Consortium of Endophytic Bacteria Application Improves Grain Yield of Rice

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Abstract. The use of beneficial endophytic bacteria is an attractive strategy to control pathogens, improve plant growth and yield. The objectives this research were to investigate the effect of consortium application of endophytic bacteria on plant growth, grain yield, and to increase plant resistant to pathogens. The research was carried out on paddy fields located at Kembaran Banyumas with three treatments and nine replications. The treatments consisted of application of the endophytic bacteria consortium, synthetic bactericide and control (without endophytic bacteria consortium or bactericide). The treatments were applied five times with an interval of seven days starting at 30 days after planting. The variables observed included growth components such as the number of tillers per clump, productive tillers, and the yield components such as weight of grain yield per clump and weight of 1000 grains. Result showed that the consortium endophytic bacteria application increased the number of tillers, productive tillers, and weight of grain yield per clump by 37.85%, 52.76%, 39.50%, respectively. The consortium of endophytic bacteria promoted the progress of plant resistance to pathogens by increasing phenol 105 mg GAE/100g.

Keywords: Endophytic bacteria · Plant resistance · Promote · Rice yield

1 Introduction

The character of the antagonistic mechanism against plant pathogens is possessed by endophytic bacteria. This endophytic bacteria are benefits to plants because they are not pathogenic and help plants in increasing resistance to pathogens, it can produce compounds that help plant growth. Endophytic bacteria reach sufficient populations and are capable of producing plant growth-promoting compounds such as IAA (indole acetic acid) and gibberellins. Endophytic bacteria are associated with plants to help the absorption of nutrients because they are able to dissolve phosphate into a form available to plants [1, 2]. The consortium of endophytic bacteria was made by mixing five isolates of rice root endophytic bacteria from different regions, and compatibility tests were carried out [3]. These endophytic bacteria both single isolate and the consortium have been tested for their ability to produce IAA, siderophores, HCN, phosphate solubility, chitinase and protease as well as their ability to inhibit rice pathogens [3–5].

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Like the rhizobacteria, according to [6], they are able to act as biostimulants (produce phytohormones), biofertilizers (to help absorb nutrients for plants), and bioprotectants (suppress disease). They are able to produce siderophore to chelate iron into iron-siderophore bonds available to plants. Endophytic bacteria indirectly affect plants as a bioprotectant or biocontrol to several plant diseases [7, 8].

Endophytic bacteria stimulate plant growth and will have a positive effect on yield. The application of endophytic bacteria, either singly or in a consortium, was able to improve plant growth and yield as previous research was able to enhance the number of fruit per plant, weight of fruit, and quality of fruit [9].

Efficient strains of bacteria are a rich sources of natural products that might improve crop yield in numerous biological ways, such as nitrogen fixation, hormone production, mobilization of insoluble nutrients, and mechanisms related to plant biotic and abiotic stress alleviation. Additionally, these microorganisms also exhibit great potential for the biocontrol of phytopathogens and pest insects. The endophytic and rhizospheric microorganisms associated with tropical plants is a sustainable alternative to control diseases and to enhance food production to minimize ecological damage in tropical ecosystems [10].

The objectives this research were to investigate the effect of consortium of endophytic bacteria application on plant growth and grain yield, and plant resistant to pathogens.

2 Materials and Methods

2.1 Inoculum Preparation of Endophytic Bacteria Consortium

The endophytic bacteria used in this study were single endophytic bacterial isolates from Petanahan Kebumen (isolates A5, A6), Karangwangkal Banyumas (KR4, KR7), and Sumbang Banyumas (SB3). The consortium of endophytic bacteria were combination of five isolates of endophytic bacteria. Which have been tested for compatibility [3]. Enrichment of single isolate endophytic bacteria was carried out by growing the bacteria on NA (nutrient agar) medium for two days. Then a suspension was made by inoculating one loop in 100 ml NB medium (nutrient broth), then shaking for 1 x 24 h in a KBLee 3001 orbital shaker with a speed of 150 rpm, at room temperature.

Suspension of each isolate is taken in equal volumes to be mixed in the form of consortium stock. For its application, a liquid formula of 10 ml consortium suspension of endophytic bacteria was prepared in 1000 ml of sterile water.

2.2 Application of Endophytic Bacteria Consortium to Improve Plant Growth and Grain Yield of Rice

The application of the endophytic bacteria consortium was carried out by soaking the seeds into a liquid formula for 1x24 hours and pouring 50 ml of liquid formula per polybag at the age of 20, 30, 40 and 50 days after planting. The bacterial density used was $\mathbf{10}^8$ cfu/ml.

Observations on growth components included plant height, number of leaves and number of tillers, fresh and dry plant weight. The yield component of rice was indicated by the number of panicles per clump, dry grain weight and 1000 seed weight.

2.3 The Effect of Endophytic Bacteria Consortium Application to Increase Plant Resistant to Pathogens

Study on increasing plant resistance to pathogens is carried out by observing resistance components such as the content of phenolic compounds in leaf tissue at the end of the vegetative phase of the plant. In addition, the plants were inoculated naturally because the field was in the category of endemic disease and was previously planted with rice. The disease intensity of rice was also observed using formula as follow:

$$\mathrm{DI} = \frac{\Sigma(\mathrm{n_i}.\mathrm{v_i})}{\mathrm{N.V}} X\,100\% \tag{1}$$

where

DI = disease intensity, n_i = the number of plants that show symptoms with a certain category, v_i = scale value with a certain symptom category, N = number of plant sample, V = the highest score for the symptom category.

The score of the symptom category (v) as follows: 0 = no symptoms, 1 = 1–20% infected plant parts, 2 = 21–40% infected plant parts, 3 = 41–60% infected plant parts, 4 = 61–80% infected plant parts, 5 = 81–100% infected plant parts.

The analysis of total phenol content refers to the method [12]. The rice leaves were extracted using 96% ethanol and then 10% Folin-Ciocalteu reagent and 7.5% Na_2CO_3 were added. The solution was incubated and the absorbance was measured using a spectrophotometer at a wavelength of 765 nm. Calculation of total phenol content is calculated based on [13].

$$\text{Total phenol} = \frac{x.V.Fp}{BS} \tag{2}$$

where

x = sample consentration (µg/mL), V = volume of sample (mL), Fp = dilution factor of sample, BS = weight of sample (g).

3 Result and Discusion

The treatment of endophytic bacteria consortium showed the best on the variables of plant height, number of leaves, and plant fresh weight, and the dry weight of plants was significantly different from the bactericide treatment and control. It indicates that the endophytic bacterial consortium is able to trigger plant growth and is able to compete with bactericidal treatment, so that it can substitute for bactericide applications in the field. The application of bactericides is still used as a control of plant diseases, but if the application is not wise, it can cause harm to the environment, and other useful microbes. The utilization of endophytic bacteria as plant pathogen biocontrol agents has been reported to be able to suppress disease development and promote plant growth [3, 11, 14, 15].

The increase in rice yield after the application of the endophytic bacteria consortium was indicated by number of tillers, grain weight per tiller and weight of 1000 seeds. The consortium endophytic bacteria is the best treatment base of four variables (Fig. 1). This

Table 1. Plant growth component after consortium endophytic bacteria application

Treatment	P.height (cm)	NoL	Pfw (g)	Pdw (g)
B	101.22b	22.90b	44.82b	11.04b
C	104.42a	25.18a	46.33a	11.60a
K	98.40c	22.41c	32.76c	7.96c

Note: B: Bactericide; C: Consortium endophytic bacteria; K: control (water); numbers followed by the same letter in the same column show no significant difference in the 5% LSD test. P.height: plant height; NoL: number of leaves; Pfw:plant fresh weight; Pdw: plant dry weight

Table 2. Plant resistance response and increase phenol content after consortium of endophytic bacteria application

Treatment	DI Sheath Blight (%)	DI Bacterial Leaf Blight (%)	Resistance level	Phenol total (mg GAE/100g)	Increase of phenol total (mg GAE/100g)
B	10.2	27.9	Mod. Resist	236.4	23.4
C	8.26	26.6	Mod. Resist	318	105
K	20.6	42.5	susceptible	271.2	58.2

Note; B: Bactericide; C: Consortium endophytic bacteria; K: control (water

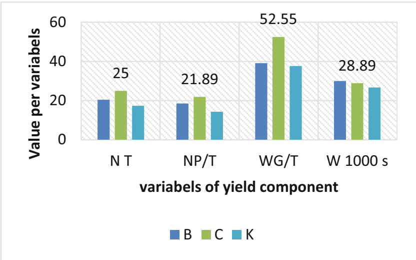

Fig. 1. Component of rice yield after consortium application.

result is in accordance with the statement: Consortium of *Azotobacter, Bacillus, Enterobacter*, and *Xanthobacter* can increase 18% of plant yield. Consortium of *Anabaena variabilis*, *Tolypothrix tenuis*, *Nostoc muscorum*, and *Aulosira fertilissima* can increase grain yield of rice more than 37% [16].

Disease intensity of sheath blight and bacterial leaf blight (Fig. 2) showed that the consortium treatment is the best for decrease disease intensity.

The increase of phenol total showed by consortium application at 56%, while the bactericide and control were 13% and 31% respectively (Fig. 3).

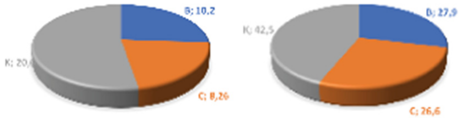

Fig. 2. Disease intensity of sheath blight and bacterial leaf blight of rice.

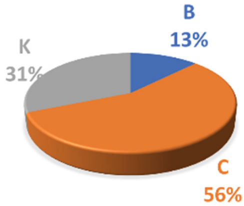

Fig. 3. The increase of phenol content.

The endophytic bacteria consortium treatment was able to increase the content of phenolic compounds by 105 mg GAE/100g. This indicates that the consortium of endophytic bacteria composed of five isolates is compatible in producing phenolic compounds which play a role in supporting plant resistance (Table 2). In accordance with the opinion [17] that the increase in phenolic compounds in bhendi crops as a defense against pathogens attacks and enhance the vigour and tolerance under salt stress. The phenolic compound such as peroxidase and catalase produce by consortium as antioxidant activity.

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Authors' Contributions. NP was in charge of planning and responsible for the implementation of the research stages. HAD carried out the endophytic bacteria application to improve plant growth and yield. PL contributed for prepare the manuscript. Author's agree with the final manuscript.

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