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Potential of rhizobacterial consortium in increasing area and weight of mulberry leaves (*Morus indica*)

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Abstract. *Mustaka Z, Patandjengi B, Alam G, Melina. 2022. Potential of rhizobacterial consortium in increasing area and weight of mulberry leaves (*Morus indica*). Biodiversitas 23: 1368-1373.* Rhizobacteria are a group of bacteria that live saprophytically in the rhizosphere area. Some of them can act as plant growth promoters and as biocontrol agents against diseases to increase crop yields. This study was conducted to find potential of the rhizobacteria consortium in various treatments to increase the area and weight of mulberry (*Morus indica* L.) leaves. Results showed that the rhizobacteria consortium formulation had a significant effect on increasing leaf area and weight. The percentage increase in leaf area in the treatment using the MKR15 rhizobacteria consortium was better at 39.28% (2,800 mm^2) when compared to the control MK0t (1,700 mm^2), and the percentage increase in MKR15 leaf weight (74.80 g) was 54.54% higher compared to control MK0t (34 g). With these results, it is clear that the rhizobacteria consortium as PGPR affects plant growth and nutrition in a very specific way involving bacterial components that induce plant responses. Molecules of PGPR can affect plants in complex mechanisms, sometimes affecting plant growth and nutrition, and resistance simultaneously.

Keywords: *Bombyx mori*, cocoon, consortium, mulberry, rhizobacteria

INTRODUCTION

The economic value of silkworms in South Sulawesi is quite high and has good prospects for development. The economic prospect of silkworms begins with cocoons that enclose the pupa as a source of silk fiber. South Sulawesi Province is the largest silk producer in Indonesia, with about 90% of the production of silk thread for the whole country. For the government of South Sulawesi, this commodity is a priority and gets the main attention in developing the economy of rural communities, eradicating poverty, and raising their welfare by opening job opportunities through employment, increasing economic growth, and obtaining regional income (Sadapotto et al. 2021).

Silkworm farming in South Sulawesi is an activity that has been practiced by people in South Sulawesi since the 1950s. The availability of extensive land, good weather, and socio-cultural communities support the cultivation of caterpillars. So that this business can be said to be a legacy that has been passed down from generation to generation. The silk company is one of the people's economic activities of South Sulawesi, which is important to be developed through small and medium enterprises (SMEs). This silk is also part of economic life, tradition, and culture (Iwang and Sudirman 2020).

Silkworm farming cannot be separated from the cultivation of mulberry plants. Mulberry plants (*Morus* sp.) are the only food for the *Bombyx mori* L silkworm, and caterpillars fed with mulberry leaves with good nutrition will resist disease and produce better cocoons (Andadari et al. 2017). The availability of mulberry plants that meet in terms of quality and quantity is one of the determining factors for the continuity of silkworm maintenance (Nurhaedah et al. 2015). The production of mulberry leaves determines the growth and health of the caterpillars and affects the quality of the cocoons produced. So that directly or indirectly will affect the quality and quantity of silk thread produced, in addition to other factors such as caterpillar seeds, methods and means of maintenance, and environmental conditions.

The quality of mulberry leaves is determined by the leaves' surface area and the mulberry leaves' weight. Caterpillars fed mulberry leaves with good nutrition will be more resistant to disease and produce more cocoons. The quality and quantity of mulberry plants as feed greatly affect the growth of silkworms (Murthy et al. 2013). One way to improve the quality of mulberry leaves is by fertilizing them. Mulberry leaves directly affect the quality of the cocoons produced, while the quality is closely influenced by the quality of the cocoons spun (Kumar et al. 2014). Fertilization with organic matter will improve the structure, aeration, and water holding capacity of the soil, regulate soil temperature, and increase the availability of nutrients to maintain growth and encourage additional plant leaf production.

Plant growth-promoting rhizobacteria (PGPR) are bacteria that live and colonize in the rhizosphere and can stimulate plant growth. PGPR can improve health and fitness and increase crop yields (Elango et al. 2013; Singh et al. 2013). PGPR availability in the soil is very important in increasing plant growth and production (Selviana 2020). PGPR extracellular products can directly stimulate plant genetic and molecular pathways, leading to increased plant growth and induction of plant resistance and tolerance (Rosier et al. 2018). Research efforts in developing microbial formulations that can provide benefits to plants and the environment are widely supported by agricultural companies (Dessaux et al. 2016).

This approach can allow the presence of synergistic products to provide benefits and a positive response to the recovery of rapidly changing environmental conditions (Timmusk et al. 2017). The rhizosphere can also act as plant protectors, increase legumes and non-legumes and stimulate plant defenses (Subramanian et al. 2016). PGPR has become an invaluable finding for plant defense that is more effective than synthetic derivatives (Bektas and Eulgem 2014; Wiesel et al. 2014). In connection with the above, this study was intended to examine the potential of the rhizobacteria consortium in improving the quality of mulberry leaves, namely leaf area and weight.

MATERIALS AND METHODS

The research was carried out from June to September 2021 at the Social Forestry and Environmental Partnership Center of South Sulawesi Province, Gowa Regency and the Plant Disease Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia, for the manufacture of a microbial consortium and measurement of plant area and weight.

The materials used in this study were mulberry (*Morus indica* L.), sterilized growing media, a consortium of microbes consisting of *Lactobacillus* sp., *Bacillus subtilis*, *Actinomyces* sp., *Azotobacter* sp., *Bacillus polymyxa*, *Pseudomonas fluorescens*, and *Rhizobium* originating from the plant disease laboratory, Faculty of Agriculture, Hasanuddin University.

The soil used was first sterilized by transferring it in a sterile box and kept at 100°C for 1 hour. Then left it for 1 day and sterilized again in some condition. Then the soil was put into a polybag measuring 35 x 35 cm as much as 5 kg.

Prosedur

The experimental design used was a Randomized Block Design with 5 (five) treatments, namely:

MK0t = Control, without fertilization
MKR5 = 5% rhizobacteria consortium
MKR10 = 10% rhizobacteria consortium
MKR15 = 15% rhizobacteria consortium
MKR20 =. 20% rhizobacteria consortium

Each treatment consisted of 10 (ten) replications, so the total was 50 plants. The length of the cuttings used was 30 cm.

Observed parameters: (i) Leaf area increase rate: measurement of leaf area increases every week. (ii) Leaf area of plants: measurement of leaf area by taking the largest leaf. Measurement of leaf area every week. (iii) Plant leaf weight: weigh all the mulberry leaves from each fresh sample plant.

Observations were made from the first day after planting. To see the effect of the treatment, an analysis of variance was performed on the collected data. If the results of the ANOVA showed a significant effect on the variables being tested, then the data analysis continued to test the average difference of each treatment with Duncan's multiple significant difference test.

RESULT AND DISCUSSION

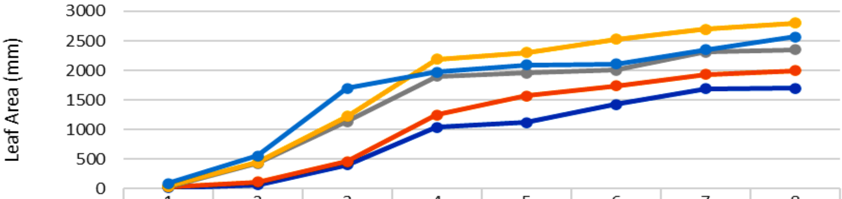
Mulberry leaf area increase rate per week

Based on observations that have been made on plant growth, it can be seen that there is a significant difference in the treatment using a consortium of rhizobacteria and controls. The comparison of plant growth can be seen in Figure 1.

All treatments applied by the rhizobacteria consortium showed a faster leaf shoot release rate than the control. Plants synthesized all 20 amino acids common to protein synthesis. The main elements of amino acids are carbon, hydrogen, oxygen, and nitrogen. The N compounds transported from roots to shoots and leaves via xylem are nitrates and amino acids. The percentage of nitrate-N in xylem sap depends on the plant species, growth stage, and environmental conditions. A root environment that contains lots of nutrients for plant roots will stimulate plant growth (Elango et al. 2013). The use of PGPR is an alternative to reduce the use of chemical fertilizers and their impact on the environment, improving soil quality and crop productivity (Sudewi et al. 2020). PGPR can be used as a biostimulant, biological fertilizer, and biocontrol agent in increasing plant growth and is an attractive strategy to replace synthetic fertilizers that are environmentally friendly and inexpensive.

Based on the tests that have been carried out, the rhizobacteria consortium treatment has a significant effect on the variables of leaf area and leaf weight. The treatment of the rhizobacteria consortium significantly affected the growth variables of the mulberry plant.

Figure 1. Comparison of the growth of mulberry plants that were applied by (A) the rhizobacteria consortium compared to (B) the control at the observation 1 week after planting



	1	2	3	4	5	6	7	8
MK0t	19,4	69,33	401,6	1043	1120,67	1426,23	1689,67	1700,21
MKR5	35,86	111,13	460,26	1246	1570,23	1740,32	1933,23	2000,66
MKR10	36,53	425,46	1133,33	1903,53	1960,67	2001,67	2314,66	2350
MKR15	44,26	447,2	1225,4	2192,86	2300	2524,86	2699	2800
MKR20	89,73	560,33	1700,23	1973,6	2091,3	2110	2353,3	2566,67

Figure 2. The rate of increase in leaf area of mulberry plants with rhizobacteria consortium treatment for 8 weeks of observation

Figure 2 shows that until the third week of observation, the highest rate of leaf area increase was found in the MKR20 treatment. However, in the fourth week of observation, the rate of leaf area increased from the MKR15 treatment was higher than the other treatments. And this lasted until the eight week observation. This was caused by the interaction of plant roots with the given rhizobacteria. Rhizobacteria is a functional group as a provider of nutrients in the soil, fixing nutrients and facilitating the availability of nutrients in the soil.

The percentage increase in leaf area in the treatment using the MKR15 rhizobacteria consortium when compared to the control was 39.28%. Rhizobacteria applied to the treatment acted as Plant Growth Promoting Rhizobacteria (PGPR). PGPR is able to produce plant hormones such as auxin, gibberellins and cytokinins as well as phosphate solvents and nitrogen-fixing bacteria converting free nitrogen from the atmosphere into ammonia (Ahmad and Husain 2017; Sukmawati et al. 2020). Nitrogen fixing bacteria that live in the rhizosphere are microorganisms that can increase the availability of N for plants in a non-symbiotic manner. Nitrogen-fixing bacteria also support growth through the production of growth hormones (Haerani et al. 2021)

As a biofertilizer for plants, PGPR is a biological fertilizer capable of providing nutrients for plant growth. Plants can grow optimally and have resistance to pests, diseases, and stresses from the environment. In addition, the mechanism between PGPR and plants can also influence plants to produce exudate around the rhizosphere. Zhou et al. (2016) reported that the mechanism of PGPR in its interaction with plants is to stimulate plants to exudate the metabolites needed by microbes as a producer of nutrients for plants. This mechanism indirectly serves to provide benefits for plants in inviting rhizobacteria as PGPR around the roots.

Mulberry leaf weight

The results of analysis of variance showed significant differences in the value of area and weight of mulberry leaves. Figure 3 shows that the treatment with the rhizobacteria consortium had a higher leaf weight value than the control. More number of leaves and wider leaf area. This is because the rhizobacteria consortium used contains *Lactobacillus*, *B. subtilis*, *Actinomyces*, *Azotobacter*, *B. polymyxa*, *P. fluorescens*, and *Rhizobium*.

Bacillus sp. has many potentials that are capable of producing IAA, dissolving phosphate, secreting siderophores, and acting as a biocontrol agent by inducing plant immune systems and producing antibiotics. Prashar et al. (2014) reported that cyclic lipopeptide bacterial antibiotics, especially surfactin, were produced in excess by *Bacillus* spp. *Bacillus* groups are found in the rhizosphere up to 95%. Many *B. subtilis* and *B. amyloliquefaciens* groups produced sufficient surfactin to provide disease protection through ISR (Cawoy et al. 2014).

The Actinomycetes group can also dissolve Fe-P and Al-P. These phosphate solubilizing bacteria also play a role in energy transfer, protein preparation, coenzymes, nucleic acids, and other metabolic compounds that can increase P uptake activity in P-deficient plants.

Azotobacter can stimulate plant growth because of its ability to fix nitrogen, it turns out that these two microbes can also produce growth hormones such as auxins, gibberellins, and cytokinins. Each hormone produced greatly affects the life of the plant. Anindya and Zulaika (2016) reported that the isolates of Azotobacter A1b, A3, A6, A9, and A10 isolated from the ITS Eco Urban Farming Land were isolates that had potential as biofertilizers. Hindersah et al. (2018) reported that the application of N-fixing rhizobacteria Azotobacter increased growth, thereby reducing the use of NPK fertilizers.

Figure 3. Comparison of mulberry leaf weight in control (MK0t), treatment with 15% rhizobacteria consortium (MKR15), and treatment with 20% rhizobacteria consortium (MKR20)

The rhizobacteria consortium used also contains *Pseudomonas* sp., which is a bacterium capable of fixing N and dissolving P and *Rhizobium* bacteria which are expected to stimulate growth and produce phytohormones. Bhattacharyya and Jha (2012) stated that rhizobacteria inoculation contributed to 20-50% of the total nitrogen requirement of plants from the N2 fixation process.

Majeed et al. (2018) reported that of the many rhizobacteria, the following genera that have shown potential as biological fertilizers or growth promoters are *Azospirillum, Azotobacter, Pseudomonas, Acinetobacter Beijerinckia, Derxia, Herbaspirillum, Burkholderia, Gluconacetobacter, Enterobacter, Bacillus, Rahnella, Alcaligenes*, *Klebsiella*, *Lysobacter*, and *Paenibacillus*. Several groups of bacteria were reported to fix N and dissolve P, namely *Pseudomonas, Bacillus, Serratia, Paenibacillus,* and *Micrococcus* (Kenneth et al. 2019).

The use of PGPR has proven to be an environmentally friendly way to increase crop yields by facilitating plant growth. It also reduces production costs and environmental impacts associated with chemical fertilization. Testing the effect of giving a consortium of rhizobacteria on leaf weight is presented in Table 1.

The results of the observations show that mulberry leaf weight has differences in each treatment. The highest leaf weight was Treatment MKR15 with a weight of 74.80 grams, and the lowest was control with a leaf weight of 34.00 grams. This difference was significant where the treatment with the MKR15 rhizobacteria consortium gave an increase in leaf weight of 54.54% higher than the control. This answers the hypothesis that providing a consortium of rhizobacteria can strengthen the quality of mulberry leaves as feed for silkworms.

Discussion

Siahaan et al. (2013) reported that the use of microbial consortia tends to give better results than the use of single isolates because it is expected that the enzyme work of each type of microbe can complement each other in order to survive using the nutrient sources available in the carrier media. The rhizobacteria consortium is a mixture of microbial populations in the form of communities that have cooperative, commensal, and mutualistic relationships. Community members who have a relationship will associate, so that they are more successful in degrading chemical compounds than single isolates.

The rhizobacteria consortium in this case produces a role for plant growth that is in synergy, so that it can support the growth of single isolates and others. Bacterial consortium is a collection of bacteria that work together to form a community, to produce significant products (Arora 2014). The consortium is a combination of several biological agents that are mutually compatible and synergistic. The advantage of a consortium of biologic agents is that the mechanisms are more diverse and mutually supportive (Munif et al. 2019). The relationship between the consortium bacteria in a state of sufficient substrate will not interfere with each other, but synergize with each other to produce higher reform efficiency during the plant growth process. The mechanism of synergism between isolates in the consortium was due to several factors, including: (i) one member of the genus was able to provide one or more nutritional factors that other members of the genus could not synthesize, (ii) one member of the genus was unable to degrade organic matter. Certain species will depend on members of the genus that are able to provide the results of the degradation of the organic matter, (iii) one member of the genus protects other members of the genus that are sensitive to certain organic materials by reducing the concentration of toxic organic matter by producing specific and non-specific protective factors (Deng and Wang 2016).

Plant growth which includes cell division, cell elongation, cell formation, and the formation of new tissues requires carbohydrates where carbohydrate synthesis is heavily influenced by the ability of plants to carry out photosynthesis. All metabolic processes that occur in plants are influenced by the availability of nutrients. Application of rhizobacteria to mulberry plants consistently increased plant growth. Tuhuteru et al. (2019) reported the results of their research that rhizobacteria played a role in increasing the number of roots and root weight of shallots. This role causes the absorption of roots to be wider and able to reach farther places. So that nutritional needs are met more quickly, and the use of artificial fertilizers can be reduced.

Plants evolve together with microorganisms in a symbiotic relationship to survive in the ecosystem (Werner et al. 2014). The interactions that occur between plants and endophytic bacteria are mutualistic interactions (Afzal et al. 2019; Firdous et al. 2019). The selected Rhizobium bacteria stimulated growth and produced phytohormones, namely cytokinins and auxins. Rhizobia mutualism symbiosis in fixing nitrogen for plants, produces auxin as the main regulator of growth, development, plant response to stress, and produces phytohormones (Udvardi and Poole 2013; Liu et al. 2014; Spaepen 2015).

Table 1. The results of the measurement of the potential of rhizobacterial consortium fertilizers in optimizing the quality of mulberry leaves

Code	Treatment	Average Leaf Weight (g)
MK0t	Control	34,00a
MKR5	Rhizobakteria Consortium 5%	54,00b
MKR10	Rhizobakteria Consortium 10%	65,00c
MKR15	Rhizobakteria Consortium 15%	74,80d
MKR20	Rhizobakteria Consortium 20%	68,80c

Note: The rhizobacteria consortium applied at 8 weeks after planting. Value means St Dev from different applications. The same letter in the same column does not differ significantly at the 0,05 level.

Mardiah et al. (2016) reported that the application of rhizobacteria to seeds increased the growth and yield of red chili (*Capsicum annum* L.). The application of rhizobacteria was also able to increase the growth of bud chips in sugarcane (*Saccharum officinarum* L.), increase growth and control anthracnose disease in chili plants (Sulistyoningtyas et al. 2017; Wiyono et al. 2019). Plant interactions with microorganisms play an important role in plant vigor and survival (Busby et al. 2017). Plant phytohormones regulate various developmental stages to coordinate defense responses (Thaler et al. 2012). Phytohormones such as salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) are responsible for plant defense responses such as systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is induced by microbial associated molecular patterns (MAMP). When plants recognize the presence of microbial components through compounds such as flagella or chitin, plants will create a line of defense through the membrane pattern recognition receptor (PRR), then MAMP will trigger immunity. If this fails, the next line of defense is effector triggered immunity (ETI), leading to programmed cell death (Fu and Dong 2013). Based on the greenhouse test, six isolates of *Bacillus* sp. can suppress nematode penetration by up to 85% in coffee plants. In addition, two rhizobacteria, namely *Pseudomonas diminuta* and *B. subtilis* isolated from coffee plants can also suppress *Pratylenchus coffeae* populations by up to 50% in arabica coffee plants (Yulitaasary et al. 2017). Biological agents used as biological control generally come from endophytic bacteria and rhizobacteria (Oliveira et al. 2019).

The application of a consortium of rhizobacteria, where one of the bacteria used is a phosphate solvent, which can help the absorption of nutrients by plant roots. One of the physiological characteristics of rhizobacteria related to their role as plant growth promoters is their ability to dissolve phosphate and produce growth hormones (Budiyani et al. 2018; Sugianto et al. 2019; Asria et al. 2020).

Nitrogen, P and K are essential macronutrients for plant growth and increased production. However, it is not freely available to plants (Meena et al. 2014). The largest reservoir of N is in the form of atmospheric N (78%), but in a biological form it is not available to plants (Ali 2016). Phosphorus in soil is present as P in solution, insoluble organic P and insoluble inorganic P. The availability of phosphate in the soil is low because it binds to iron, aluminum and calcium as insoluble P. The concentration of dissolved K in the soil is very low (1% to 2%) because K is mainly present as insoluble rock, silicate minerals, and other deposits (Parmar and Sindhu 2013). Rhizobacteria are able to convert forms that are not available to those that are available for plant absorption (Meena et al. 2014). Phosphate is indispensable in the metabolic process of plants, among others, to stimulate plant growth, root development, fruit growth, improve quality and strengthen resistance to pests and diseases (Handayani et al. 2019). Bacteria that have the ability to dissolve P have the potential as biological fertilizers (Pudjiwati et al. 2019).

The conclusion was that the percentage increase in leaf area in the treatment using the MKR15 rhizobacteria consortium was better at 39.28% (2,800 mm^2) when compared to the control MK0t (1,700 mm^2) and the percentage increase in leaf weight MKR15 (74.80 g) reached 54.54% higher than the control MK0t (34 g). With these results, it is clear that the rhizobacteria consortium as PGPR affects plant growth and nutrition in a specific way involving bacterial components that induce plant responses. Molecules of PGPR can affect plants in complex mechanisms, sometimes affecting plant growth and nutrition and resistance simultaneously.

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