Physiological Response of Shallots (*Allium ascalonicum* L.) to Inoculation of Diazotrophic Bacteria

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Abstract. This research was aimed to study the effect of diazotrophic bacteria application on nitrogen uptake, chlorophyll content and rate of assimilation in shallot. The research was carried out in a screen house Experimental Farm and the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto from February 2021 to April 2021. This study was arranged using a Randomized Completely Block Design (RCBD). The treatment in this research consisted of I1 = LCR3, I2 = LAR5, I3 = LBR1, I4 = LAZ2, I5 = LAZ3, I6 = LCA1, I7 = LAR3, I8 = LAA5, and I9 = LAA4. There are 9 treatments and a control each repeated 3 times. The observed variables consisted of leaf area, shoot dry weight, root dry weight, total root length, root volume, root shoot ratio, chlorophyll content, relative growth rate. The data obtained was analyzed using Analysis of Variance (ANOVA), if they were significantly different then further tested using DMRT at the 95 percent confidence level. The result showed that application of diazotroph bacteria had a significant effect on variables such as net assimilation rate, and nitrogen uptake. The treatment of isolat I2 (LAR5) resulted in the highest number of leaf area and nitrogen uptake variables.

Keywords: Shallots · Nitrogen · Diazotrophic · Bacteria

1 Introduction

Shallots (*Allium ascalonicum* L.) are one of the vegetable commodities that have been intensively cultivated by farmers for a long time [1]. This is because red onions have many benefits. Besides being used as a kitchen spice, shallots can also be used as traditional medicine [2]. The productivity of shallots in Indonesia in 2012–2018 tends to be static and does not increase significantly. National production of shallots in 2018 reached 1.50 million tons. Shallot production in Central Java is 0.45 million tons, in East Java 0.37 million tons, in the West Southeast 0.21 million tons, in West Java 0.17 million tons, and in West Sumatra 0.11 million tons [3].

The growth and yield of shallots are closely related to their growth environment, especially regarding soil conditions [4]. The factor causing low productivity is the low level of soil fertility [5]. Efforts to increase the production of shallots can be done by

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applying nitrogen fertilizer [6]. The use of N fertilizer is still less efficient on agricultural land because the nitrogen element has a fairly high solubility, so that the N loss is greater than other nutrients [7]. Excessive or inappropriate use of N fertilizer in the long term can disrupt the environment [8]. Another effort to increase production and reduce the use of inorganic nitrogen fertilizers is the application of nitrogen fixing bacteria. Efforts to utilize diazotroph bacteria (N_2 fixing) as biological fertilizers have been widely carried out, but due to the inadequate understanding of microbial ecology that has been applied, so far these efforts have not provided consistent results [9].

N-fixing bacteria are often called diazotrophic bacteria which are able to use air N as a source of N for their growth. The role of bacteria in fixing air nitrogen has a large influence on the economic value of agricultural land [10]. The use of nitrogen-fixing diazotrophic bacteria has the potential to reduce the need for synthetic N fertilizers, increase production and farm income at a lower price [11]. This research was aimed to study the effect of diazotrophic bacteria application on nitrogen uptake, chlorophyll content, and rate of assimilation in shallot.

2 Materials and Methods

The research was carried out from February to April 2021 in a screen house at an altitude of ±110 m above sea level and the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Jenderal Soedirman University.

This study was a polybag experiment using a Completely Randomized Block Design with treatment of 9 types of diazotrophic bacteria isolates and 1 control which was repeated 3 times. The treatment consisted of 9 types of bacterial isolates, namely I1 = LCR3, I2 = LAR5, I3 = LBR1, I4 = LAZ2, I5 = LAZ3, I6 = LCA1, I7 = LAR3, I8 = LAA5, and I9 = LAA4. Based on this treatment, 30 experimental units were obtained. The variables observed were leaf area, shoot dry weight, root dry weight, root/shoot ratio, root volume, total root length, relative growth rate, net assimilation rate, chlorophyll content, and nitrogen uptake.

The research data were analyzed using Analysis of Variance (ANOVA). If the ANOVA results show differences between treatments, then further tested using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

3 Results and Discussion

The results showed that the leaf area parameters at the age of 14 and 42 days were significantly different among the 9 types of diazotrophic bacterial isolates used, while at the age of 28 days there was no significant difference among the 9 types of diazotrophic bacterial isolates used (Table 1). Bacterial isolates I2 (LCR3) and I7 (LAR3) from the genus *Rhizobium* sp. proven to have the best effect in increasing the leaf area of shallots compared to the genera *Azospirillum* sp. and *Azotobacter* sp. This is because *Rhizobium* sp. given is *Rhizobium* sp. effective so that it is able to provide nitrogen nutrients needed by shallot plants to carry out their metabolic activities which results in an increase in the total leaf area, where with increasing leaf area the photosynthesis process can take place properly. The genus *Rhizobium* sp. classified as symbiotic organisms with legumes, but Rhizobium can act as a non-symbiotic diazotroph in the plant rhizosphere [12]. *Rhizobium* strains from the rhizosphere of rice plants are capable of endophytic colonization of the cortex and vascular system of lateral roots. Cortical regions provide favorable conditions for diazotrophs for fixation of dinitrogen with low concentrations of oxygen and available nitrite [13]. Nitrogen is needed by the leaves so that if the N element is sufficient, the plant leaves become wider, greener, and of high quality [14].

Observations 14 day after planting (DAP), 28 DAP, and 42 DAP application of the best diazotrophic bacterial isolates for root length parameters were treatment I7 (LAR3) of the genus Rhizobium and control treatment without bacterial isolates showed the lowest results (Tables 1 and 2). One of the factors associated with plant root length is the hormone IAA. The concentration of IAA produced by LAR3 isolates was 3.51 ppm [15]. Diazotrophic bacteria are able to produce IAA phytohormones or endogenous auxins that play a role in root development and elongation [16]. IAA directly plays a role in the elongation and division of soybean plant cells [17]. The results of [18] Rhizobium sp. which was isolated from rubber plantation soil, was able to produce IAA with a concentration of 2.86 ppm, *Azospirillum* isolates at 0.0429 ppm, and *Azotobacter* isolates at 0.0517 ppm. Another factor that affects the spread of roots is the availability of water and the amount of nutrients available in the soil [19]. The results of the research [20] showed that inoculation with *Rhizobium* sp. able to increase leaf greenness, total root length, and total biomass in rice plants.

The results showed that the treatment of 9 types of diazotrophic bacterial isolates gave a significant effect on the shootdry weight the ages of 14 DAP, 28 DAP, and 42 DAP (Table 3). The shoot dry weight was influenced by the fresh weight of the shoot and the number of leaves because the leaves were the site of accumulation of plant photosynthetic results [21]. The presence of diazotrophic bacteria isolates in plant tissue is able to provide optimum and stable intake of N, producing phytohormones which also increase resistance to pathogens [9]. The presence of N is very important in plant growth because of its position in plant biochemical processes as an essential element in cell formation, protein preparation, cytoplasm, chlorophyll, and other cell components [9]. The positive effect obtained from inoculation of diazotrophic bacteria on plant dry weight was related to the ability of diazotrophic bacteria to fix nitrogen and solubilize phosphate [22].

The results showed that the treatment of 9 types of diazotrophic bacteria had a significant effect on root dry weight at the age of 14 DAP, 28 DAP, and 42 DAP (Table 3). The ability of bacteria to fix nitrogen free from the air, produce ammonia, and dissolve phosphate in the growing media can increase the dry weight of shoots and roots of soybean plants. The longer the root, the higher the dry weight of the root because the dry weight of the root is a collection of organic compounds related to the growth of root length [23].

The average relative growth rate of shallot plants in the treatment of diazotrophic bacterial isolates showed that all treatments were not significantly different or relatively the same (Table 4). This is presumably due to factors that are above the ground that can affect growth, including light. The climatic factors that affect plant growth include temperature, light, humidity, gas composition in the atmosphere, air movement, air pressure, and precipitation [24].

Table 1. Effect of application of diazotrophic bacterial isolates on leaf area and root length

Treatments	Leaf area at 14 DAP (cm^2)	Leaf area at 28 DAP (cm^2)	Leaf area at 42 DAP (cm^2)	Root lenght at 14 DAP (cm)	Root lenght at 28 DAP (cm)	Root lenght at 42 DAP (cm)
Control	82.01 b	190.21 a	262.60 c	58.46 e	117.54 b	151.51 cd
I1	128.01 b	379.65 a	535.27 ab	91.19 cd	122.90 b	145.54 d
I2	258.42 a	402.04 a	488.54 b	110.75 bc	142.25 ab	176.21 abc
I3	119.12 b	348.17 a	485.81 b	115.49 ab	146.98 ab	180.95 ab
I4	112.88 b	352.94 a	471.15 b	101.08 bcd	132.78 b	166.74 bcd
I5	115.52 b	364.59 a	504.74 ab	100.46 bcd	131.95 b	165.92 bcd
I6	152.23 b	397.81 a	493.05 b	112.19 bc	144.31 ab	178.27 abc
I7	128.92 b	782.58 a	654.92 a	134.22 a	165.51 a	199.48 a
I8	117.98 b	489.95 a	567.66 ab	91.19 cd	122.28 b	156.25 bcd
I9	99.86 b	437.94 a	558.68 ab	89.14 d	121.04 b	155.01 bcd

Note: The numbers followed by the same letter in the same column was not significantly different according to DMRT 5%

Table 2. The effect of the application of diazotrophic bacterial isolates on the root volume of shallot plants

Treatments	Root volume at 14 DAP (ml)	Root volume at 28 DAP (ml)	Root volume at 42 DAP (ml)
Control	0.20 a	0.37 a	1.07 a
I1	0.30 a	0.57 a	1.34 a
I2	0.53 a	1.03 a	2.00 a
I3	0.27 a	0.73 a	1.53 a
I4	0.30 a	0.80 a	1.73 a
I5	0.17 a	0.77 a	1.80 a
I6	0.23 a	0.57 a	1.20 a
I7	0.47 a	0.87 a	1.67 a
I8	0.23 a	0.67 a	1.47 a
I9	0.23 a	0.60 a	1.43 a

Note: The numbers followed by the same letter in the same column was not significantly different according to DMRT 5%

Table 3. Effect of application of diazotrophic bacterial isolates on shoot dry weight and root dry weight of shallots

Treatments	Shoot Dry Weight at 14 DAP (g)	Shoot Dry Weight at 28 DAP (g)	Shoot Dry Weight at 42 DAP (g)	Root Dry Weight at 14 DAP (g)	Root Dry Weight at 28 DAP (g)	Root Dry Weight at 42 DAP (g)
Control	0.32 b	0.78 b	1.26 b	0.02 b	0.09 b	0.2 d
I1	0.61 a	1.43 a	2.26 a	0.07 a	0.16 ab	0.27 bcd
I2	0.69 a	1.29 a	2.47 a	0.08 a	0.18 a	0.34 ab
I3	0.61 a	1.41 a	2.41 a	0.06 a	0.19 a	0.25 bcd
I4	0.65 a	1.28 a	2.36 a	0.05 ab	0.20 a	0.27 bcd
I5	0.55 ab	1.23 a	2.14 a	0.05 ab	0.18 a	0.31 abc
I6	0.74 a	1.39 a	2.43 a	0.05 ab	0.21 a	0.33 ab
I7	0.54 ab	1.27 a	2.37 a	0.05 ab	0.18 a	0.38 a
I8	0.58 a	1.26 a	2.29 a	0.07 a	0.21 a	0.31 abc
I9	0.49 ab	1.22 a	1.87 ab	0.06 a	0.16 ab	0.21 cd

Note: The numbers followed by the same letter in the same column was not significantly different according to DMRT 5%

Table 4. The effect of the application of diazotrophic bacterial isolates on the relative growth rate and root/shoot ratio of shallots

Treatments	Relative Growth Rate (g/g/week)	Root/Shoot Ratio at 14 DAP	Root/Shoot Ratio at 28 DAP	Root/Shoot Ratio at 42 DAP
Control	0.64 a	17.50 a	10.18 a	6.49 a
I1	1.00 a	13.72 a	9.07 a	8.27 a
I2	1.07 a	8.84 a	7.53 a	6.99 a
I3	1.07 a	11.58 a	7.69 a	10.16 a
I4	1.06 a	16.25 a	7.46 a	8.84 a
I5	1.02 a	11.26 a	7.24 a	7.36 a
I6	1.06 a	17.05 a	6.71 a	7.74 a
I7	1.13 a	11.72 a	10.85 a	6.28 a
I8	1.06 a	8.43 a	6.14 a	8.00 a
I9	0.88 a	9.04 a	8.30 a	9.11 a

Note: The numbers followed by the same letter in the same column was not significantly different according to DMRT 5%

Table 5. Effect of application of diazotrophic bacterial isolates on chlorophyll content, net assimilation rate and nitrogen uptake of shallot plants

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Net Assimilation Rate (g/cm^2/week)	Nitrogen Uptake (g/palnt)
Control	0.73 a	0.27 a	7.58 b	0.03 c
I1	0.84 a	0.32 a	14.90 a	0.07 ab
I2	0.73 a	0.27 a	16.09 a	0.07 a
I3	0.80 a	0.30 a	15.44 a	0.07 ab
I4	0.76 a	0.28 a	15.10 a	0.07 ab
I5	0.70 a	0.29 a	14.30 a	0.06 ab
I6	0.80 a	0.32 a	15.86 a	0.07 ab
I7	0.63 a	0.30 a	16.86 a	0.07 ab
I8	0.75 a	0.32 a	15.37 a	0.06 ab
I9	0.69 a	0.28 a	12.20 ab	0.06 ab

Note: The numbers followed by the same letter in the same column was not significantly different according to DMRT 5%

The results showed that the application of 9 types of diazotrophic bacterial isolates did not significantly affect the content of chlorophyll a and b in shallot plants (Table 5). Statistically there was no significant difference in the treatment of bacterial isolates on the chlorophyll content of shallot plants. This is thought to be due to the light factor. The light affects plants in photosynthesis, chlorophyll synthesis, phototropism, and stomata opening [24]. This is not in accordance with the research conducted by [22] that the application of diazotroph bacteria can increase the chlorophyll content of corn plant leaves. The content of chlorophyll a and b is not only influenced by light intensity. Genetic factors also affect the optimal rate of plant photosynthesis. Each type of plant responds differently to the intensity of light received with a different range of photosynthetic rates. The intensity of light affects the activation of light harvesting genes, causing each plant species to have a different response in activating the harvesting pigment genes according to the quantity of light received [25].

The net assimilation rate was associated with leaf area and dry matter resulting from a certain period [26]. The research data showed the leaf area of large shallot plants. This is related to the role of N which further stimulates plant growth so that plants are larger in size. Plants with a larger crown size will produce more photosynthate because they have photosynthetic active leaves and roots that relatively absorb more nutrients and water than smaller plants [19].

Based on the analysis of N uptake, the treatment given by diazotrophic bacteria from the genera *Rhizobium, Azotobacter* and *Azospirillum* was proven to be able to meet the N needs of shallot plants. It is known from the value of nitrogen uptake in the treatment given diazotroph bacteria showed that the N uptake of shallot plants was included in the high and very high categories except the control had a low nitrogen uptake. This is in accordance with [15] that *Rhizobium*'s N_2 fixing ability is higher than *Azotobacter* and

Azospirillum, which is 87.15–88.55 ppm. *Azotobacter* 3.50–6.65 ppm, and *Azospirillum* were able to fix nitrogen at 7.00–14.00 ppm. The application of biofertilizers with endophytic bacteria *Gluconacetobacter diazotrophicus* could meet the N needs of sugarcane plants with high N uptake values [7]. Another study also stated that high concentrations of endophytic bacteria in biofertilizers significantly increased N uptake in plants [27].

4 Conclusion

Based on the results of the study, it can be concluded that the Application of diazotroph bacteria can increase the rate of nitrogen assimilation and uptake, but does not increase leaf chlorophyll levels in shallot plants. The treatment of isolate I2 (LAR5) from the genus *Rhizobium* resulted in the highest number of nitrogen uptake variables, which was 0.07g/plant.

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