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Physiological and Anatomical Responses of Red onion (*Allium cepa* L.) to Drought Stress after Biofertilizer Application

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Abstract. *Red onion (Allium cepa) is a plant that requires sufficient water and is susceptible to drought stress. The use of biofertilizer is employed because it contains microorganisms that enhance nutrient availability and assist plant growth under abiotic stress conditions. This study aims to analyze the physiological and anatomical responses of red onion bulbs following the application of biofertilizer under drought stress. The doses of biofertilizer used were 0; 10;15; and 20 L/ha with field capacity levels of 25; 50; 75 and 100%. The parameters tested were bulb diameter, reducing sugar content number of bulb layers, anatomy of root cortex thickness, anatomy of root metaxylem diam Red onion eter, and fresh weight of red onion roots. The method used in determining the level of reducing sugar is the Nelson-Somogyi method. The method used for preparing the red onion root specimens was the embedding method. The results showed that the largest red onion bulb diameter was obtained from treatment A1B2, without the addition of biofertilizer at 75% field capacity. Meanwhile, the highest reduced sugar content was found at a dose of 15 L/ha with 50% field capacity.*

Keywords: *Allium cepa L. biofertilizer, drought stress, red onion*

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INTRODUCTION

Red onion (*Allium cepa*) is a horticultural plant from the Alliaceae family. Red onion are widely cultivated worldwide, including in Africa, Europe, Asia, and America (Fitriana & Susandarini, 2019). In Indonesia, red onion is the highest vegetable crop produced. National production data shows that red onion production in 2020 was 1,815,445 tons (Cahyani et al., 2022). However, the productivity of red onions in Indonesia is still very low compared to several other Asian countries. Red onion are widely cultivated due to their high nutritional value. The nutritional value in 100 g of red onion includes 79.80 g of water, 0.290 mg of vitamin B5, 60 mg of phosphorus, 334 mg of potassium, and 37 mg of calcium (Utari et al., 2023).

One of the challenges in red onion cultivation is its susceptibility to abiotic stress such as drought. Indonesia is a tropical country but often experiences extreme climate changes, such as prolonged dry seasons that cause drought and stress plants, reducing their productivity. Red onion plants have a shallow root system and are vulnerable to water shortages, requiring adequate irrigation to maintain plant growth. A decrease in soil water content by up to 60% will cause drought stress in red onion plants (Cahyani et al., 2022). Drought stress in red onions disrupts the plant's physiology and biochemistry. Drought stress hampers plant growth and productivity due to disturbed metabolism (Siswanti & Riesty, 2021).

Water is an abiotic component that plays an important role in plant growth. Plant responses to drought are divided into 3 parts, namely (tolerance) where plants can tolerate air vulnerability or are able to survive with low air potential, (escape) where plants complete their life cycle before drought occurs to maintain part of their reproductive process, and (avoidance) where plants can survive with low water potential (Manavalan & Nguyen, 2017). One of the causes of low production is environmental factors (Lathifah & Siswanti, 2021)

The application of biofertilizers can be a solution to counteract drought stress in plants as they can enhance growth under abiotic stress. Biofertilizers are organic fertilizers containing microorganisms that provide nitrogen and essential nutrients for plants (Khairunnisa & Siswanti, 2021). The biofertilizer materials used are manure, cow urine, bacterial starters consisting of *Bacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp., IAA-producing bacteria. This product plays a role in increasing plant production through direct or indirect mechanisms. Therefore, the use of biofertilizer products is very important because it minimizes negative impacts on the environment due to its environmentally friendly nature (Siswanti, 2015). The use of biofertilizers helps plant growth under stress conditions and improves the physical, chemical, and biological properties of the soil compared to the use of chemical fertilizers on groundnuts (Arachis hypogeaea) (Pangestuti & Siswanti, 2021).

Based on research conducted by Lumbantoruan & Anggraini (2021) on the application of biofertilizers to stimulate corn plant growth under drought stress conditions, it was found that the addition of biofertilizer formulations was able to increase stem diameter under drought stress. In addition, the application of biofertilizers also showed an increase in the number of leaves and leaf area compared to treatments without biofertilizers. Another study by Saputra et al. (2024) showed that the application of biofertilizers affected the height of curly red chili plants at 42 days after planting, which is the maximum vegetative phase.

Research on drought stress is conducted to understand the impacts of drought and effective adaptations using biofertilizers. This research is expected to help create sustainable and environmentally friendly agricultural practices. Based on the background, research on the effect of biofertilizers under drought stress conditions on red onion (*A. cepa*) plants has not been widely conducted. Therefore, this study aims to examine the effect of biofertilizer application on the physiological and anatomical responses of red onion bulbs under drought stress.

MATERIALS AND METHODS

This study was conducted from October 2023 to February 2024. Field research was carried out at the Sawitsari Research Station, Faculty of Biology, Gadjah Mada University. Laboratory research was conducted at the Biochemistry Laboratory and Plant Development Structure Laboratory, Gadjah Mada University, Yogyakarta. The following are the procedures for this research.

Research Design

The study was conducted using an experimental design with a completely randomized design (CRD). With two factors, namely biofertilizer concentrations, and variations in drought stress

The following research design is used (Table 1. Research Design.)

Explanation :

The variations in biofertilizer concentration used are as follows:

A1: Biofertilizer at a dose of 0 L/ha (control)

A2: Biofertilizer at a dose of 10 L/ha

A3: Biofertilizer at a dose of 15 L/ha

A4: Biofertilizer at a dose of 20 L/ha

The variations in drought stress levels used are as follows:

B1: Without drought stress (0%) (with 100% field capacity)

B2: 75% field capacity

B3: 50% field capacity

B4: 25% field capacity

The primary parameters observed were the diameter and number of red onion bulbs, stele thickness, metaxylem thickness. Meanwhile, reduced sugar content, root wet weight function as supporting parameters.

Table 1. Research Design

Field Capacity	Biofertilizer Dosage			
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15L/ha)	A4 (20 L/ha)
B1 (100%)	A1B1	A2B1	A3B1	A4B1
B2 (75%)	A1B2	A2B2	A3B2	A4B2
B3 (50%)	A1B3	A2B3	A3B3	A4B3
B4 (25%)	A1B4	A2B4	A3B4	A4B4

Anatomy of the Root : Stele Thickness, and Metaxylem Thickness

The root anatomy parameters of red onions are analyzed using the embedding method. Plant root samples are processed through fixation, dehydration and dealcoholization, infiltration, wrapping, slicing, coloring, and receiving (Sutikno, 2006). Firstly, the roots are cut transversely. Secondly, the red onion roots are fixed by immersion in FAA solution. Thirdly, the FAA solution is drained, and replaced with 70% alcohol, left to stand for 30 minutes. Fourthly, after 30 minutes, the 70% alcohol is drained and replaced with 1% safranin, and left to stand for 24 hours. Fifthly, washing and dehydration are carried out (Sutikno, 2006).

Sixth, de-alcoholization is performed. Seventh, the xylene solution is replaced with a xylene-paraffin mixture at a ratio of 1:9, then placed in an oven at 57°C for 24 hours. Eighth, infiltration is carried out, wherein the xylene-paraffin mixture is replaced with pure paraffin. Ninth, embedding is carried out. Tenth, sectioning is done using a rotary microtome. Eleventh, mounting is performed. Twelfth, staining is carried out with 1% safranin in 70% alcohol. Successive slides are immersed in xylene, xylene, alcohol/xylene 1:3, alcohol/xylene 1:1, alcohol/xylene 3:1, 100% alcohol, 100% alcohol, 95% alcohol, 80% alcohol, and 70% alcohol, with each immersion lasting for 3 minutes. Afterward, the slides are immersed in 1% safranin solution in 70% alcohol for 1 hour. Thirteenth, sealing is performed, where the slices are covered with Canada Balsam. Then, the preparations are observed using an optical microscope (Sutikno, 2006; Palupi & Siswanti, 2023).

Diameter and Number of Red Onion Bulbs

The red onion bulb is cut into two parts, after which the number of layers and the diameter of the red onion bulb are counted. This process is repeated three times.

Analysis of Reduced Sugar Content

The main parameters observed were the diameter and number of red onion bulbs. Meanwhile, the reduced sugar content, stele thickness, metaxylem thickness, and others function as supporting parameters. The main parameters observed were the diameter and number of shallot bulbs. Meanwhile, reducing sugar content, stele thickness, and metaxylem thickness function as supporting parameters. 1.25 grams of red onion bulbs are ground, added to 20 mL of water, and filtered with filter paper. Subsequently, 1 mL of the filtered solution is added to 50 mL and topped with water to the mark. Then, 1 mL of the sample solution and 1 mL of Nelson-Smogyi reagent are added. The same procedure for preparing the glucose standard curve is followed after that.The data obtained were further analyzed using the formula below :

$$\text{Reducing sugar} = \frac{\frac{mg}{L}\, curve \times fp}{sample\ weight(gr) \times 1000} \times 100\%$$

(Ardiansyah et al., 2018).

Wet Weight of Red onion Roots

The procedure for measuring the wet weight of red onion roots involves cutting the red onion plant at the base of the roots and then weighing the roots using an analytical balance.

Data Analysis

After the observations are made, the data is then analyzed using analysis of variance (ANOVA) at a 95% confidence level or α = 0.05 to determine the effect of the treatments. If significant differences exist, the analysis is followed by the Duncan Multiple Range Test (DMRT) at a 95% confidence level. This data analysis is conducted using SPSS 25 software.

RESULTS AND DISCUSSION

Environmental Conditions

Based on the results in Figure 1, the pH values in each treatment are not significantly different, meaning that the treatment of different doses of biofertilizer and field capacity does not affect the pH value. However, there is a significant difference in terms of humidity parameters. In Figure 1, it can be seen that the humidity value will decrease as drought stress increases. Environmental conditions during red onion cultivation were conducted inside a greenhouse to minimize bias towards other undesired treatments.

Red onion plants require soil conditions with a friable texture and good water drainage levels (Sansan et al., 2024). Soil pH will affect the activity of microorganisms and the availability of nutrients. Soil with acidic pH will have more microelements compared to soil with neutral to alkaline pH (Gentili et al., 2018). The soil pH in red onion plants subjected to drought stress ranges from 6.7 to 6.85. The highest pH values tend to occur under drought stress conditions. This proves that under drought stress conditions, the pH becomes more alkaline, meaning that drought inhibits bacterial function. Under drought stress conditions, microorganisms are not optimal in their growth because they cannot metabolize properly (Siebielec et al., 2020). In general, the soil pH range in the research medium is still tolerable for red onion plants. Based on the results obtained, the humidity value will decrease as drought stress increases (Zhang et al., 2023). This is consistent with the analysis of pH measurement environmental parameters. The humidity value will decrease as drought stress increases. This is consistent with the analysis of pH measurement environmental parameters where under drought stress conditions, it creates an unsupportive environmental condition.

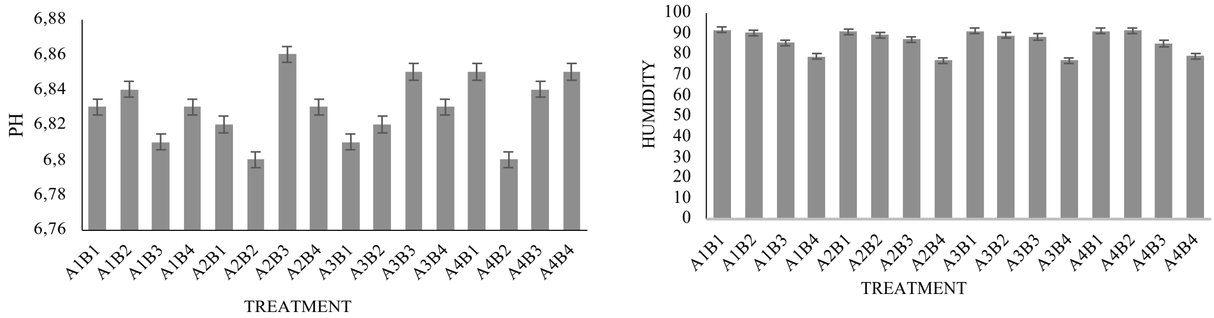

Figure 1. (a) Enviromental parameters of pH and (b) Enviromental Humadity Parameter (b) *Allium cepa* L.

Red Onion Bulb Diameter

Measurements of bulb diameter revealed that treatment A1B2, without biofertilizer application at 75% field capacity, yielded the best results (Figure2). The addition of biofertilizer (A2B1, A2B2) significantly decreased bulb diameter (Table 2). This suggests that for the specific conditions of this experiment, the application of biofertilizer did not enhance bulb size.

Table 2. Bulbs diameter in drought stress treatment and biofertilizer application.

Field Capacity	Biofertilizer Dosage (L/ha)				Average (cm)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	0.93 ± 0.06[abcd]	0.97 ± 0.12[abcd]	0.83 ± 0.06[ab]	0.87 ± 0.06[abc]	0.90 ± 0.06[xy]
B2 (75%)	1.07 ± 0.21[d]	1.03 ± 0.06[cd]	1.00 ± 0.1[bcd]	0.87 ±0.06[abc]	0.99 ± 0.09[y]
B3 (50%)	0.80 ± 0.1[a]	0.93 ±0.06[abcd]	0.87 ± 0.06[abc]	0.87 ± 0.12[abc]	0.87 ± 0.05[x]
B4 (25%)	0.90 ± 0.1[abcd]	0.90 ± 0.0[abcd]	0.93 ± 0.15[abcd]	1.00 ± 0.1[bcd]	0.93 ± 0.05[xy]
Average	0.93 ± 0.15[o]	0.96 ± 0.08[o]	0.91 ± 0.11[o]	0.90 ± 0.09[o]	

*) Numbers followed by the same letter in a column and row are not significantly different in the DMRT test at a significance level of 95% (0.05).

Based on research results, a field capacity of 75% provides the most optimal results in increasing the bulb diameter of red onion compared to the treatments of 50% and 25% field capacity. This is in line with the literature by Sansan et al. (2024), which states that red onion requires sufficient air and are not tolerant to air accumulation or drought conditions, as these can cause the plants to experience abiotic stress. Adequate air availability and proper fertilization can increase plant weight, plant height, the number of shoots, bulb diameter, and fresh bulb weight (Polakitan et al., 2022) as in the case of basil pant, which experiences a decrease in growth under drought stress with a capacity of 25% (Riyadi & Siswanti, 2022).

In drought trials, biofertilizers helped red onion plants maintain a bulb diameter equal to or greater than the control treatment (without drought stress). According to research by Hardiansyah & Guritno (2022), the diameter of red onion bulbs increases with the application of nitrogen fertilizer at doses of 100 kg/ha and 200 kg/ha. This is because nitrogen fertilizer is a vital macronutrient required for the growth of red onion plants. Nitrogen deficiency leads to a reduction in chlorophyll biosynthesis, resulting in decreased photosynthesis in the leaves. However, excessive nitrogen application also disrupts the nutrient balance and can lead to soil toxicity (Piri & Niserin, 2020).

Number of Layers of Red Onion Bulbs

The results of the experiment indicated that the application of biofertilizer had a positive impact on red onion growth. Observations of the number of layers in red onion bulbs indicated that the treatment with 10 L/ha biofertilizer at 75% field capacity (A1B2) resulted in the highest number of layers, although not significantly different from treatment A2B2 with 10 L/ha biofertilizer at 75% field capacity. This finding aligns with the results for bulb diameter, suggesting that treatment A2B2 with biofertilizer at 75% field capacity was beneficial in terms of both bulb diameter and number of layers.

Based on the results in Table 3. The number of layers of red onion bulbs is in line with the measurement of the diameter of the red onion bulbs, this is evidenced by the average field capacity showing the highest results at a field capacity of 75% and the average optimum biofertilizer administration for the number of bulb layers is a dose of 10 L/ha. This shows that the measurement of the number of bulb layers is in accordance with the literature from Zakari et al. (2017) where the number of layers of red onion bulbs is influenced by the diameter of the bulb layers, the larger the diameter of the bulb, the more layers of the bulb there will be in it.

Wet Weight of Red onion Roots

Based on Table 5, the most optimal biofertilizer dose for increasing red onion's wet root weight is 20 L/ha. This is consistent with the literature, which states that biofertilizers containing *Bacillus* sp. bacteria can produce IAA (Indole Acetic Acid), thus increasing wet root weight (Irawan et al., 2022). At 75% field capacity, the results were not significantly different from the control treatment. This indicates that biofertilizer helps the plants tolerate drought stress, producing similar results to those without stress (control).

Based on research results, the optimal biofertilizer dose for increasing the fresh root weight of red onion is 20 L/ha. This aligns with literature stating that biofertilizers containing *Bacillus* sp. bacteria can produce IAA (Indole Acetic Acid), which increases root fresh weight (Irawan et al., 2022). At 75% field capacity, the results were not significantly different from the control treatment. This indicates that biofertilizers help plants endure

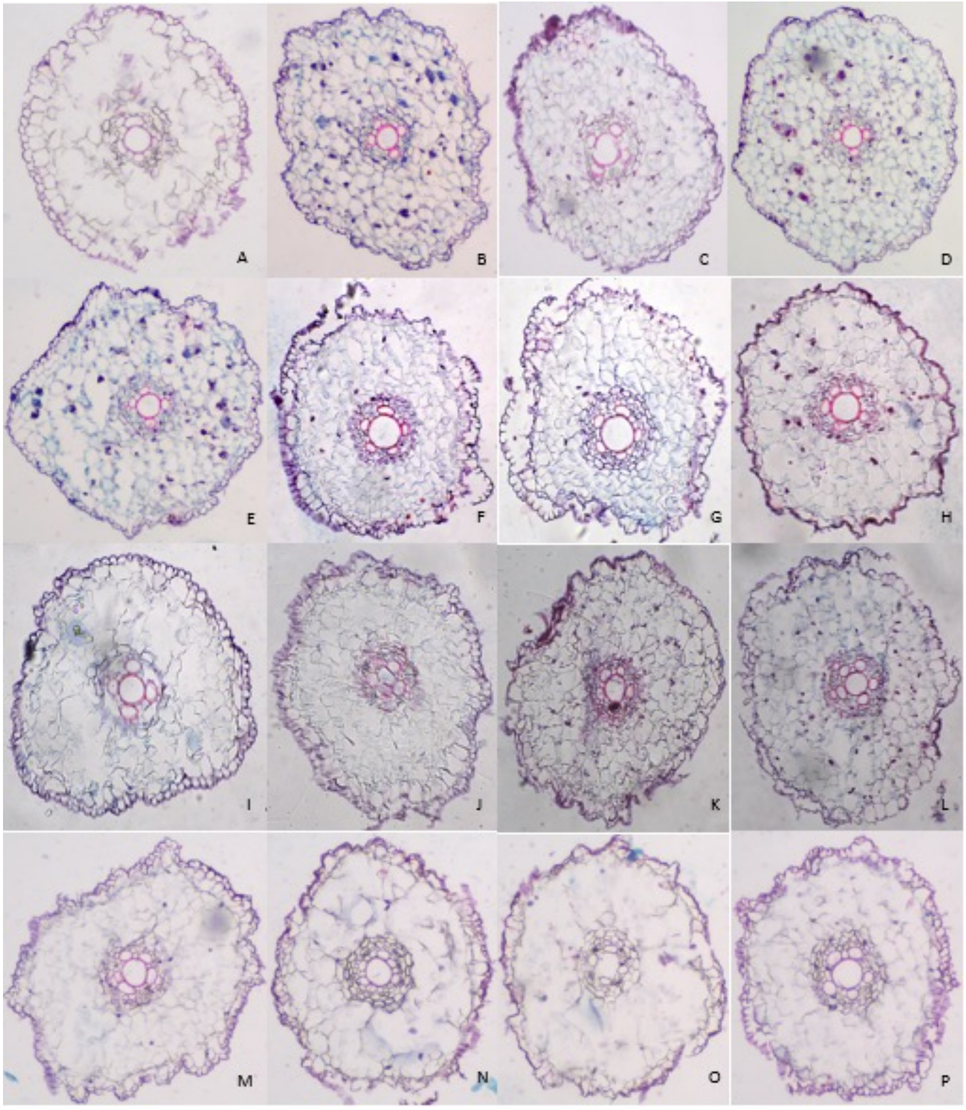

Figure 2. *Allium cepa* L. root anatomy

A : Biofertilizer at a dose of 0 L/Ha with 100% field capacity (control)
B : Biofertilizer at a dose of 10 L/Ha with 75% field capacity
C : Biofertilizer at a dose of 15 L/Ha with 50% field capacity
D : Biofertilizer at a dose of 20 L/Ha with 25% field capacity
E : Biofertilizer at a dose of 10 L/Ha with 100% field capacity
F : Biofertilizer at a dose of 10 L/Ha with 75% field capacity
G : Biofertilizer at a dose of 10 L/Ha with 50% field capacity
H : Biofertilizer at a dose of 10 L/Ha with 25% field capacity
I : Biofertilizer at a dose of 15 L/Ha with 100% field capacity
J : Biofertilizer at a dose of 15 L/Ha with 75% field capacity
K : Biofertilizer at a dose of 15 L/Ha with 50% field capacity
L : Biofertilizer at a dose of 15 L/Ha with 25% field capacity
M : Biofertilizer at a dose of 20 L/Ha with with 100% field capacity
N : Biofertilizer at a dose of 20 L/Ha with 75% field capacity
O : Biofertilizer at a dose of 20 L/Ha with 50% field capacity
P : Biofertilizer at a dose of 20 L/Ha with 25% field capacity

drought stress, resulting in outcomes similar to those of plants not experiencing drought stress (control). According to research by Ali & Sulaiman (2023), biofertilizers significantly enhance root growth. This is due to the positive effects of *Bacillus* sp., *Pseudomonas* sp., and *Azotobacter* sp., which promote growth by modifying root structure, increasing the number of roots and root hairs. Additionally, t these bacteria improve soil fertility by solubilizing soil nutrients, making them more easily absorbed by plant roots (Meena et al., 2017).

Table 3. Number of red onion bulbs in drought stress treatment and biofertilizer application.

Table	Biofertilizer Dosage (L/ha)				Average (cm)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	4.33 ± 0.58[abc]	5.33 ± 1.16[d]	4.00 ± 0.0[ab]	4.00 ± 0.0[ab]	4.42 ± 0.79[xy]
B2 (75%)	4.67 ± 0.58[abc]	5.33 ± 0.57[d]	5.00 ± 0.0[bc]	4.33 ±0.57[abc]	4.83 ± 0.57[y]
B3 (50%)	3.67 ± 0.58[a]	4.67 ±0.57[abc]	4.00 ± 0.0[ab]	4.33 ± 0.57[abc]	4.17 ± 0.57[x]
B4 (25%)	4.00 ± 0.0[ab]	4.33 ± 0.57[abc]	4.33 ± 0.57[abc]	4.33 ± 0.57[abc]	4.25 ± 0.45[x]
Average	4.17 ± 0.43[o]	4.92 ± 0.5[p]	4.33 ± 0.47[o]	4.25 ± 0.17[o]	

Table 4. Root wet weight in drought stress treatment and biofertilizer application.

Table	Biofertilizer Dosage (L/ha)				Average (cm)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	0.28 ± 0.04[cdef]	0.34 ± 0.09[ef]	0.14 ± 0.09[ef]	317.33 ± 258.54[ef]	265.33 ±73.75[y]
B2 (75%)	0.41 ± 0.10[f]	0.18 ± 0.05[abcd]	0.18 ± 0.05[avcd]	255.67 ±71.23[def]	306.67 ±71.29[y]
B3 (50%)	0.15 ± 0.06[abc]	0.26 ± 0.00[bcde]	0.26 ± 0.00[bcde]	113.2 ± 61.08[abc]	202.47 ± 85.94[x]
B4 (25%)	0.08 ± 0.01[a]	0.19 ± 0.06[abcd]	0.19 ± 0.06[abcd]	279.33 ± 208.71[cdef]	203.33 ± 56.73[x]
Average	0.23 ± 0.14[p]	0.24 ± 0.08[p]	0.24 ± 0.08[p]	241.38 ± 89.15[p]	

Table 5. Stele diameter (μm) of roots in drought stress treatment and biofertilizer application.

Table	Biofertilizer Dosage (L/ha)				Average (cm)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	114.99 ± 4.44[bc]	119.29 ± 1.16[bcd]	109.48 ± 2.24[ab]	118.88 ± 10.84[bcd]	115.66 ±6.56[xy]
B2 (75%)	99.56 ± 1.88[a]	128.32 ± 3.39[cde]	100.01 ± 3.44[a]	120.9 ± 4.39[bcd]	112.20 ± 13.56[x]
B3 (50%)	141.03 ± 7.45[ef]	108.32 ± 5.93[ab]	118.32 ± 6.44[bcd]	117.97 ±2.05[bcd]	121.57 ± 13.46[y]
B4 (25%)	148.83 ± 11.6[f]	113.08 ± 5.2[ab]	149.12 ± 9.78[f]	130.28± 20.34[de]	135.33 ± 19.11[z]
Average	126.10 ± 21.59[p]	117.25 ± 8.64[o]	119.39 ± 19.96[op]	122.01 ± 11.26[op]	

Table 6. Root metaxylem diameter (μm) in drought stress treatment and biofertilizer application.

Table	Biofertilizer Dosage (L/ha)				Average (cm)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	59.04 ± 1.94[cde]	54.17 ± 2.01[cd]	45.93 ± 3.55[b]	56.63 ± 3.66[cde]	53.94 ± 5.7[x]
B2 (75%)	53.04 ± 5.14[bcd]	68.24 ± 1.98[fg]	35.94 ± 0.05[a]	55.83 ± 4.16[cde]	53.26 ± 13.3[x]
B3 (50%)	74.75 ± 2.52[gh]	63.85 ± 3.00[ef]	50.70 ± 8.47[bc]	54.02 ± 3.39[cd]	60.83 ± 10.83[y]
B4 (25%)	76.44 ± 1.07[h]	59.29 ± 1.78[de]	56.82 ± 3.61[cde]	61.32 ± 10.49[def]	63.47 ± 8.84[y]
Average	65.82 ± 10.79[r]	61.39 ± 5.79[q]	47.35 ± 9.01[o]	56.95 ± 3.11[p]	

Tabel 7. Reducing sugar content of red onion bulbs in field capacity treatment and application of biofertilizer

Table	Biofertilizer Dosage (L/ha)				Average (cm)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	24.54±0.06^{cd}	23.56 ± 1.32^{ab}	25.79 ± 0.56^{f}	23.98 ± 0.12^{bc}	24.47 ± 0.97^{x}
B2 (75%)	25.58 ± 0.17^{ef}	27.23 ± 0.52^{g}	25.67 ± 0.25^{ef}	25.70 ± 0.12^{ef}	25.05 ± 0.79^{x}
B3 (50%)	27.58 ± 0.09^{g}	23.62 ± 0.12^{ab}	28.49 ± 0.12^{h}	25.74 ± 0.28^{ef}	26.36 ± 2.15^{x}
B4 (25%)	24.06 ± 0.27^{bc}	23.06± 0.27^{a}	24.98 ± 0.03^{def}	24.94± 0.09^{de}	24.26 ± 0.91^{x}
Average	25.44 ± 1.42^{o}	24.37 ± 1.85^{o}	26.23 ± 1.43^{q}	25.09 ± 0.76^{p}	

*) Numbers followed by the same letter in a column and row are not significantly different in the DMRT test at a significance level of 95% (0.05).

Root Anatomy: Stele Diameter and Metaxylem Diameter of Red onion Roots

Red onion root anatomy

Based on Table 6. the application of 20 L/ha biofertilizer resulted in the most optimal increase in the stele diameter of red onion roots. The application of 20 L/ha biofertilizer showed no significant difference compared to the 15 L/ha treatment. Biofertilizer application increased stele diameter.

Based on Table 7. applying a 10 L/ha dose of biofertilizer results in the most optimal metaxylem diameter compared to doses of 15 L/ha and 20 L/ha. The biofertilizer application at 10 L/ha. 15 L/ha. and 20 L/ha showed significant differences compared to the control treatment. The average field capacity optimal for increasing metaxylem diameter is at the 25% field capacity treatment. The 25% field capacity treatment did not significantly differ from the 50% treatment but showed a significant difference compared to the control and the 75% field capacity treatments. This aligns with the literature. which states that metaxylem diameter will increase significantly as the drought ratio increases.

Stele diameter (µm)

Based on the research. the application of 20 L/ha biofertilizer resulted in the most optimal increase in the stele diameter of red onion roots (Figure 2). The application of 20 L/ha biofertilizer showed no significant difference compared to the 15 L/ha treatment. Biofertilizer application increased stele diameter. which is consistent with the literature by Salem et al. (2024). The microbial treatment enhanced the width of the cortex and stele of lemon roots. thereby increasing the diameter of lateral roots. The microbial treatment increased the levels of phytohormones. which can enhance the number and size of cells. leading to an increase in root diameter. The Bacillus sp. strain produces phytohormones such as auxin. gibberellin. ABA. cytokinin. ethylene. brassinosteroids. strigolactones. and jasmonates in the root zone. which directly act on the meristem for cell division. elongation. and differentiation (Bhardwaj et al.. 2014). Additionally. Bacillus sp. strains improve soil physicochemical properties. thereby increasing nutrient balance in the soil and the plant parts (Ennab. 2016).

Root metaxylem diameter (µm)

Based on the research. the application of a 10 L/ha dose of biofertilizer results in the most optimal metaxylem diameter compared to doses of 15 L/ha and 20 L/ha. The application of biofertilizer at 10 L/ha. 15 L/ha. and 20 L/ha showed significant differences compared to the control treatment. This is consistent with the literature from Grover et al. (2021). which states that biofertilizers produce rhizobacteria that enhance root length and diameter. as well as the expansion of the cortex. protoxylem. and metaxylem. in addition to increasing the total phenol content and flavonoid content in the roots.

The average field capacity that is most optimal for increasing metaxylem diameter is at the 25% field capacity treatment. The 25% field capacity treatment did not significantly differ from the 50% treatment but showed a significant difference compared to the control and the 75% field capacity treatments. This aligns with the literature. which states that metaxylem diameter will increase significantly as the drought ratio increases. Metaxylem (Figure 3) functions in the efficient transport of water and nutrients from the roots to all parts of the plant. leading to an increase in diameter in response to drought conditions. This is aimed at improving water transport efficiency by reducing hydraulic resistance so that the plant can maintain its water supply even when soil water availability decreases. The increase in metaxylem diameter allows for a greater and more efficient flow of water through the wider xylem vessels. helping plants cope with drought stress (Bibi et al.. 2022).

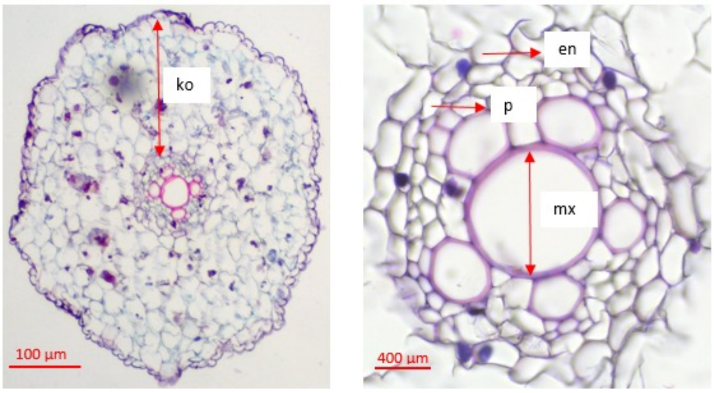

Figure 3. Transversal section of *Allium cepa* L. root : ko - kortex; en – endodermis; p – pericycle; mx – metaxylem

Reducing Sugar Content of Red onion Bulbs

Based on the results in Table 4. The average dose of biofertilizer showed that a dose of 15 L/ha produced the most optimum reducing sugar levels compared to other treatments. This indicates that the application of biofertilizer influences the increase in the reducing sugar content of red onion bulbs.

Based on the research. the average dose of biofertilizer showed that a dose of 15 L/ha produced the most optimum reducing sugar levels compared to other treatments. This shows that the administration of biofertilizers has an effect on increasing the reducing sugar levels of red onion bulbs. This is in accordance with the literature written by Gupta et al. (2024) which explains that in the biochemical study of garlic bulbs there was an increase in reducing sugar levels after the addition of biofertilizers. The addition of biofertilizers stimulates increased activity in the shikimate pathway and phenylalanine ammonia lyase (PAL) which are enzymes that play a role in phenol synthesis so that phenol production increases. The increase in reducing sugar is due to the addition of biofertilizers which have the potential to balance the nutrients needed during the growth period (Thakur and Kumar. 2018).

Jurnal Biodjati 9(2):359-372, November 2024

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CONCLUSION

Based on the research conducted. it can be concluded that the application of biofertilizer affects bulb diameter at a dose of 10 L/ha at 75% field capacity. The increase in reducing sugar content in red onion bulbs under drought stress with a dose of 15 L/ha and is effective at 50% field capacity. Meanwhile. observations of the number of layers in red onion bulbs showed that treatment with 10 L/ha biofertilizer at 75% field capacity (A1B2) produced the highest number of layers.

AUTHOR CONTRIBUTION

N.I.L : conducted research. collected and analyzed data. revised. edited. wrote the manuscript. **D.U.S**:developed research ideas and designs. edited. revised. reviewed the manuscript. and approved the final manuscript.

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CONFLICT OF INTEREST

The authors assert that there are no conflicts of interest or personal connections that might have affected the results reported in this paper.

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