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ADAPTATION TEST OF THE CONSORTIUM OF PHYLLOSPHERE MICROBES FM48 AND RHIZOSPHERE MICROBES R15 TO STIMULATE GROWTH RICE PLANT IN PADDY FIELDS

ARIS AKSARAH*, ARFAN, LISA INDRIANI AND ARFANDI

Agrotechnology Study Program, Faculty of Agriculture, Alkhairaat University,
Diponegoro Street 39 Palu 94221, Indonesia

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Abstract-Previous research has succeeded in obtaining a consortium of Phyllosphere microbes Fm48 and a consortium of rhizosphere microbes R15 which are the most effective in increasing the growth and yield of rice plants in experimental pots. The consortium of microbes of Phyllosphere Fm48 and R15 microbes of rhizosphere have the ability to fix N_2 from the air, dissolve P and secrete growth hormones. This study aims to examine the effectiveness of the consortium of Phyllosphere microbes and rhizosphere microbes in adapting to the rice field environment and their ability to stimulate the growth of rice plants in paddy fields. The research was conducted in nurseries and transplanted plantings, using a completely randomized design consisting of four treatments, namely: control, giving the consortia of Phyllosphere microbe Fm48, giving consortium of rhizosphere microbesR15 and the combination of consortium s of Phyllosphere microbe Fm48 and consortium of rhizosphere microbesR15. The data obtained were processed by analysis of variance and if there was a significant difference, it was continued with the Honest Significant Difference (HSD) test at the 1% level. The consortium of phyllosphere microbes Fm48 and consortium of rhizosphere microbes R15 had no effect on rice growth in nurseries, but they were able to adapt to the rice field environment and stimulate rice plant growth even though its process was slow. The consortium of phyllosphere microbes Fm48 and consortium of rhizosphere microbes R15 and their combination stimulated the growth of the number of leaves and the number of rice tillers.

INTRODUCTION

A new environmentally friendly approach is needed to increase plant biomass production, namely the use of plant growth-promoting bacteria as a biotechnological tool to increase growth in various environments. The obstacle faced in the effort to develop the use of microbes in cultivated plants is the ability of the applied microbes to adapt to a new environment and compete with local microbes. Each different habitat provides different diversity. Microbes cannot be separated from the biotic and abiotic environment of an ecosystem.

Utilizing Phyllosphere and rhizosphere microbes in the form of a consortium that has the ability to fix N_2, dissolve inorganic P and secrete plant growth hormones is one of the efforts aimed at reducing the use of inorganic fertilizers and improving environmental quality. Generally, the management of paddy fields in Indonesia, to achieve high rice production, farmers still continue to use synthetic fertilizers to meet the nutritional needs of plants. Intensive use of synthetic fertilizers on agricultural land in the long term can cause damage to soil structure, decrease in soil organic content, and environmental pollution. Microbes associated with plants are involved in the growth, development, and tolerance of plants to various stresses. The plant microbiome can be used as a functional part to increase overall plant productivity. Strategies for using microbial inoculants for agro-management practices prevent negative impacts on the environment (Sharma *et al*., 2021).

Previous research has succeeded in obtaining a consortium of Phyllosphere microbes Fm48 and a consortium of rhizosphere microbes R15 which are the most effective in increasing the growth and yield of rice plants in experimental pots. The application of a consortium of Phyllosphere microbes and rhizosphere microbes has the potential to reduce the use of N_2fertilizer by half the dose without reducing yields (Pas *et al*., 2015[a]). Pas *et al.* (2015[b]) reported that a consortium of Phyllosphere microbes Fm48 and rhizosphere microbes R15 had the ability tofix N_2 from the air, dissolve P and secrete growth hormone.

This study aims to examine the effectiveness of the consortium of phyllosphere microbes and rhizosphere microbes in adapting to the rice field environment and their ability to stimulate the growth of rice plants in paddy fields.

MATERIALS AND METHODS

Place and time

The research was carried out from August to September 2021. Laboratory experiments were carried out at the Laboratory of the Faculty of Agriculture, Alkhairaat University, Palu. The field experiment was carried out in rice fields, Boya Baliase Village, Marawola District, Sigi Regency, Central Sulawesi Province, Indonesia.

Materials and tools

The materials used consisted of: Inpari7 variety rice seeds, isolates from the Fm48 consortium and the R15 consortium, labels, sterile distilled water. Materials for the manufacture of culture media. The tools used consist of: Tools for making culture media, meters and tools for cultivation techniques.

Methods

The experiment used a one-factor Completely Randomized Design (CRD). The treatment consisted of four treatments, namely:without giving the consortium isolates (control); Provision of isolates from the Fm48 Phyllosphere microbial consortium; Provision of isolates from the consortium of rhizosphere microbes R15 and the combination of isolates from the consortium of Phyllosphere microbes Fm48 and the consortium of rhizosphere microbes R15. Each treatment was repeated 3 times, so there were 12 experimental plots.

A consortium of Phyllosphere microbes and rhizosphere microbes was grown in sterile NB medium. Each 1 ose of the consortium was put into a 125 ml erlenmeyer containing 50 ml of medium aseptically, and then shaken with a shaker for ± 3 - 4 days (72 hours). Then the optical density was seen using a spectrophotometer, so that the total population equivalent to a wavelength of 620 nm can be seen (Gusmaini 2005).

Before planting, rice seeds are sterilized first, that is, soaked in alcohol for 3-5 minutes, then soaked in 3% chlorotic for 3-5 minutes, cleaned with distilled water until the smell is gone. The seeds for treatment with the rhizosphere microbial consortium R15 isolate were soaked in the isolate solution for 24 hours. Rice seeds were sown on the prepared nursery media, one week after planting, each isolate of the consortium was inoculated according to treatment, namely by spraying it on the plants and soil of each plant. The frequency of inoculants was done every two weeks as much as 15-20 ml/plant. The total population of the consortium that was applied was 10^9 cfu.ml^{-1}.

Furthermore, inoculants were given every two weeks as much as 15-20 ml/plant until the plants were 28 days after planting (DAT). Then transplanting was carried out until the age of 42 DAT.

During the growth period, one day after application of the treatment, the growing medium was flooded with water according to the conditions for growing rice. Water is given as high as ± 2 cm from the ground surface. Water conditions were maintained to moist soil conditions, before the next application. At the time of application the soil conditions were in a moist state. The growth variables observed included: plant height, number of leaves, wet weight and dry weight of plants aged 28 DAP, plant height, number of leaves and number of tillers aged 21 and 42 DAT.

Data analysis

The data obtained was processed by analysis of variance and if there was a significant difference, it was continued with the Honest Significant Difference (HSD) test at the 1% level. The data were processed using the computer program SAS (Statistical Analysis System) for windows version 9.1 (Matjik and Sumertajaya, 2006).

RESULTS

Nursery

Inoculation of consortium isolates of phyllosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on plant height, number of leaves, wet weight and dry weight of plants aged 28 DAP. The average plant height, number of leaves, wet weight and dry weight of plants aged 28 DAP are presented in Table 1.

Description : The mean followed by the same letter in the same column shows a non-significant difference based on the BNT test at a significance level of 0.01. DAP = Day after planting

Transplanting

Inoculation of consortium isolates of phyllosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on plant height at 21 and 42 days after transplanting and leaf number at 42 days after transplanting, but significantly affected the number of leaves at the age of 21 days after transplanting and the number of tillers at the age of 42 days after transplanting. The average plant height, number of leaves and number of tillers are in Table 2.

The treatment of inoculation of the R15 rhizosphere microbial consortium and the combined inoculation of the Fm48 and R15 rhizosphere microbial consortium had a better effect than other treatments.

DISCUSSION

The results showed that the inoculation treatment of the rhizosphere microbial consortium and the combination of the Fm48 and R15 rhizosphere microbial consortium could stimulate the growth of rice plants after transplanting, although it has not had an effect at the time of sowing. Microbes need time to adapt to the rice field environment. Sri Sudewi *et al*. (2021) reported that seed soaking treatment with rhizosphere microbial solution stimulated germination, root length and germination height of rice. Bacterial isolates sprayed on plants showed differences with control plants including vegetative growth characteristics, such as increased plant height, number of tillers and increased yield (Pati, 1992).

Knief *et al*. (2012) reported that so far, bacterial isolates from the rice phyllosphere have been identified and their potential beneficial interactions with rice plants, such as promoting plant growth, fixing nitrogen or plant hormone production and protection against pathogens have been identified. Plant growth and productivity depend on the complex and dynamic interactions between plant roots and soil microorganisms. Microbes with plant growth-promoting properties have great potential to increase plant biomass and crop yields. Beneficial plant growth improvement mechanisms include increased availability of nutrients (N, P, K, Zn and S), phytohormonal secretion, phytopathogen biocontrol and improvement of biotic and abiotic stresses. These plant-microbial interactions are indispensable for sustainable agriculture and these

Table 1. Average plant height, number of leaves, wet weight and dry weight of plants at 28 DAP.

Treatment	Plant height (cm)	Number of leaves (sheet)	Wet weight (g)	Dry weight (g)
Control	28,23a	5,22a	0,34a	0,14a
Microbes of phyllosphere Fm48	28,32a	5,36a	0,43a	0,16a
Microbes of rhizosphere R15	38,57a	5,44a	0,37a	0,15a
Microbes of phyllosphere Fm 48 + microbes of rhizosphere R15	28,25a	5,32a	0,42a	0,16a

Table 2. Average plant height, number of leaves, number of tillers at the age of 21 and 42 days after transplanting (DAT)

Treatment	Age 21 DAT			Age 42 DAT	
	Plant height (cm)	Number of leaves (sheet)	Plant height (cm)	Number of leaves (sheet)	Number of tillers
Control	39.06a	20,11b	56,97a	54,16a	12,17b
Microbes of phyllosphere Fm48	38.69a	20,69b	53,89a	59,42a	16,67ab
Microbes of rhizosphere R15	37.33a	28,25a	53,72a	63,97a	17,58a
Microbes of phyllosphere Fm 48 + microbes of rhizosphere	38.44a	21,19ab	57,76a	63,33a	18,56a
BNT 1 %		6,96			4,99

Description : The mean followed by the same letter in the same column shows a non-significant difference based on the BNT test at a significance level of 0.01. DAT = Day after transplanting microbes can play an important role as an ecological regulator to reduce the use of chemical fertilizers (Kumar *et al*., 2021). Phyllosphere microbes can increase plant resistance to stress conditions (Azevado *et al.* 2000), increase plant growth (Sturs and Nowak 2000), produce phytohormones (Morris 2001) and N_2 fixation (Bodenhausen *et al*., 2014; Werner *et al*., 2005).

The presence of N_2 fixation from the applied microbes, stimulates plant growth. Gardner *et al*. (1991) in addition, the ability of microbes to dissolve P increases the availability of P for plant needs. The association between plants and N_2-fixing microbes and P solvents is very important, because N and P are essential macronutrients needed by plants. Nitrogen is a building block for amino acids, amides, nitrogenous bases such as purines and proteins as well as the building blocks of chlorophyll. N deficiency limits cell enlargement and cell division and interferes with the growth process which causes stunted plants, yellowing and reduced dry weight of crop yields.

Research result Olenska *et al*. (2020), plant growth promoting rhizobacteria (PGPR) can interact with plants directly by increasing the availability of essential nutrients (e.g. nitrogen, phosphorus, iron), production and regulation of compounds involved in plant growth (e.g. phytohormones), and stress hormonal status (eg levels of ethylene by ACC-deaminase). Rhizobacteria can also indirectly affect plants by protecting against disease through competition with pathogens for very limited nutrients, biocontrol of pathogens through production of aseptic activity compounds.

Plants have a very complex, diverse, and distinctive microbial community which are symbiotically associated with each other and regulate the physiological activities of each other directly or indirectly. Hundreds of microbial species are found inhabiting different plant microenvironments such as the rhizosphere, phyllosphere, and endosphere. Microbes are the main determinants of plant growth and plant physiological processes under different environmental conditions. Microbes of the phyllosphere are the most dynamic inhabitants of the various microbes that regulate different physiological processes between plants, microorganisms, and the atmosphere. Microbial phyllosphere can trigger new dimensions in sustainable agricultural strategies such as the development of microbial inoculants as biofertilizers for plant growth and development as well as increased yields, increased mobilization of nutrients and biocontrol agents to induce systemic resistance to phytopathogens, or stress resistance mechanisms to overcome various biotic stress conditions. and abiotic (Parasuraman *et al*., 2019).

CONCLUSION

The consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15 had no effect on rice growth in nurseries, but they were able to adapt to the rice field environment and stimulate rice plant growth even though it was slow. The consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15 and their combination stimulated the growth of the number of leaves and the number of rice tillers.

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