Effectiveness of Seed Biopriming Using Indigenous Rhizobacteria From The Rhizosphere of Upland Rice In Improving The Growth of Local Upland Rice On Ultisol Soil

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Abstract.
Ultisol soils are widely distributed in Southeast Sulawesi and are characterized by low fertility, acidic pH, low organic matter content, and limited availability of essential nutrients. These constraints contribute to the low productivity of local upland rice and require sustainable technologies that can improve plant growth with lower dependence on synthetic inputs. This study aimed to evaluate the effectiveness of seed biopriming using indigenous rhizobacterial isolates from the rhizosphere of upland rice on the growth of local upland rice cultivated on Ultisol soil. The experiment was arranged in a randomized block design consisting of ten treatments: nine rhizobacterial isolates, namely KNW01, KNW03, KNW05, KNW09, KNW12, BTN15, KNS8, MN06, and BMB01, and a non-inoculated control. Each treatment was replicated three times. The observed variables included plant height, number of leaves, leaf area, number of tillers, root length, plant dry weight, and relative growth rate. The results showed that seed biopriming with indigenous rhizobacteria improved the growth performance of upland rice compared with the control. Isolate KNW05 consistently produced the best response across most growth variables, including plant height of 120.7 cm, leaf area of 1,622 cm², root length of 37.9 cm, plant dry weight of 24.3 g, and relative growth rate of 2.5 g/g/week. KNW05 also increased the number of tillers by 87% compared with the control. These findings indicate that indigenous rhizobacteria, particularly isolate KNW05, have potential as biofertilizer candidates to support sustainable upland rice cultivation on Ultisol soil.

Keywords: *Rhizobacteria, Upland Rice, Seed Biopriming, Indigenous Isolates, Ultisol Soil and Plant Growth.*

I. INTRODUCTION

Rice productivity in Indonesia, particularly in Southeast Sulawesi, still faces various constraints, including high agricultural input costs, limited availability of production facilities, inefficient use of chemical fertilizers and pesticides, and the conversion of paddy fields into residential and industrial areas. However, dryland rice cultivation has not been optimized despite Indonesia's extensive dryland resources. In Southeast Sulawesi, approximately 60.3% of agricultural land is dominated by Red-Yellow Podzolic soils and Ultisols, which are characterized by low fertility due to acidic pH, low organic matter content, and limited availability of macronutrients such as nitrogen, phosphorus, and potassium as a result of leaching. These conditions contribute to low rice productivity, particularly for local upland rice, when soil fertility improvement strategies are not applied [9][29].

One environmentally friendly strategy to improve the productivity of Ultisol soils is the use of beneficial microbial bioagents, such as phosphate-solubilizing rhizobacteria, Azotobacter, Rhizobium, and arbuscular mycorrhizal fungi. These microorganisms play important roles in increasing nutrient availability through phosphate solubilization, nitrogen fixation, and the formation of symbiotic associations that enhance nutrient uptake by plants [2][14]. In addition, bioagents can improve soil structure, suppress soil-borne pathogens, produce phytohormones such as indole-3-acetic acid (IAA), gibberellins, cytokinins, and ACC deaminase, and increase plant tolerance to various environmental stresses, including drought [36][37]. Therefore, rhizosphere engineering through the use of beneficial microorganisms is a sustainable innovation for improving plant growth and productivity on suboptimal land with lower external input requirements [25].

The use of bioagents is in line with the concept of Low External Input Sustainable Agriculture (LEISA), which integrates biofertilizers, organic fertilizers, and inorganic fertilizers in a balanced manner to maintain productivity and agroecosystem sustainability. This approach can improve nutrient-use efficiency, enhance soil health, and reduce dependence on synthetic fertilizers and pesticides without reducing crop yield. Therefore, applying indigenous rhizobacteria as biofertilizers in upland rice cultivation on Ultisol soil may provide an effective strategy to overcome biotic and abiotic constraints, improve plant growth and yield, and support productive and sustainable agriculture.

II. METHODS

Preparation of Rhizobacterial Bioagent Isolates

The rhizobacterial isolates used in this study were stock cultures from the Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University, Kendari. Before use, ten isolates were subcultured on sterile tryptic soy agar (TSA) medium. Subculturing was carried out aseptically in a laminar airflow cabinet. Subsequently, one loopful of each rhizobacterial isolate was rejuvenated on TSA medium and incubated for 48 hours at room temperature. After incubation, the bacterial isolates were suspended in Erlenmeyer flasks containing 100 mL sterile distilled water and shaken at 150 rpm for 24 hours.

Preparation of Planting Media

The planting medium consisted of Ultisol soil, rice husk charcoal, and manure. The soil was sieved using a coarse aluminum sieve with a 10 mm mesh size to remove soil clods, roots, and stones. The soil, rice husk charcoal, and manure were separately placed in heat-resistant plastic bags and sterilized using an autoclave for 1 hour for three consecutive days. The planting medium was prepared by mixing Ultisol soil, rice husk charcoal, and manure at a ratio of 4:1:1 and then placing the mixture into polybags measuring 40 cm × 30 cm.

Seed Preparation and Planting

Seeds of the upland rice variety Paebiu Kolopua were treated with rhizobacterial biopriming by soaking them in bacterial suspension for 12 hours [5]. Two seeds were planted in each polybag containing the prepared planting medium. The planted polybags were arranged according to the experimental design and placed in a screen house. Watering was carried out evenly in the morning and afternoon, depending on weather conditions. Plant maintenance was performed manually, including weed removal from the polybags and pest and disease control when required.

Observation Variables

Sample plants were selected randomly for each treatment. The observed variables were plant height, leaf area, root length, relative growth rate, number of leaves per clump, plant dry weight, and number of tillers per clump.

Data Analysis

All observational data were analyzed using analysis of variance at a 95% confidence level. When significant treatment effects were detected, mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

III. RESULT AND DISCUSSION

Performance of Plant Height and Number of Leaves of Upland Rice Treated with Indigenous Rhizobacterial Biopriming

The results showed that inoculating upland rice seeds with rhizobacterial isolates through biopriming affected plant growth. Seeds treated with rhizobacteria isolated from the rhizosphere of upland rice showed better growth than the control. This finding indicates the potential of local microbial resources from Southeast Sulawesi to support the development of environmentally friendly and sustainable upland rice cultivation technology.

In general, local upland rice benefited from the application of rhizobacteria as biological agents. Based on its effectiveness, isolate KNW05 showed the best overall performance compared with the other isolates. Plant height under the KNW05 treatment reached 120.7 cm, while the control reached only 103.9

cm. However, KNW05 was not significantly different from several other isolates, namely KNW01, KNW03, KNW09, KNW12, BTN15, KNS8, MN06, and BMB01 (Fig. 1a).

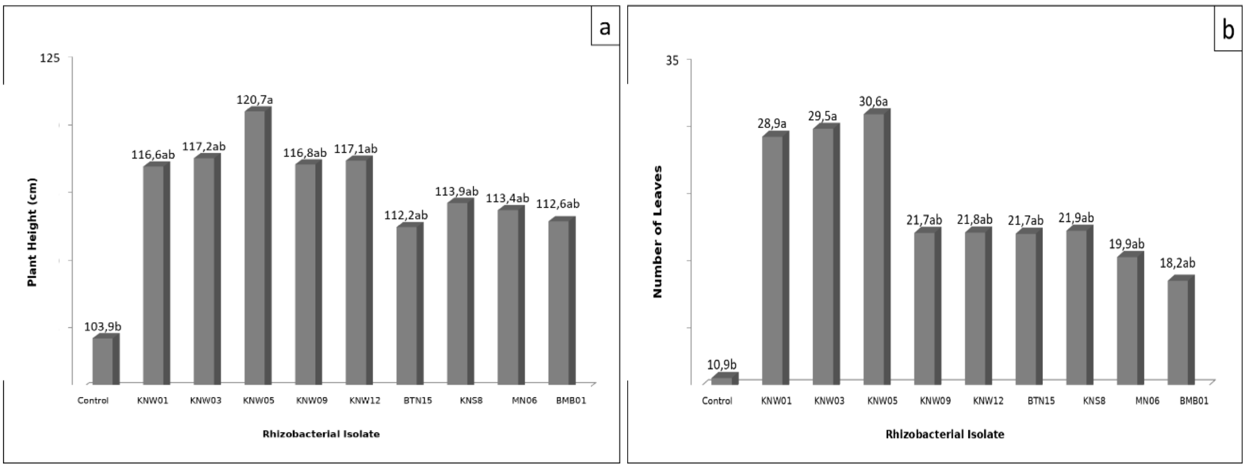

Fig. 1. (a) Plant height and (b) number of leaves of upland rice under different rhizobacterial biopriming treatments

The application of rhizobacterial isolates significantly increased the number of leaves of upland rice compared with the control. Isolates KNW01, KNW03, and KNW05 produced significantly higher numbers of leaves than the control, whereas isolates KNW09, KNW12, BTN15, KNS8, MN06, and BMB01 did not show significant differences. The number of leaves in the KNW01, KNW03, and KNW05 treatments reached 29, 30, and 31 leaves, respectively, while the control produced only 10 leaves (Fig. 1b). Thus, the effectiveness of these three isolates in increasing the number of leaves reached 165%, 171%, and 181%, respectively, compared with the control. These results indicate that several rhizobacterial isolates had greater ability to stimulate vegetative growth of upland rice, particularly leaf formation.

The increase in the number of leaves is consistent with previous studies reporting that biological agents based on rhizobacteria can improve vegetative growth and crop yield [3][17][41]. The high growth response in the KNW05 treatment was likely related to its ability as a plant growth-promoting rhizobacterium (PGPR), through mechanisms such as phosphate solubilization, nitrogen fixation, and indole-3-acetic acid (IAA) production. These mechanisms act synergistically to increase nutrient availability and stimulate plant growth. One important mechanism is the ability to solubilize phosphate that is initially unavailable to plants into forms that can be readily absorbed. Phosphate-solubilizing bacteria can transform phosphate compounds bound to iron and aluminum oxides, such as Fe-P and Al-P, into available phosphate through the production of organic acids and other biological mechanisms [18][41]. Increased phosphorus availability is essential for vegetative growth because phosphorus plays a role in energy transfer, cell division, new tissue formation, and leaf development. Conversely, phosphorus deficiency can inhibit vegetative growth due to disrupted metabolism and limited energy availability for growth [19]. Therefore, the ability of KNW05 to solubilize phosphate, supported by nitrogen fixation and IAA production, was presumed to be a major factor contributing to the increased number of leaves in upland rice in this study.

Inoculation of upland rice seeds with rhizobacterial isolates significantly increased leaf area and number of tillers compared with the control. For leaf area, isolate KNW05 produced the best response, reaching 1,622 cm^2 and differing significantly from the control as well as isolates KNW12, BTN15, MN06, and BMB01; however, it was not significantly different from KNW01, KNW03, KNW09, and KNS8. In comparison, the leaf area of the control reached only 498 cm^2 (Fig. 2a), indicating that the effectiveness of KNW05 in increasing leaf area reached 226% compared with the control. Similarly, for the number of tillers, KNW05 produced an average of 8.6 tillers per plant, which was significantly different from the control and isolates KNS8 and MN06, but not significantly different from KNW01, KNW03, KNW09, KNW12, BTN15, and BMB01. The control produced only 4.6 tillers (Fig. 2b), so the effectiveness of KNW05 in increasing the number of tillers reached 87% compared with the control. These results indicate that KNW05 had the highest potential to stimulate vegetative growth in upland rice.

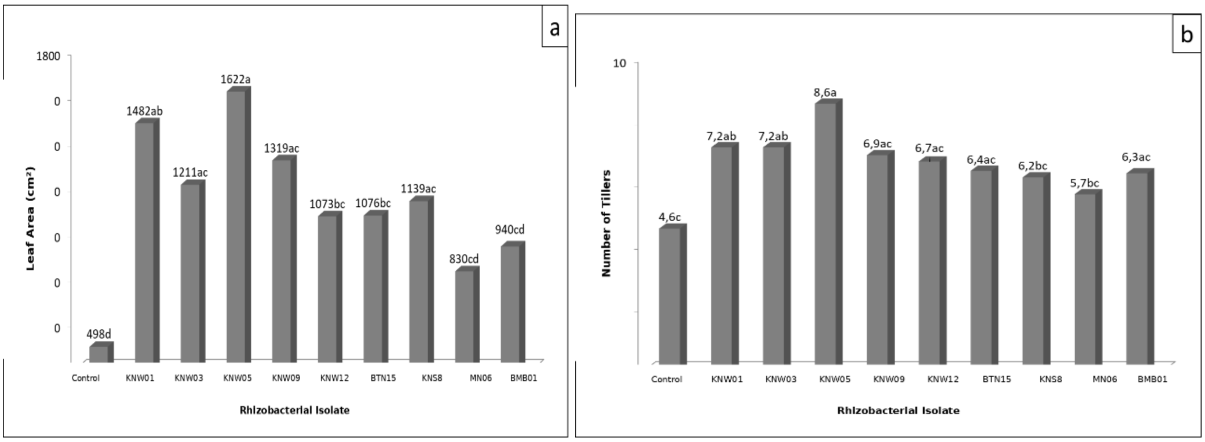

Fig. 2. (a) Leaf area and (b) number of tillers of upland rice under different rhizobacterial biopriming treatments

The increase in leaf area and number of tillers was likely associated with the ability of KNW05 as a plant growth-promoting rhizobacterium to increase nutrient availability, particularly phosphorus and nitrogen. One of the main mechanisms involved is the bacterial ability to solubilize unavailable soil phosphate into forms that can be absorbed by plants. When natural phosphate compounds are present in insoluble forms, phosphate-solubilizing bacteria produce various organic acids, such as formic, acetic, and lactic acids, which can transform phosphate into organic or inorganic forms that are more available to plants. In addition to soil minerals, organic phosphate is also found in phospholipids, phytin, and nucleic acids derived from plant and microbial tissues. Therefore, phosphate solubilization is an important physiological trait supporting the function of rhizobacteria as plant growth promoters [1][7][28]. Increased phosphorus availability supports the formation of new tissues, expands leaf development, enhances photosynthetic activity, and stimulates the formation of more tillers.

In addition to increasing phosphorus availability, isolate KNW05 was also presumed to contribute through its ability to fix atmospheric nitrogen. Although nitrogen-fixing bacteria are often associated with plants that form root nodules, the results of this study indicate that rhizobacteria living in the root zone of local upland rice in Southeast Sulawesi also have potential as nitrogen-fixing bacteria. Rhizosphere bacterial communities are an important source of nitrogen in dryland rice cultivation systems. Bacteria classified as PGPR can biologically fix atmospheric nitrogen, thereby increasing nitrogen availability for plants [33][21]. Rhizobacterial inoculation has been reported to provide approximately 20–50% of total plant nitrogen requirements through N_2 fixation [1][22]. Increased nitrogen availability plays a role in the synthesis of chlorophyll, proteins, and enzymes that support cell division and enlargement, thereby optimizing leaf growth and tiller formation. Thus, the ability of KNW05 to solubilize phosphate and fix nitrogen was presumed to be a key factor supporting the increase in leaf area and number of tillers of upland rice in this study.

Performance of Root Length and Plant Dry Weight of Upland Rice Treated with Indigenous Rhizobacterial Biopriming

Application of rhizobacterial isolates through seed inoculation significantly increased upland rice root length. Compared with the control, application of isolate KNW05 produced the best root length and was significantly different from the control as well as isolates KNS8, MN06, and BMB01, but not significantly different from isolates KNW01, KNW03, KNW09, KNW12, and BTN15. Root length under the KNW05 treatment reached 37.9 cm, while the control reached only 18.8 cm (Fig. 3a). The effectiveness of KNW05 in increasing root length reached 102% compared with the control. In addition to improving root system development, rhizobacterial application also significantly affected plant biomass accumulation, as indicated by the increase in plant dry weight under the same treatment.

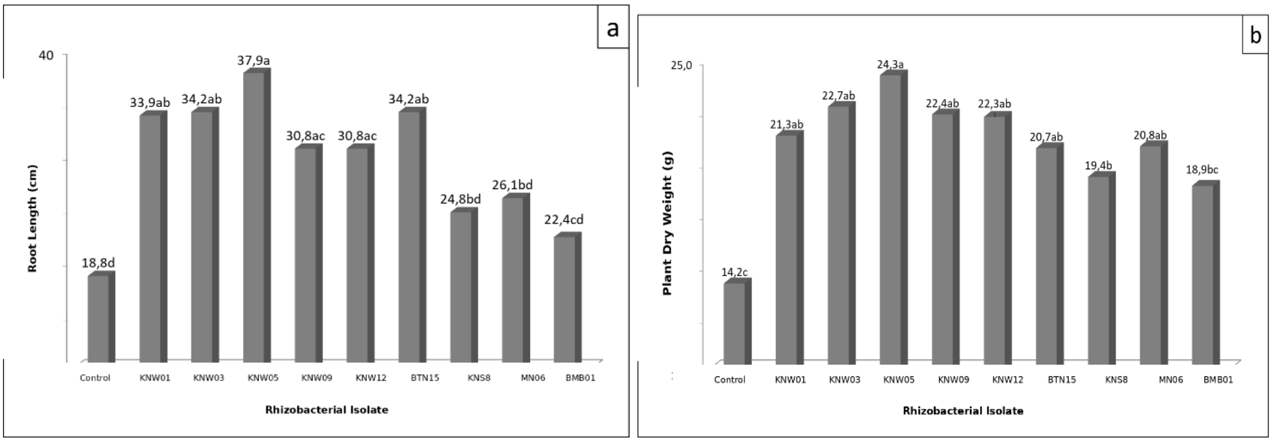

Fig. 3. (a) Root length and (b) plant dry weight of upland rice under different rhizobacterial biopriming treatments

Application of rhizobacterial isolates through seed inoculation also significantly increased plant dry weight. Compared with the control, plant dry weight under the KNW05 treatment was significantly different from the control, KNS8, and BMB01, but not significantly different from KNW01, KNW03, KNW09, KNW12, BTN15, and MN06. Plant dry weight under the KNW05 treatment reached 24.3 g, while the control reached only 14.2 g (Fig. 3b). The effectiveness of KNW05 in increasing plant dry weight reached 71% compared with the control. The increase in root length followed by increased plant dry weight indicates that KNW05 improved the ability of plants to absorb water and nutrients, thereby supporting vegetative growth and biomass accumulation more optimally.

The increase in root growth and plant dry weight was likely related to the ability of rhizobacteria to produce plant growth-promoting compounds. One important trait of rhizobacteria is their ability to produce indole-3-acetic acid (IAA), in addition to solubilizing phosphate and fixing nitrogen [24]. The amount of IAA produced by each isolate is influenced by the ability of bacteria to utilize the amino acid tryptophan present in root exudates and by their ability to colonize the plant root zone [26]. IAA produced by soil microbes functions as a phytohormone that stimulates cell division, elongation, and differentiation, thereby accelerating root and vegetative growth.

Isolates belonging to the genera Bacillus sp. and Pseudomonas fluorescens are known for their ability to produce auxin [35]. Bacterial IAA production contributes to root formation and elongation, thereby increasing the root absorption area for water and nutrients. This condition ultimately supports improved plant growth, as reflected in greater root length and higher dry weight. Therefore, rhizobacterial isolates capable of producing IAA, solubilizing phosphate, and fixing nitrogen have potential to be developed as biofertilizers to improve plant growth while reducing dependence on chemical fertilizers, thereby supporting a more sustainable and environmentally friendly cultivation system [39].

Performance of Relative Growth Rate of Upland Rice Treated with Indigenous Rhizobacterial Biopriming

Inoculation of upland rice seeds with rhizobacterial isolates also significantly increased the relative growth rate of plants. Compared with the control, application of isolate KNW05 produced the highest relative growth rate and was significantly different from the control and isolate BMB01, but not significantly different from isolates KNW01, KNW03, KNW09, KNW12, BTN15, MN06, and KNS8. The relative growth rate of upland rice under the KNW05 treatment reached 2.5 g/g/week, while the control reached 2.0 g/g/week (Fig. 4). The effectiveness of KNW05 in increasing the relative growth rate reached 25% compared with the control.

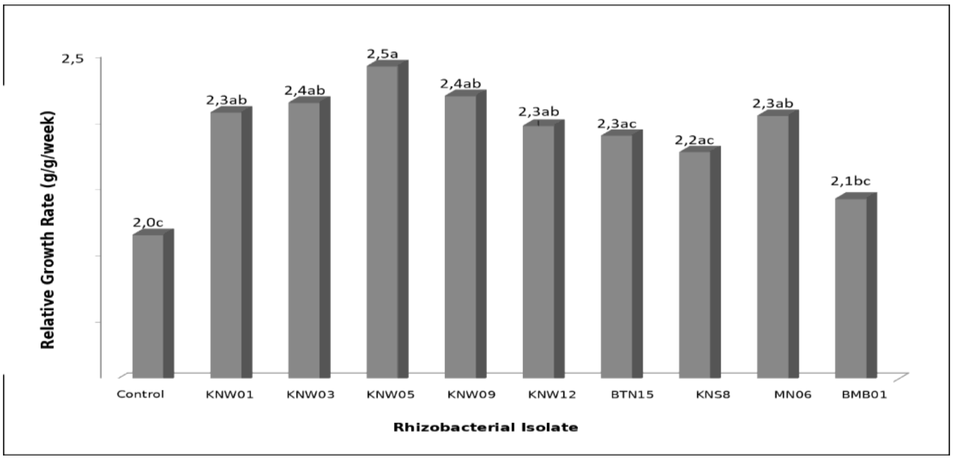

Fig. 4. Relative growth rate of upland rice under different rhizobacterial biopriming treatments

Seed germination is a critical transition phase in the early life of plants because it determines subsequent growth and development. The germination process includes three main events: tissue hydration, metabolic activation, and mobilization of food reserves. Metabolic activity begins with the reactivation of enzymatic systems to repair damage to DNA, RNA, and proteins. Reactivation of metabolism during germination promotes the initiation of radicle growth. At this stage, seeds begin to lose their tolerance to desiccation and enter the post-germination and seedling development phases [12].

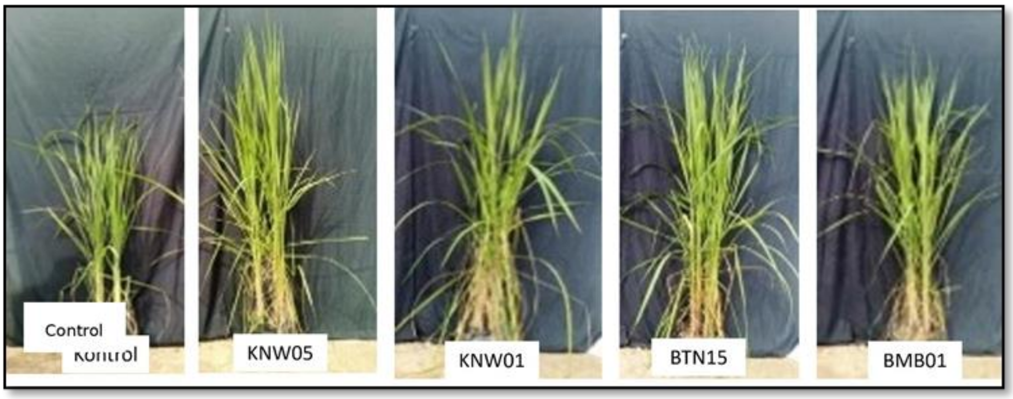

Fig. 5. Growth performance of upland rice under different rhizobacterial biopriming treatments

The transition phase during germination requires special handling so that seeds can maintain their capacity to grow optimally. Seed biopriming is a seed treatment using beneficial microbial suspensions that can induce germination while providing protection against biotic and abiotic stresses during early growth. Biopriming can also improve the effectiveness of seed hydration, thereby accelerating imbibition and increasing the germination rate [32].

The application of rhizobacteria to upland rice seeds through biopriming is an effort to improve plant resistance to biotic and abiotic stresses while supporting plant growth, yield, and survival. Through a controlled hydration procedure, seeds are treated with a suspension of beneficial microbes in the form of PGPR rhizobacterial isolates [10][13][31][36]. Improved growth and plant tolerance to environmental stresses are likely related to the activation of repair systems during the pre-germination stage. In addition, seed biopriming can increase early protein and DNA synthesis and support more effective mitochondrial development [10][15][32].

IV. CONCLUSION

Seed biopriming using indigenous rhizobacterial isolates from Southeast Sulawesi improved plant height, leaf area, root length, plant dry weight, number of tillers, and relative growth rate of local upland rice cultivated on Ultisol soil. Among the tested isolates, KNW05 was the most effective isolate in improving the overall growth of upland rice. The increase in growth based on the number of tillers reached 87% compared with the control.

V. ACKNOWLEDGEMENTS

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