Res. Crop. **23** (4) : 737-744 (2022) DOI : 10.31830/2348-7542.2022.ROC-901
Printed in India

Evaluating diazotrophic endophytic bacteria consortium on the physiology of various varieties of rice (*Oryza sativa*) in rainfed lowlands

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(Received : September 20, 2022/Accepted : November 11, 2022)

ABSTRACT

Rainfed land is usually poor in nutrients including nitrogen nutrients. A consortium of endophytic bacteria can fix nitrogen from the air so that it is expected to improve the physiology of lowland rice varieties. This study was conducted during June 2022 at rainfed rice fields in Demangan, Sambi, Boyolali, Central Java, Indonesia to determine the analysis of the growth of paddy varieties at various doses of the endophytic bacteria consortium. This research used a completely randomized block design with two factors and three replications. The first factor was a consortium of endophytic bacteria with a dose of 0, 20, 30, and 40 L/ha/application, while the second factor was varieties paddy *i.e.*, Situbagendit, Ciherang and Mekongga. The results showed that the dose of endophytic bacteria consortium 40 L/ha/application showed an increase in leaf area index (LAI), leaf area duration (LAD), net assimilation rate (NAR), and crop growth rate (CGR) compared to doses of 0, 20 and 30 L/ha/application. The implication of this research is that in rainfed rice fields to increase LAI, LAD, NAR and CGR of rice varieties, it is better to use a consortium dose of diazotroph endophytic bacteria 40 L/ha/ application and can use Situbagendit, Ciherang or Mekongga varieties.

Key words : Crop growth rate, crop physiology, endophytic bacteria, rainfed rice

INTRODUCTION

Rainfed land in Indonesia is 3,292,578 ha and 24% of it is for rice cultivation in 2009 as per Ministry of Agriculture of Republic of Indonesia. This rainfed land uses rainwater for irrigation and is different from irrigated rice fields. Rainfed rice fields have a low available P content due to groundwater leaching (Meng *et al.*, 2018). In general, improper agricultural management, long-term application of chemical fertilizers, and inefficient fertilizer use decrease the soil productivity of rice fields.

Drought stress is one of the most destructive abiotic stresses affecting plant growth and development. Drought stress affects physiological processes, biochemical changes, formation of secondary metabolites, significantly accumulates endogenous reactive oxygen species (ROS), and increases toxin levels (Hasanuzzaman *et al.,* 2017).

Drought stress greatly reduces rice grain yields and vegetative growth (Ahadiyat *et al.*, 2014; Maisura *et al.*, 2014). Water-scarce conditions generally reduce grain size, grain weight, and seed formation rates in crops (Raman *et al.*, 2012; Rahil Golfam *et al.*, 2021). Drought stress during the booting, flowering, and terminal stages can interfere with floret initiation, cause grain sterility, lower grain weight, and ultimately lower grain yield (Acuña *et al.*, 2008). The rate of grain yield loss depends on the duration of water scarcity, plant growth stage, and stress intensity (Gana, 2011).

One of the efforts to overcome drought stress is microbial-based technology, such as a consortium of endophytic bacteria. Endophytic bacteria are found in the host plant (Kumar *et al.*, 2017). This type of bacteria creates a complex relationship with the host plant where it acts as a plant growth promoter. The role of plant-associated bacteria in increasing crop production and soil fertility Glick (2020). Microbial components in the plant endosphere and rhizosphere form beneficial associations with plants that can increase crop productivity (Ali, 2017; Aghajan Bahadori *et al.*, 2021). These bacteria increase plant resistance to various abiotic and biotic factors that limit growth and production (Kumar and Verma, 2018). These microbes can live both internally and externally in host plant tissues. For example, rhizosphere bacteria inhabit plant roots in the soil, and epiphytic bacteria inhabit the leaf surfaces of plants.

Rhizobacteria refers to the plant growth promoting bacteria present in the rhizosphere. The rhizosphere consists of a narrow zone of soil that is influenced by the plant root system where maximum microbial activity occurs (Verma *et al.*, 2019; Chebotar *et al.*, 2021). The rhizosphere zone is an ecological niche that provides a rich source of nutrients and energy for plant growth. Rhizobacteria are abundant plant partners in the rhizosphere, but they differ in their role in the promotion of plant growth. Various interactions occur between plants and rhizobacteria in the rhizosphere. These interactions are equally important and involve signals between rhizobacteria and plant roots that regulate their biochemical activity (Bhattacharyya and Jha, 2012). Rhizobacteria are essential in the rhizosphere for nutrient cycling, carbon sequestration, and ecosystem functions that promote plant growth, yield and nutrition. Various genera of bacteria have been used as plant growth-promoting rhizobacteria (PGPR), including Burkholderia, Pseudomonas, Arthrobacter, Bacillus, Serratia, Micrococcus, Chromobacterium, Erwinia, Azospirillum, Caulobacter, Agrobacterium, and Azotobacter (Verma *et al.*, 2019).

Rhizobacteria produce plant growth-regulating phytohormones such as ethylene, gibberellins, and auxins. Other important metabolites include the production of siderophores, enzymes, organic acids, antibiotics, biosurfactants, nitric oxide, and osmolytes. Metabolites are responsible for increasing nutrient absorption, tolerance to abiotic stress, nitrogen fixation, suppression of pathogenic organisms (Pii *et al.*, 2015).

In addition, this trait is inherited and can be transferred through seeds, making it more suitable and effective in promoting plant growth (Verma *et al.*, 2019). These heritability factors are important in selecting adaptive and effective endophytes associated with certain crops that are important for agriculture, especially in plant breeding and addressing challenges related to climate change. Their capacity to tolerate and induce resistance to biotic and abiotic stresses in plants can be harnessed to solve the associated edaphic and pathogenic challenges facing the crop production sector. According to AfzalI *et al.* (2019), the various benefits associated with endophytes can be more striking when plants are subjected to adverse environmental stress. Habitat-induced stress triggers plant microbial signaling, which forms complex communications.

Endophytic bacteria positively affect host plant development without significant harm while suppressing pathogens that may attack the plant (Zhang *et al.*, 2019). In return, endophytic microbes benefit and use the plant endosphere as a unique and safe haven that is not disturbed by harsh climatic conditions that can harm and affect its function (Le Cocq *et al.*, 2017). Moreover, most of the endophytic bacteria exhibit a biphasic life cycle in which they alternate between soil and plant environment, thus surviving between seasons (Kumar *et al.*, 2017). Other bacteria form symbiotic structures such as nodules from beans that harbor various strains of bacteria. Only the rhizobia responsible for nitrogen fixation are well known, while other endophytic bacteria are poorly studied (AfzalI *et al.*, 2019).

The purpose of this study was to determine the leaf area index, leaf area duration, net assimilation rate and crop growth rate on various varieties of lowland rice due to the administration of a consortium of diazotropic endophytic bacteria in paddy fields on rain-fed areas.

MATERIALS AND METHODS

Study Area

Research was conducted in Demangan, Sambi, Boyolali, Central Java, Indonesia, from June to September 2022 with alfisol soil. A geographical position was between 110° 22'-110° 50' east longitude and between 7°7'-7°36' south latitude with a height of 184 m above sea level (ASL). The average rainfall and temperature were 139 mm per month and 26-32°C, respectively.

Experimental Design

This research was arranged in a

randomized completely block design (RCBD) with two factors and three replications. The first factor was the dose of a consortium of endophytic bacteria consisting of four levels, *i.e.,* doses of 0, 20, 30, and 40 L/ha/application. The second factor was the second factor was varieties of rice fields *i.e.*, Situbagendit, Ciherang and Mekongga. In this study, there were 12 treatment combinations. Each treatment combination was three times replications, and each replication consisting of five plant samples.

Research Procedures

Before the research, the team conducted a chemical analysis of the soil used for the research substrate. The results showed an H_2O pH of 6.52 (slightly sour), C concentration of 1.34% (low), organic matter concentration of 2,28% (low), total N concentration of 0.22% (low), available P of 9.49 ppm (very high), available K of 0.28 me/100 g (high).

The media used was Alfisol soil. The length and width of the experimental plots were 500 cm and 200 cm, respectively. The water level was 5 cm deep, with the plants spaced 20 cm × 20 cm apart. The experimental field was weeded at 2 and 4 weeks after planting and controlled pests and diseases using organic pesticides. Urea, NPK Phonska and SP-36 fertilizers at a dose of 200, 100 and 75 kg/ha, respectively, were applied at planting time and five weeks after planting. The harvest criterion was the seed shells above the panicle being clean and firm.

Measurement

The parameters observed were the leaf area index (LAI), leaf area duration (LAD), net assimilation rate (NAR), and crop growth rate (CGR). The data observation was conducted in 6 and 8 WAP. LAI was calculated from the ratio between the total leaf surface area per unit ground area. LAI was determined by the intensity of radiation intercepted divided planting spacing. LAD is the time a leaf could last on the plant. LAD was calculated from leaf area (cm^2) divided by time (week).

NAR is the ability of plants to produce dry materials that assimilate each unit of leaf area at each unit of time, which is stated in Eq. 1.

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\ln LA_2 - \ln LA_1}{LA_2 - LA_1}, \text{ (in g/cm}^2\text{/weeks)} \quad \text{(Eq. 1)}$$

CGR is the ability of plants to produce dry materials that assimilate each unit of land area at each unit of time, which is stated in

$$CGR = \frac{1}{G} \times \frac{W_2 - W_1}{t_2 - t_2}, \text{ (in g.m}^{-2}\text{/weeks)} \quad \text{(Eq. 2)}$$

Where, W_1 = total dry weight per plant at the time of t_1. W_2= Total dry weight per plant at the time of t_2. LA_1= Total leaf area per plant at the beginning. LA_2 = Total leaf area per plant at the time of t_2. G = the area of land overgrown with plants. t_1 = harvest time in the beginning. t_2 = harvest time in the end.

Statistical Analysis

Observational data were analyzed using analysis of variance (ANOVA) with the SAS 9.1 program. If the treatment had a significant effect, then to know the difference between treatments was done using Duncan's new multiple range tests (DMRT) at 5% significance level (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Analysis of Variance

Based on the analysis of variance, the dose factor of the endophytic bacteria consortium had a significant difference (Table 1) in Leaf area index (LAI), Leaf area duration (LAD), Net assimilation rate (NAR) and Crop Growth Rate (CGR), but there was no significant difference in the variety of rice varieties. There was no interaction between the dose of the endophytic bacteria consortium and the varieties tested on LAI, LAD, NAR and CGR.

Leaf Area Index (LAI)

Lea area index is the ratio between the leaf surface area and the land surface area overgrown with plants (Yoshida, 1981; Gardner *et al.*, 1991). Based on Table 2, the leaf area index with a consortium of 20 and 30 L/ha/application doses of endophytic bacteria was not different from that without a diazotroph

Table 1. Analysis of variance of leaf area index (LAI), leaf area duration (LAD), net assimilation rate (NAR) and crop growth rate (CGR).

Parameters	Endophytic bacteria consortium (D)	Varieties of paddy (V)	D x V
LAI	4.31**	0.63ns	2.20ns
LAD	6.66**	0.79ns	2.18ns
NAR	5.40**	0.14ns	1.12ns
CGR	3.29**	0.21ns	1.17ns

*and**: Significant at P=0.05 and P=0.01 levels of significance, respectively, ns = Not Significant at P=0.05.

endophytic bacteria consortium, while the dose of 40 L/ha/application achieved the largest leaf area index and was different from other treatments. This is because a consortium of endophytic bacteria is able to fix N_2 so that the leaf size is maximized. Kumar and Rao (2012), Diazotrophic endophytic bacteria are able to reduce N2 to ammonia (NH3). Endophytic bacteria are microbes that live in plant tissues (Rao, 2007). A consortium is a mixture of two or more bacterial isolates of different types. Bashan (1998) stated that a synergistically interacting microbial consortium was able to give better results than a single microbial consortium. Colonization of endophytic rhizobia in plants can increase the growth and yield of rice, wheat, corn and barley (Chi *et al.*, 2010).

Rao (2007) added that the nitrogen fixing process was initiated by the presence of aerenchyma in rice plants to transfer air from the atmosphere to the rhizosphere. The air transferred to the root zone contains sufficient N_2 for N_2-fixing activity by bacteria in the rhizosphere. These bacteria belong to the genera Beijerincka, Azotomonas, Pseudomonas, Flavobacterium, Azozpirillum, Azotobacter.

Leaf Area Duration (LAD)

Leaf area duration is the length of time the leaves last on a plant. Based on the Duncan 5% test (Table 2), the leaf area duration with the dose of the endophytic bacteria consortium 40 L was the highest and different from the dose of the other endophytic bacteria consortium and the doses of 10 and 20 L/ha / application were not different from the control (0 L/ha/application). This is because the endophytic bacterial consortium acts as a plant growth regulator so that the leaves are not easily degraded by chlorophyll. Hasanuzzaman *et al.* (2017), the presence of endophytic bacteria in plant tissue is known to stimulate plant growth. The ability of bacteria to penetrate the internal tissues of plants can be caused by the presence of extracellular enzymes in the form of cellulases produced by these bacteria. After penetrating, endophytic bacteria will colonize thus inhibiting the growth of pathogenic bacteria through the mechanism of competition for space and nutrients (Pal *et al.*, 2012).

Glick (2020) added that this type of bacteria creates a complex relationship with the host plant where this bacterium acts as a plant growth promoter. These bacteria are associated with plants in increasing crop production and soil fertility. Ali (2017) stated that microbial components in the plant endosphere and rhizosphere form beneficial associations with plants that can increase plant productivity.

The ability of endophytic bacteria to penetrate into the internal tissues of plants

Table 2. Leaf area index, leaf area duration, net assimilation rate and crop growth rate of various dosage of endophytic bacteria consortium of paddy at 6-8 WAP.

Dosage of endophytic bacteria consortium (per ha/application)	Parameter			
	Leaf area index	Leaf area duration (cm^2/week)	Net assimilation rate (g/cm^2/week)	Crop growth rate (g/cm^2/week)
0 L	8.64b	6838.6b	3272b	1704b
20 L	8.09b	6463.3b	2732b	1847b
30 L	9.08b	7266.3b	2515b	1817b
40 L	10.90a	8731.7a	4756a	2663a

Figures in the same column followed by the same letters indicate no significant difference based on DMRT at P=0.05 level of significance; WAP: Week after planting.

can be caused by the presence of extracellular enzymes in the form of cellulases produced by these bacteria (Hasanuzzaman, 2017). After penetrating, endophytic bacteria will colonize thereby inhibiting the growth of pathogenic bacteria through the mechanism of competition for space and nutrients (Pal *et al.*, 2012).

Net Assimilation Rate (NAR)

Net assimilation rate (NAR) is dry matter production per unit leaf area per unit time. This gives an understanding that leaves and light are the determining factors in the formation of assimilation results. The wider the leaf area and the more light that can be absorbed will determine the amount of assimilation results.

Based on the Duncan 5% test (Table 2), the net assimilation rate with the dose of the endophytic bacteria consortium 40 L/ha/ application was the highest and different from the dose of the other endophytic bacteria consortium and the doses of 10 L and 20 L were not different from the control (0 L/ha/ application). This is because the higher the dose given, the more bacteria that play a role in the uptake of N and P which are useful in the assimilation process. Bodenhausen *et al.* (2014) reported that the application of a consortium of diazotroph endophytic microbes increased N uptake, which was followed by an increase in P and K uptake in rice plants. The presence of N_2 fixation from the applied microbes, increases plant growth. Gardner *et al.* (1991) stated that the relationship between bacteria and higher plants has the capacity to reduce atmospheric N2 to ammonia (NH3).

In addition, the ability of microbes to dissolve P will increase 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 the building block of amino acids, amides, nitrogenous bases such as purines and proteins and 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.

Knief *et al.* (2012) reported that bacterial isolates from the rice phyllosphere have been identified and have potential beneficial interactions with rice plants, such as promoting plant growth, fixing nitrogen or producing plant hormones. Phylosphere microbes can increase plant growth, produce phytohormones (Morris, 2001) and bind N_2 (Bodenhausen *et al.*, 2014).

Meanwhile Chi *et al.* (2010) reported that apart from being a biological binder, the presence of rhizobia in roots has many other benefits, namely the production of plant hormones such as IAA and GA. This hormone stimulates the expansion of the surface and root architecture, thereby increasing the growth strength of rice seedlings, the efficiency of phosphate absorption, phosphate solubilization, and increasing root respiration. Hasanuzzaman *et al.* (2017) showed that the microbial consortium produced the hormones auxin (IAA), gibberellins (GA3) and cytokinins. The phytohormones contained in the microbes that make up the consortium stimulate the formation of roots, so that nutrient uptake is more effective. Naturally, roots act as channels to supply nutrients and water from the soil to plants and are the site of synthesis and exchange of a number of hormones in plants. Normal root growth ensures normal shoot development.

Endophytic bacteria can act as biological fertilizers, rhizoremediators, phytostimulators and protect plants from abiotic stress and stress. Endophytic bacteria assist the availability of nutrients for their hosts through nitrogen fixation and the ability to solubilize phosphate (Lugtenberg and Kamilova, 2009), provide Fe elements through siderophores, and produce phytohormones such as IAA, gibberellins and cytokinins (Miller and Berg 2009).

Crop Growth Rate (CGR)

Crop growth rate (CGR) is the increase in plant weight per unit area of land occupied by plants in a certain time (Gardner *et al.*, 1991). Based on the Duncan 5% test (Table 2), the crop growth rate with the dose of the endophytic bacteria consortium 40 L/ha/ application was the highest and different from the dose of the other endophytic bacteria consortium and the doses of 10 L and 20 L were not different from the control (0 L/ha/ application). The increasing dose of endophytic

Table 3. Leaf area index, leaf area duration, net assimilation rate and crop growth rate of various varieties of paddy at 6-8 weeks after planting.

Varieties	Parameter			
	Leaf area index	Leaf area duration (cm^2/week)	Net assimilation rate (g/cm^2/week)	Crop growth rate (g/cm^2/week)
Situbagendit	9.21a	7373a	3276a	1936a
Mekongga	9.48a	7594a	3205a	1969a
Ciherang	8.84a	7007a	3475a	2117a

Figures in a column followed by the same letters indicate no significant difference based on DMRT at .05 significance level.

bacteria consortium will increase the rate of growth of rice plants. Eljounaidi (2016) said that endophytic bacteria increase growth by establishing synergistic interactions with host plants or antagonistic interactions with soil pathogens. Endophytic bacteria are also known as plant growth-promoting rhizobacteria (PGPR). Several studies have defined endophytic bacteria as bacteria that do not harm plants but can be isolated on surface sterilized plant materials (Liu, 2017).

As plant growth promoters, endophytic bacteria can act as biological fertilizers, rhizoremediators, phytostimulators and protect plants from abiotic stress and stress (Induced Systemic Tolerance). Endophytic bacteria assist the availability of nutrients for their hosts through nitrogen fixation and the ability to solubilize phosphate (Lugtenberg and Kamilova 2009), provide Fe elements through siderophores, and produce phytohormones such as IAA, gibberellins and cytokinins (Miller and Berg, 2009).

Endophytic bacteria as biological control agents have advantages over other biological control agents because of their presence in plant tissues, so they are able to withstand biotic and abiotic stresses (Hallmann *et al.*, 1997). Several types of endophytic bacteria, apart from being biological control agents, are also plant growth promoters, such as *Burkholderia cepacia*, *P. fluorescens* and *Bacillus* sp. *Burkholderia* sp. PsJN strain was able to stimulate the growth of grapevines (*Vitis vinifera* L.) (Compant *et al.*, 2005). *Bacillus* sp. can induce resistance of cotton plants to sprouting disease caused by *Rhizoctonia solani* by increasing plant defense enzymes (Rajendran and Samiyappan, 2008).

Based on Duncan's test with a significance level of 5% (Table 3), the leaf area index, leaf area duration, net assimilation rate and crop growth rate of the three varieties, namely situbagendit, mekongga and ciherang were not differed, because all parameters were more influenced by the genetic nature of a variety than by environmental factors (external factors), namely a consortium of endophytic bacteria. According to Gardner *et al.* (1991) the character of a variety is more determined by genetic factors compared to the influence of external factors, namely environmental factors including the provision of a consortium of endophytic bacteria.

CONCLUSION

In conclusion, our study found that the dosage of endophytic bacteria consortium 40 L/ha/application increase leaf area index, leaf area duration, net assimilation rate and crop growth rate for lowland rice cultivated in rainfed rice fields, can use the Situbagendit, Mekongga and Ciherang varieties. In future research on rice cultivation in rainfed rice fields, it is better to use the dosage of endophytic bacteria consortium 40 L/ha/ application with application every two weeks starting at the age of 2 weeks after planting until entering the generative phase.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of National Education of the Republic of Indonesia, which has provided funding for this research through the Research Grant of the Directorate General of Higher Education of the Ministry of National Education of the Republic of Indonesia Decree Number 158/E5/PG.02.00.PT/2022 with Contract Agreement number 007/ LL6/ PB/AK.04/2022.

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