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Effects of Cow Urine Biofertilizer on Anatomical and Physiological Traits of *Allium cepa* L. in Drought Stress

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ABSTRACT

Biofertilizers from cow urine contain various nutrients that can increase soil fertility, overcome soil nutrient deficiencies, and provide plant nutrients. In addition, it also includes multiple microorganisms that can reduce the impact of drought stress on shallot plant growth. The study aimed to analyze the effect of biofertilizers by observing the physio-anatomical characteristics of shallot plants under drought-stress conditions. This study was conducted using a factorial completely randomized design. The treatments were drought stress (25%, 50%, 75%, and 100% field capacity) and urine-based biofertilizer application (0, 10, 15, and 20 L/Ha). The results showed a significant interaction, where the overall physio-anatomical characteristics of shallot plants were concurrently influenced by the combined biofertilizer and drought treatments. Specifically, the biofertilizer application increased carotenoid content (0.065 mg/g), total chlorophyll (1.342 mg/g), canopy and root water content, tuber dry matter, mesophyll thickness (181.06 µm), and vascular bundle diameter under drought stress. In contrast, flavonoid content and the number of metaxylems decreased with increasing biofertilizer dosage within this interaction. Overall, the application of biofertilizer made from cow urine suppressed the negative impact of drought stress.

Keywords: anatomy, biofertilizer, drought, flavonoid, shallot

INTRODUCTION

Drought is one of the abiotic stresses that can reduce crop yields globally. When extreme drought conditions occur, the amount of soil water needed by plants will continue to decrease, and as a result, plants will die prematurely (Gull *et al.* 2019; Umar *et al.* 2022). The impact of drought is a serious problem for agricultural production, prompting the development of various studies on plant mechanisms in response to drought. Reduced plant growth is the main impact of drought, which is related to cell division and enlargement (Seleiman *et al.* 2021; Wach & Skowron 2022).

Research conducted by Yang and Li (2023) on corn plants showed that drought stress reduces plant height, leaf area, and basal diameter. Another study by Siswanti and Rachmawati (2011) also showed that drought stress reduced the number of leaves, tillers, panicles, grains, and plant height of the IR-64 rice cultivar. In addition, drought disrupts plant nutrient absorption and triggers the formation of ROS. The accumulation of excess ROS, such as superoxide anions (O_2^-), hydrogen peroxide, and hydroxyl radicals (OH), results in protein denaturation, DNA or RNA damage, and inactivates metabolic enzymes (Macar & Ekmekçi 2008; Kapoor *et al.* 2020). Research by Hu *et al.* (2023) showed that chlorophyll content and stomatal conductance of yellow horn plants decreased under drought-stress conditions.

Plants also make anatomical changes in response to drought stress. Stomatal closure is a typical response of plants in drought stress (Agurla *et al.* 2018; Shen *et al.* 2021). Research by David *et al.* (2017) showed that xylem tissue, vascular bundles, mesophyll cells, and bundle sheaths in the wheat cultivar Cettia were more extensive than other cultivars in the face of drought stress. Underwater deficiency conditions, the decrease in stomatal conductance is triggered by increased ABA content in guard cells. This ABA-mediated stomatal closure aims to reduce water loss through transpiration but reduces CO_2 supply, which decreases the photosynthesis rate (Pirasteh-Anosheh *et al.* 2016; Gu *et al.* 2024).

Shallot (*Allium cepa* L.) has shallow roots that limit water absorption in the soil, making it vulnerable to drought stress. Reduction in bulb size and weight is one of the responses of shallot plants when subjected to drought (Sansan *et al.* 2024). Biofertilizer is one of the effective strategies in increasing plant resistance to drought stress (Eliaspour *et al.* 2020; Najafi *et al.* 2021). Research by Siswanti and Rachmawati (2011) showed that adding biofertilizer to IR-64 rice plants with drought stress treatment can reduce plant stress. Biofertilizer is an organic fertilizer derived from animals, plants, or beneficial microorganisms that can increase

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plant productivity by providing the nutrients plants need. The role of biofertilizers in improving soil fertility is fixing nitrogen in the air, dissolving phosphorus, and decomposing organic matter or producing hormones to increase plant growth (Chaudhary *et al.* 2022; Mosa *et al.* 2014).

In this study, the biofertilizer used came from a mixture of cow urine and microbial starter with a ratio of 49 : 1. This microbial starter contains nine bacteria, such as *Streptomyces* sp., *Azotobacter* sp., *Bacillus* sp., *Saccharomyces* sp., *Lactobacillus* sp., *Azospirillum* sp., *Pseudomonas* sp., *Rhizobium* sp., and IAA hormone-producing bacteria (Siswanti 2015). Several previous studies have reported the effectiveness of this biofertilizer in increasing plant growth and productivity. Biofertilizer is proven to be able to increase the growth of *Amaranthus tricolor* L. plants (Siswanti and Umah 2021), chlorophyll content of rice plants (Siswanti and Agustin 2014), and productivity of groundnuts plants (Pangestuti & Siswanti 2021).

Cow urine in biofertilizers contains macro and microelements that can increase soil fertility, effectively overcoming soil nutrient deficiencies and providing nutrients for plants (Kgasudi & Mantswe 2020; Meena *et al.* 2019). Biofertilizers can produce hormones, such as gibberellins, cytokinins, abscisic acid, and IAA can increase the growth of root length, total surface area, root hair formation and lateral roots, there by improving water absorption (Chaudhary *et al.* 2022).

The growth and capsaicin content in curly red chili plants increased after applying biofertilizer with an optimum dose of 10 L/Ha (Siswanti *et al.* 2019). Research by Najafi *et al.* (2021) reported that biofertilizers effectively improve agronomic traits, crop yield, grain yield, and reduce oxidative damage to medicinal pumpkin plants from drought stress conditions. Biofertilizers are one of the most effective strategies for sustainable agriculture (Azizi *et al.* 2024). However, few studies have addressed the impact of biofertilizers made from cow urine on shallot plants under drought-stress conditions. This research aims to study the effect of biofertilizer made from cow urine on shallot plant growth by measuring physio-anatomical characteristics under drought stress conditions. It is hypothesized that the application of cow urine-based biofertilizer can mitigate the adverse effects of drought stress on shallot plants. Thus, this study is expected to contribute to the development of sustainable agricultural strategies for shallot cultivation under drought-prone conditions.

METHODS

Experimental Design

The research was conducted at the Sawitsari Research Station Greenhouse, Faculty of Biology, Gadjah Mada University, Yogyakarta, Indonesia. The study used a completely randomized design factorial with two factors: four doses of biofertilizer (0, 10, 15, and 20 Liters/Ha) and four levels of drought stress (25%, 50%, 75%, and 100% field capacity). There were 16 treatment combinations with three replications of each treatment. Biofertilizer application was conducted seven days after planting (DAP) and applied every ten days. Meanwhile, drought stress was applied by giving field capacity after the plants were 7 days after planting (DAP).

Measurement of Environmental Parameters

Environmental parameters include air temperature, light intensity, soil moisture, and soil pH. Temperature and light intensity were measured using a lux meter, while soil moisture and pH were measured using a pH-moisture meter. Measurements were taken periodically every ten days until harvest time.

Flavonoid Content

Measurement of flavonoid content was carried out according to the method of Pourmorad *et al.* (2006) (with modifications). The test solution derived from the extraction results with methanol was taken as much as 0.5 mL. Then added 1.5 mL methanol, 0.1 mL $AlCl_3$ 10%, 0.1 mL CH_3COONa 1 M, and distilled water 2.8 mL. The solution was then allowed to stand for 30 minutes and the absorbance was measured using a spectrophotometer at a wavelength of 413 nm. Total flavonoid content was expressed based on the number of milligrams of quercetin equivalent (QE) per gram of sample (mgQE/g).

Chlorophyll and Carotenoid Content

Fresh leaves were used as much as 0.1 gram, mashed, then mixed with 80% acetone as much as 10 mL. The mixed solution was filtered with filter paper, the filtrate was put in a cuvette to measure the absorbance. The spectrophotometer was set at a wavelength of 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids. The absorbance measurement data was calculated using the following formula (Siswanti and Riesty 2021):

$$\text{Chlorophyll Total (mg/g)} = \frac{(20.2 \times A645 + 802 \times A663) \times V}{(1000 \times W)}$$

$$\text{Carotenoid (mg/g)} = \frac{\left((A470 + (0.114 \times A663) - (0.638 \times A645)\right) \times V}{(1000 \times W)}$$

where *V* is volume of extract and *W* is weight of leaf sample.

Tuber Dry Matter

The tubers were dried in an oven at 40-45°C for a week until they reached a constant weight. Dry matter content (DM) was calculated based on the formula (Rodríguez *et al.* 2023):

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$$\text{Dry Matter (\%)} = \frac{\text{dry weight}}{\text{fresh weight}} \text{ x } 100$$

Canopy and Root Water Content

Plant canopies and roots were harvested and weighed using an analytical balance. Before weighing the wet weight, the canopies and roots were cleaned and dried. After that, they were dried in an oven at 40-45°C until they reached a constant weight. The wet weight and dry weight of the canopies and roots were used to calculate the moisture content. The moisture content of the canopies and roots was calculated based on the formula (Marulanda *et al.* 2007):

$$\text{Water Content (\%)} = \frac{(\text{fresh weight} - \text{dry weight})}{\text{fresh weight}} \text{ x } 100$$

Leaf Anatomical Characters

Transverse preparations of shallot leaves were made using the embedding method. On the first day, the leaves were cut around the center of 1 cm long. The leaves were fixed with the FAA in a 24-hour flask. On the second day, the FAA solution was discarded, replaced with 70% alcohol for 30 minutes, and then stained with 1% safranin in 70% alcohol. The leaves were washed and dehydrated on the third day with graded alcohols (70, 80, 95, 100, and 100%), alcohol was removed with xylol alcohol (3:1, 1:1, and 1:3), pure xylol for 30 min each. Samples were incubated in xylol:paraffin 1:9 for 24 hours at 57ºC. Leaf samples were infiltrated two times with pure paraffin. Xylol:paraffin was discarded and replaced with pure paraffin. The casing was done, paraffin was allowed to harden. Tape preparations were made with a rotary microtome. Preparations were stained and dripped with canada balsam for closure.

Data Analysis

Observational data were processed in Microsoft Excel and calculated manually prior to statistical analysis. To assess the treatment effects, the data were subjected to ANOVA ($p<0.05$). Whenever a significant difference was detected, mean comparisons were further analyzed using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

RESULTS AND DISCUSSION

Environmental Conditions

Shallot (*Allium cepa* L.) is a seasonal crop whose growth can be affected by various factors, such as light, temperature, and soil conditions. This plant require an optimum temperature of about 15–20 °C for vegetative growth and bulb formation of about 20–27 °C (Sansan *et al.* 2024). The air temperature at the research site averaged 38.7 °C with a light intensity of about 1,126–1,892 lux (Table 1). This air temperature is relatively high because the weather in Yogyakarta, Indonesia from October to November, tends to be hot. However, shallot plants can still tolerate this temperature and continue growing.

In addition, for soil conditions, shallot plants are suitable for planting with a pH between 6.0 and 7 (Sansan *et al.* 2024). In this study, soil pH ranged from 6.7–6.9 (Table 1), which shows that the pH range is still at the standard limit for shallot growth. Soil with a pH that is too acidic will be toxic and stunt plants. Meanwhile, soil moisture in this study was around 72–92% (Table 1). This soil moisture affects root growth, so water availability in the soil must be maintained for optimal shallot growth (Karim *et al.* 2013). Overall, the environmental conditions of the research site were still optimal for shallot plant growth.

Flavonoid Content

Biofertilizer application significantly reduced flavonoid content at all levels of drought stress (Figure 1a). As the dose of biofertilizer increased, the flavonoid content decreased. The interaction relationship between biofertilizer and drought stress significantly affected the flavonoid content. The highest flavonoid content was at a dose of 10 L/Ha in 25% field capacity with a value of 6.34 mg QE/g dry weight (Figure 1a). However, there was no significant difference between the control treatment at 100% field capacity and the dose of 15 L/Ha at 25% field capacity. In contrast, applying a biofertilizer dose of 20 L/Ha in 50% field capacity resulted in the lowest flavonoid content of 2.25 mg QE/g dry weight (Figure 1a).

According to Zhao *et al.* (2021) biofertilizer provides nitrogen nutrients that can affect plant secondary metabolism such as flavonoids. High doses of biofertilizer contain high nitrogen. This high nitrogen causes a decrease in flavonoids *(Deng et al. 2019; Liu et al.* 2010; Rodrigues *et al.* 2017). According to Rodrigues *et al.* (2017) low nitrogen levels can increase the enzyme PAL (phenylalanine ammonia-lyase) which plays a role in the phenylpropanoid biosynthesis pathway. These results are similar to research conducted by Ibrahim *et al.* (2012), which found that nitrogen fertilization with low concentrations can increase flavonoid levels in *Labisia pumila*. The increased flavonoid levels were also followed by decrease in field capacity. According to Jafari and Shahsavar (2021), drought stress can increase PAL enzyme activity and phenylpropanoid levels, catalyzing cinnamic acid production as a flavonoid precursor. Under drought stress, plants produce flavonoids to prevent stomatal closure by reducing H_2O_2 levels in guard cells. In addition, flavonoids also reduce ROS damage caused by drought stress (Jan *et al.* 2022; Li *et al.* 2023).

Caratenoid and Total Chlorophyll Content

Carotenoid and total chlorophyll contents increased with the addition of biofertilizer application under drought stress conditions. The increase was significant

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between all treatments (Figure 1b). The results obtained showed that the carotenoid content in the biofertilizer treatment of 15 L/Ha and 100% field capacity watering (0.065 mg/g) was not significantly different from the biofertilizer treatment of 20 L/Ha and 75% field capacity watering (Figure 1b). This indicates that less watering with a higher biofertilizer dose of 20 L/Ha was able to maintain carotenoid levels almost equivalent to the 100% field capacity watering treatment. Biofertilizers can increase the carotenoid content of shallot leaves at doses of 15 L/Ha and 20 L/Ha. Carotenoid levels tend to decrease due to an increasing deficit in watering treatment.

Giving biofertilizer to the soil will increase the amount of elements ready to be absorbed by plants. An increase in N, P, K and organic elements in the soil can increase carotenoid levels (Karabacak & Karabacak 2019). Previous research by Siswanti & Khairunnisa (2021) stated that the biofertilizer dose of 10 L/Ha produced the highest carotenoid content. Carotenoid levels tend to decrease due to an increasing deficit in watering treatment. Drought stress can affect reducing leaf pigments such as chlorophyll and carotenoids (Khan *et al.* 2023; Samaneh *et al.* 2020). Shallot plants with drought stress resistant genotypes have high carotenoid levels (Sansan *et al.* 2024).

Meanwhile, the application of biofertilizer doses of 15 L/Ha and 20 L/Ha significantly changed the chlorophyll content of shallot leaves compared to the control (Figure 1c). However, both dose treatments showed insignificant results. The results also showed that drought stress increased total chlorophyll levels. Thus, it is known that biofertilizers can maintain the chlorophyll content of shallot leaves under-treated drought stress conditions. Previous research by Siswanti (2011) reported that along with increased biofertilizer doses, the total chlorophyll content of IR-64 rice plant leaves increased under drought stress.

Table 1 Environmental conditions

Temperature (°C)	Light intensity (lux)	Soil pH	Soil moisture (%)
34.1–39.7	1,126–1,892	6.7–6.9	73–92

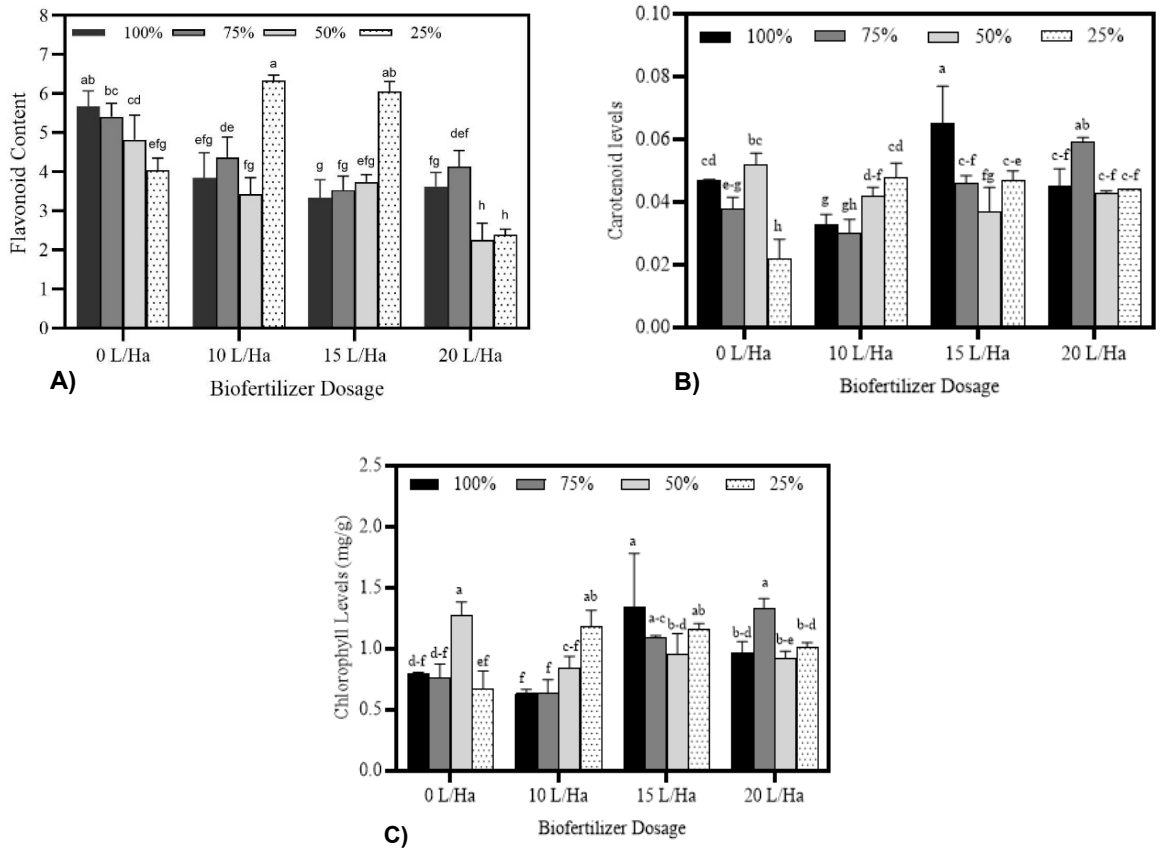

Figure 1 Interaction between biofertilizer doses and drought stress treatment for (A) flavonoid content, (B) carotenoid content, and (C) chlorophyll total in shallot plant. Histogram and error bars represented by means ± standard deviation. Means values followed by different letters significantly differ between treatments (DMRT test: $p<0.05$).

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Siswanti and Umah (2021) also noted that the biofertilizer dose of 20 L/Ha increased the chlorophyll content of *Amaranthus tricolor* L. plants under salinity stress. The elements of N and P in biofertilizers are essential in the availability of chlorophyll biosynthesis (Li *et al.* 2018; Wu *et al.* 2006). As much as 70% of the N element in leaves accumulates in chloroplasts. Sufficient N elements for plants will increase chlorophyll a and b levels (Fathi 2022).

Canopy Water Content, Root Water Content, and Dry Matter of Tubers

Biofertilizer application significantly increased the canopy water content (Figure 1d), root water content (Figure 1e), and dry matter of tubers under drought stress (Figure 1f). The increase in biofertilizer dosage was in line with the increase in canopy water content, root water content, and tuber dry matter. The highest canopy water content was at a dose of 20 L/Ha with 100% field capacity at 92.85% (Figure 1d), while the highest root water content was at a dose of 20 L/Ha with 50% field capacity at 94.92% (Figure 1e). Overall, it appears that the application of biofertilizer has a positive impact and can reduce the adverse effects of drought stress on the growth and yield of shallot plants.

Plant tissue moisture content can be expressed by the amount of water per unit of wet or dry weight (González and González-Vilar 2006). Plant tissue moisture content can be influenced by various factors, one of which is mineral nutrients (Ievinsh *et al.* 2022). Increasing nutrients can significantly increase the moisture content of plant tissues (Ievinsh 2023). Biofertilizers contain various microorganisms that provide N, P, and K elements (Chaudhary *et al.* 2022). These nutrients, especially nitrogen (N), spawn plant growth, especially in the vegetative phase (Sabur *et al.* 2021). As the dose of N fertilization increases, the moisture content of the canopy and roots will also increase. However, this increase is only up to the optimal nitrogen dose for plant growth (Ievinsh 2023; Jain *et al.* 2018). According to Demir *et al.* (2023), biofertilizers can increase the absorption of water and nutrients in the soil, so biofertilizer fertilization can significantly increase canopy and root water content. As indicated in study by Mardiana (2024), biofertilizers can enhance the rates of plant height and leaf production in shallots under drought stress, thereby increasing canopy water content.

Furthermore, the application of biofertilizer enhanced tuber dry matter compared to untreated plants. The highest tuber dry matter was at a dose of 20 L/Ha with 50% field capacity (15.82%), while the lowest was in the control treatment with 25% field capacity (8.66%) (Figure 1f). Biofertilizer helps to increase the absorption of nitrogen higher so that the accumulation of dry matter is also high. In addition, microorganisms in biofertilizers also act as biostimulants in plants (Nascimento *et al.* 2020; Kumar & Ahlawat 2006). The same results were shown by the research of Nascimento *et al.* (2020), which found that applying biofertilizer can increase the dry matter of corn plants.

Drought stress also significantly affected the crown water content, root water content, and dry matter of shallot bulbs. This study showed that the higher the drought stress, the decreased canopy water content (Figure 1d), root water content (Figure 1e), and tuber dry matter (Figure 1f). The decrease in water content and dry matter is related to stomatal closure, which then has an impact on reducing the rate of photosynthesis (Lawlor 2002; Pirzad *et al.* 2011). This will inhibit the translocation of photosynthate into the tuber (Mathobo *et al.* 2017). In this study, biofertilizer was proven to reduce the impact of environmental stress on the water content and dry matter of shallot plants. Microbes contained in biofertilizers can increase the absorption of water and nutrients from the soil. Microbes can produce the hormone IAA (Indole Acetic Acid), which can modify the root system by increasing the root surface area to increase the

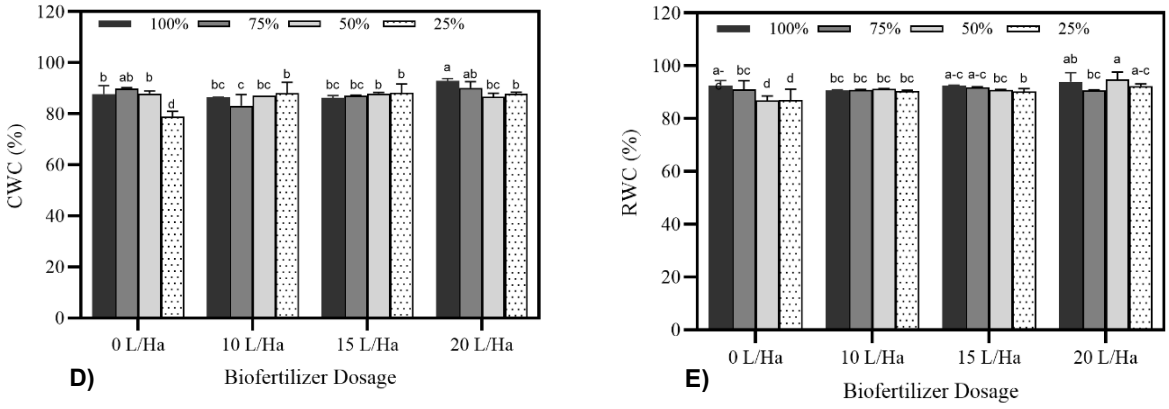

Figure 1 Interaction between biofertilizer doses and drought stress treatment for (D) canopy water content (CWC) and (E) root water content (RWC) in shallot plant. Histogram and error bars represented by means ± standard deviation. Means values followed by different letters significantly differ between treatments (DMRT test: $p<0.05$).

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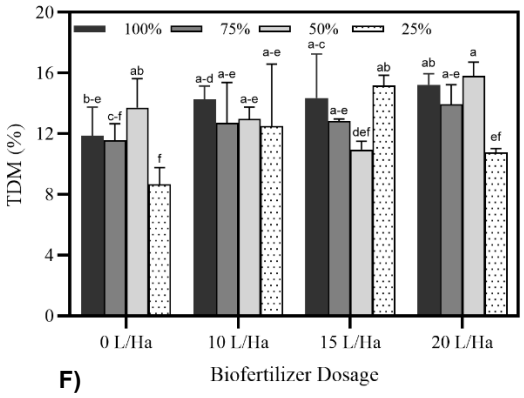

Figure 1 Interaction between biofertilizer doses and drought stress treatment for (F)) tuber dry matter(TDM) in shallot plant. Histogram and error bars represented by means ± standard deviation. Means values followed by different letters significantly differ between treatments (DMRT test: p<0.05).

absorption of water and nutrients, which can then help plants survive under drought stress (Barnawal *et al.* 2019; Daniel *et al.* 2022).

Leaf anatomical characters

The anatomical characteristics of *Allium cepa* L. leave observed in this study include the number of metaxylem, mesophyll tissue thickness, and vascular bundle diameter. Biofertilizer application based on average values significantly reduced the number of metaxylem (Figure 2a), but increased the thickness of mesophyll tissue (Figure 2b), and leaf vascular bundle diameter (Figure 2c) at all field capacities. The higher the dose of biofertilizer applied, the lower the number of metaxylem on the leaves. The highest metaxylem in the biofertilizer treatment dose of 10 L/Ha with 25% field capacity was five metaxylem per bundle sheath. It showed that biofertilizer at that dose could maintain moisture and water potential in the planting media, thus reducing the risk of damage to the metaxylem. Water use efficiency in plants is related to the diameter and number of metaxylem (Prince *et al.* 2017). Metaxylem is quite susceptible to damage when the water potential in the soil decreases so that the number can decrease during drought stress (Guet *et al.* 2015; Reeger *et al.* 2021; Sperry & Saliendra 1994).

Meanwhile, the higher the dose of biofertilizer applied, the thicker the leaf mesophyll tissue. The thickest leaf mesophyll tissue was found in the biofertilizer dose of 20 L/Ha with 75% field capacity at 181.06 µm (Figure 2b). This value was more significant than the treatment without biofertilizer at 100% field capacity of 159.44 µm. The lowest mesophyll thickness was in the treatment without biofertilizer, with 25% field capacity at 43.83 µm. The provision of biofertilizer really affects the thickness of leaf mesophyll. Mesophyll tissue is closely related to photosynthesis, and it will thicken to reduce the impact of drought (Yavas *et al.* 2024). Biofertilizers can increase mesophyll thickness. Biofertilizers contain microbes that tether the element N_2 to NH_3 derivatives that plants can use (Sharma *et al.* 2021). The effect of element N on leaf area can be observed through leaf anatomy. Leaf thickness is closely related to the thickness of mesophyll tissue. Leaf thickness will decrease when the soil has a nitrogen deficit (Onyango 2002). Research conducted by Gao *et al.* (2020), which examined changes in the anatomy of rice leaf mesophyll based on nitrogen supply with different levels, showed the results that the thickness of mesophyll tissue was getting more extensive when the supply of NH_4^+ and NO_3^- increased. Applying biofertilizer with higher doses enriches the levels of NH_4^+ and NO_3^- fixation by microbes, so the leaf mesophyll tissue is also thicker.

Biofertilizer application also significantly increased leaf vascular bundle diameter at all field capacities. The higher the dose of biofertilizer applied, the smaller the vascular-bundle diameter. The diameter of the vascular bundle without biofertilizer treatment was the smallest and significantly different among all treatments. The largest leaf vascular-bundle diameter was found in the 10 L/Ha dose of biofertilizer with 25% field capacity of 123.95 µm (Figure 2c). The lowest vascular-bundle diameter in the treatment without biofertilizer with 25% field capacity was 35.46 µm. Biofertilizers contain nutrient-fixing microbes, including nitrogen (Sharma *et al.* 2021). Based on research conducted by Khan *et al.* (2020) on the species of *F. mandshurica*, *T. amurensis*, *P. koraiensis*, and *L. gmelinii* with the addition of N showed an increase in the size of the vascular-bundle diameter in the four plant species. The diameter of the vascular-bundle is related to the number and size of vascular tissues, one of which is the xylem. Drought stress encourages plants to adapt by increasing the size of the xylem diameter and soil nutrients (Cary *et al.* 2020). The research aligns with the study by Azkiya (2024), which reported that shallot plants subjected to 75% drought stress (25% field capacity) exhibited the largest metaxylem compared to those subjected to 25% and 50% drought stress. This means that the

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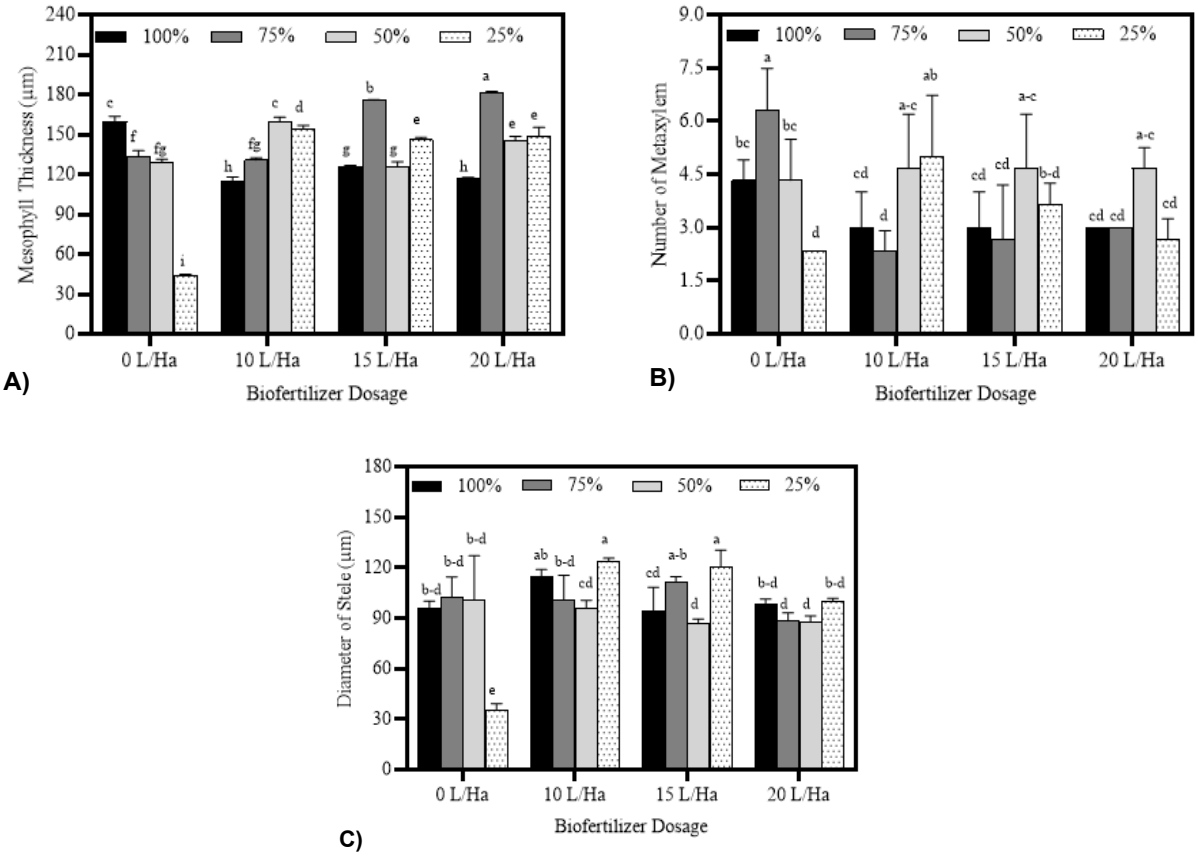

Figure 2 Interaction between biofertilizer doses and drought stress treatment for (A) number of metaxylem, (B) mesophyll thickness, and (C) diameter of stele in shallot leaf. Histogram and error bars represented by means ± standard deviation. Means values followed by different letters significantly differ between treatments (DMRT test: $p<0.05$). Not significantly different in the DMRT test at $p<0.05$ significance level. Note. C: control, ns: not significant, *: significant at $p<0.05$.

more the number and size of the diameter of each vascular network in the vascular-bundle increases, the more the diameter of the vascular-bundle increases. Biofertilizers can compensate for the increase in soil nutrients so that the diameter of the vascular-bundle increases.

CONCLUSION

The application of cow urine-based biofertilizer in shallot plants under drought stress improved various physio-anatomical traits, including canopy and root water content, leaf mesophyll thickness, tuber dry matter, and vascular bundle diameter, as well as increasing carotenoid and total chlorophyll content. In contrast, flavonoid content and the number of metaxylems decreased with increasing biofertilizer dosage. Furthermore, the significant interaction between biofertilizer dosages and drought stress levels substantially influenced the overall physio-anatomical characteristics of the shallot plants. These findings confirm that cow urine-based biofertilizer, particularly at higher doses, can help mitigate the adverse effects of drought on shallot plants by improving water retention, anatomical adaptation, and pigment stability. The results provide theoretical insight into plant adaptation mechanisms under drought and offer practical potential for using cow urine-based biofertilizer as a sustainable agricultural input in drought-prone areas.

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