicated that biopriming with rhizobacteria was able to enhance seed vigour and early vegetative growth of rice seedlings. Considerance from the seed germination variable showed that the biopriming treatment did not shown its effect on the percentage of the seed germination. It can be seen that the percentage of germination between control and other treatments did not show a significant difference, where the range of germination percentage values ranged from 93.33 percent to 100 percent. This illustrates that the physiological quality of the seeds is still good, where the harvest of seeds is only about two months. These results are in line with the results of (Madyasari et al., 2017) where the seed biopriming treatment did not have a significant effect on 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 availability of energy for the germination process so that it will cause an increase in germination speed (Chitra & Jijeesh, 2021). Mitra et al., (2021) state that living microorganisms have different multifunctional capabilities such as the production of plant growth regulators such as auxins, cytokinins, abscisic acid and gibberellins which are produced as secretions of effector molecules and secondary metabolites through modulation of various pathways, are the most suitable for the biopriming method. Murunde & Wainwright (2018) reported that biopriming treatment using *Bacillus subtilis* and *Serratia nematodiphila* resulted in increased germination of onion seeds.

Seedling growth and biomass

Seed priming treatment in this study had a positive effect on 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 root length of rice seedlings is strongly influenced by the treatment of rhizobacteria inoculation. Overall total root length increased by 83.41 percent compared to the control. The inoculation treatment of Kn1 isolate was able to reach the highest plant height much higher than the control, although between 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. The results indicated an increase in the greenness of the leaves. The greenness of the leaves reflects the total chlorophyll content in the leaves of the plant. The biopriming treatment with rhizobacteria isolates had a significant effect (p=0.0030) in increasing the greenness of the leaves with an average value of 20.07 units.

The effect of biopriming treatment is clearly visible on 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 seedling were achieved in the inoculation treatment of i 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 was able to increase the growth of rice seedling. The application of rhizobacteria was able to increase vegetative growth, which was triggered by the ability of rhizobacteria to produce auxins, especially indol acetic acid (IAA) (Chitra & Jijeesh, 2021; Chauhan et al., 2021). The ability of rhizobacteria to produce IAA will stimulate root elongation so that the 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 stimulated by hormones, thereby increasing nutrient uptake caused by the ability of rhizobacteria to dissolve nutrients such as P. Rhizobacteria have the ability to increase the availability of nutrients in the soil (N,P, K) so that nutrient uptake (N, P, K) increases, increases photosynthetic pigment, and increases photosynthetic 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, where 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 of rice seeds with rhizobacteria isolates is very clear on the vigor of the seeds. The results of the analysis of variance showed that the treatment of rhizobacteria isolates significantly affected the vigor of rice seedlings (p=0.0182). Based on observations, it was found that the highest seed vigor was achieved in the J12 isolate treatment, and the lowest was in the control (Figure 1).

Biopriming of rice seeds with rhizobacteria isolates was able to significantly increase the vigor index. It can be seen that in all rhizobacteria isolate treatments, the vogor index value increased compared to the treatment without biopriming (control). Seed biopriming was able to increase 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 control. The increase in the vigor index is not only influenced by the percentage of germination, but 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 root length had a very close correlation with 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, a hormone that stimulates cell division and also by the effect of auxin as a hormone that stimulates cell elongation (Agbodjato et al., 2016). (Roslan et al., 2020) reported that inoculation of okra seeds with *Enterobacter* sp. was able to increase the initial growth of okra seedlings compared without inoculation based on hypocotyl length, radicle, number of lateral roots and vigor index.

Conclusion

In general, it can be concluded that the biopriming treatment with rhizobacteria isolates derived from the rice rhizosphere origin from The Northern Coast of Pemalang was able to increase the rate of seed germination, seed vigor index and early vegetative growth of rice seedlings. Inoculation with isolate J12 was able to produce the higherest vigor index than the control of, 8280.01 but not significantly different with other isolates. .

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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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

Note: The number is followed by same letter in the same coloum is not different significant accorfing DMRT 5%.

Table 2. The effect of rhizobacteria inoculation to 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

Note: The number is followed by same letter in the same coloum is not different significant according DMRT 5%.

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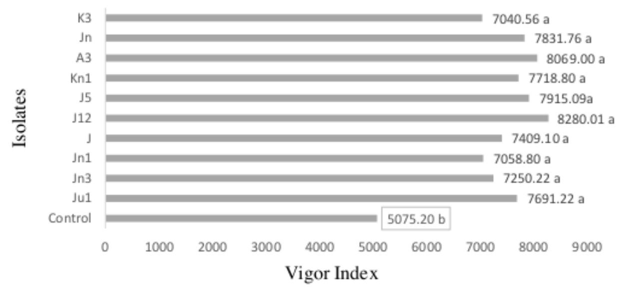

Figure 1. The effect of rhizobacteria isolates to seed vigor index

Table 3. Pearson Correlation Coefficient

	Plant Heeight	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 Greennes	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

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