PHYSIOLOGICAL RESPONSE OF SUGARCANE (*Saccharum officinarum*) TO ARBUSCULAR MYCORRHIZAL APPLICATION AND IRRIGATION INTERVALS

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ABSTRACT

Sugarcane requires water during the first four months; if drought occurs, it can lead to a decrease in photosynthetic activity, stomatal closure, and reduced protoplasmic activity. This study aims to determine the effect of mycorrhizal application and irrigation intervals on the physiological responses of sugarcane, as well as their interaction, and to identify the appropriate mycorrhizal dosage and irrigation interval for the physiological activity of sugarcane seedlings. The research was conducted at the Plant Research House, Pontianak State Polytechnic, from June to August 2022.

The study used a Split Plot design with three replications. The main plot was the irrigation interval (once every day, once every three days, and once every five days), and the sub-plot was the mycorrhizal dosage (0 grams/polybag, 10 grams/polybag, 30 grams/polybag, and 50 grams/polybag). The results showed that the mycorrhizal dosage and irrigation interval significantly affected the physiological characteristics of sugarcane seedlings in terms of stomatal density. There was an interaction between the irrigation interval and mycorrhizal dosage in terms of stomatal density. The treatment with a mycorrhizal dosage of 30 grams and an irrigation interval of once every five days showed physiological activity indicating drought-tolerant plants.

Keywords: **D**osage, Mycorrhiza, Irrigation, Sugarcane, Seedling

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is an important commodity as the main ingredient in sugar production, as nearly all parts of the plant can be processed into sugar, with varying sugar content depending on the variety, age, and processing method (Saifudin, 2010). The quality of sugarcane seedlings is a key factor in sugar production. High-quality seedlings are essential for the success of sugarcane cultivation and result in high yields, leading to increased sugar production (Nugroho, 2015).

One of the challenges in sugarcane seedling cultivation is vulnerability to water deficiency, as sugarcane requires water during the first four months (Subantoro, 2014). According to Masria (2015), the amount of water absorbed by the roots depends on soil moisture content and the rate of transpiration. In conditions where soil moisture is low or below field capacity, and when evapotranspiration exceeds water absorption, the plant experiences water stress or drought.

Water stress in plants causes a reduction in photosynthetic activity, such as a decrease in photosynthetic surface area, stomatal closure, and reduced protoplasmic activity due to dehydration. Physiologically, plants growing under drought stress conditions reduce the number of stomata to lower water loss, which is followed by stomatal closure and a reduction in net CO2 absorption in the leaves (Subantoro, 2014). Additionally, the uptake of nutrients from the soil by the roots is hindered, affecting the availability of essential elements such as nitrogen (N) and magnesium (Mg), which play a vital role in chlorophyll synthesis (Syafi, 2008).

All plants, including sugarcane, can form symbiotic relationships with other beneficial microorganisms in the soil. One form of mutualistic symbiosis is the partnership between sugarcane roots and mycorrhizae (Warouw & Kainde, 2010). Mycorrhizae, commonly referred to as soil fungi, inhabit the soil and the rhizosphere (root zone) of plants. The unique characteristic of this fungus is its ability to assist plants in absorbing nutrients, particularly phosphorus (P) (Pamuna et al., 2013). Mycorrhizae are also used as biological control agents against soil pathogens. Furthermore, mycorrhizae enhance plant drought tolerance (Kartika, 2012).

Research conducted by Halid (2016) demonstrated that mycorrhizal application can assist plants in coping with drought stress. This aligns with the role of mycorrhizae in helping plant roots maintain water availability in the root zone. Based on the above explanation, it is necessary to apply arbuscular mycorrhizae and examine the interaction between irrigation intervals and the physiological response of sugarcane. This research aims to determine the effects of mycorrhizal application and irrigation intervals on the physiological responses of sugarcane, as well as their interaction, and to identify the appropriate mycorrhizal dosage and irrigation intervals for the physiological activity of sugarcane seedlings.

2. Research Methodology

Phe research was conducted at the Plant Research House, Pontianak State Polytechnic, from May to July 2022. The study was designed using a Split Plot arrangement, with the main plot consisting of arbuscular mycorrhizal dosages: M0 = 0 grams/polybag, M1 = 10 grams/polybag, M2 = 30 grams/polybag, M3 = 50 grams/polybag, and the sub-plot consisting of irrigation intervals: P1 = once every day, P2 = once every three days, P3 = once every five days. The observed parameter was chlorophyll content, measured using a spectrophotometer, and the absorbance results were calculated using the following formula:

[1] Klorofil total (mg/g) = (20.2 × A645) + (8.02 × A663)
[2] = [1] × dilution

Stomatal density and stomatal aperture width, stomatal density is calculated using the following formula:

$$KS = \frac{Number of Stomata}{Field of View Area} mm^2$$

Transpiration Rate, observed using cobalt chloride paper and calculated using the following formula:

$$\frac{Area of Cobalt Chloride Paper}{Duration of colour change} \text{ (cm}^2\text{/second)}$$

The observation data were analyzed using analysis of variance (ANOVA), and if a significant effect was found, it was followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level.

3. Results

a. Chlorophyll Content

Chlorophyll is the green pigment found in green plants that photosynthesize. It plays a crucial role as a light receptor, specifically sunlight, which is used for the photosynthesis process in plants. The amount of chlorophyll varies between species.

Table 1. Average Chlorophyll Content of Sugarcane Plants with Mycorrhizal Application and Irrigation Intervals (g/l)

Treatments	M0	M1	M2	M3	Average
P1	0,59	0,71	0,5	0,64	0,61 a
P2	0,51	0,79	0,68	0,79	0,69 a
P3	0,51	0,68	0,81	0,67	0,67 a
Average	0,54 a	0,72 a	0,66 a	0,70 a	

Note: Numbers followed by the same lowercase letters in the same column are not significantly different based on Duncan's test at the 5% significance level. *Transformation : $\sqrt{x + 0.5}$. M0 = control, M1 = 10grams, M2 = 30grams, M3 = 50 grams and P1 = once every day , P2 = once every three days, P3 = once every five days.

The results of the analysis of variance showed that mycorrhizal application and irrigation intervals had no significant effect on the chlorophyll content of sugarcane plants. However, visual observations indicated that the application of 30 grams of mycorrhiza per plant with an irrigation interval of once every five days (P3M2) showed the highest chlorophyll content compared to other treatments. Nitrogen is one of the main components of leaf chlorophyll, accounting for about 60%, and plays a role as an enzyme and membrane protein. Mycorrhiza interacts with nitrogen-fixing bacteria, allowing mycorrhizal application to enhance the absorption of nitrogen, which is a key component of chlorophyll (Abdillah et al., 2021). The reduction in chlorophyll content is one of the physiological responses of plants to water deficiency. A decrease in chlorophyll content during water stress is related to the reduced activity of photosynthetic apparatus and lowers the plant's photosynthesis rate due to the reduced production of rubisco enzymes in the dark reaction (Mashmud and Oktavia, 2015).

According to Dwidjoseputro (2013), environmental factors surrounding the sugarcane plants also influence chlorophyll content. The variation in leaf color among treatments shows different shades of green.

b. Transpiration Rate

Transpiration is the process of water loss in the form of water vapor from the plant's body, mostly occurring through the stomata. Water can also be released through the cuticle and lenticels (Taluta et al., 2017). The transpiration process starts with the absorption of soil water by the plant roots, which is then transported through the stem to the leaves and released as water vapor into the atmosphere. The transpiration rate is influenced by factors such as vegetation characteristics, soil characteristics, the environment, and crop cultivation patterns.

Tabel 2. Rerata Laju Transpirasi Tanaman Tebu pada Pemberian Mikoriza dan Interval Penyiraman

Treatments	**M0**	**M1**	**M2**	**M3**	**Average**
P1	0.23	0.11	0.10	0.12	0.14 a
P2	0.09	0.13	0.10	0.11	0.11 a
P3	0.10	0.11	0.08	0.10	0.10 a
Average	0.14 a	0.12 a	0.09 a	0.11 a	

Note: Numbers followed by the same lowercase letters in the same column are not significantly different based on Duncan's test at the 5% significance level. *Transformation : $\sqrt{x + 0.5}$. M0 = control, M1 = 10grams, M2 = 30grams, M3 = 50 grams and P1 = once every day , P2 = once every three days, P3 = once every five days.

The application of mycorrhiza had no significant effect on the transpiration rate at various irrigation intervals. The transpiration rate is controlled by stomatal aperture. Under water deficit conditions, stomata close, and gas exchange decreases. Stomata are the main pathway for water loss and CO2 absorption during photosynthesis. In water-deficient conditions, CO2 absorption decreases, which triggers a reduction in metabolic activity, resulting in decreased plant growth and development. The ability to limit water loss through transpiration is one of the key factors in plant tolerance to water scarcity. The transpiration rate decreases as stomatal conductivity declines (Setiawan et al., 2013).

Field observations showed that the application of 30 g of mycorrhiza per plant with an irrigation interval of once every five days (P3M2) resulted in the lowest transpiration rate compared to other treatments. This is because mycorrhiza can regulate water release through stomata or transpiration, maintaining the relative water content in the leaves during drought conditions. Additionally, mycorrhiza enhances the absorption of nutrients, especially phosphorus (P). In the soil, phosphorus is often unavailable, but mycorrhizal hyphae can increase the activity of acid phosphatase enzymes, converting P into a form that is easily absorbed by the plant for ATP synthesis (Lumbantoruan et al., 2021). This

ATP is used by the plant for nutrient absorption through cell membranes, increasing nutrient uptake (Abdillah, 2021). According to Setiawan et al. (2013), when water availability is limited, and the transpiration rate is equal to or lower than the rate of water absorption by roots, plant growth is not hindered. This condition was observed in the treatment with 30 g of mycorrhiza per plant with an irrigation interval of once every five days (P3M2).

c. Stomatal Aperture Width

Stomata are part of the epidermis of plant organs, consisting of a pore surrounded by specialized cells called guard cells. Stomata are crucial for plants because their primary function is to facilitate gas and water exchange between the atmosphere and the intercellular space system in the mesophyll tissue beneath the epidermis. The structure of stomata influences their effectiveness during the photosynthesis process.

Table 3. Average Stomatal Aperture Width of Sugarcane Plants with Mycorrhizal Application and Irrigation Intervals

Treatments	M0	M1	M2	M3	Average
P1	20.03	18.75	19.63	20.93	19.84 a
P2	24.61	20.40	20.40	23.32	22.18 a
P3	21.36	19.96	20.06	19.16	20.14 a
Average	22.00 a	19.71 a	20.03 a	21.14 a	

Note: Numbers followed by the same lowercase letters in the same column are not significantly different based on Duncan's test at the 5% significance level. *Transformation : $\sqrt{x + 0.5}$. M0 = control, M1 = 10grams, M2 = 30grams, M3 = 50 grams and P1 = once every day , P2 = once every three days, P3 = once every five days.

The application of mycorrhiza and the irrigation interval had no significant effect on the stomatal openings of sugarcane plants. Based on field observations, the application of 50 g of mycorrhiza per plant with an irrigation interval of once every five days (P3M3) showed that the size of the stomatal openings was tighter compared to other treatments, particularly the treatment without mycorrhiza. The application of mycorrhiza is related to its function in retaining water, thus optimal mycorrhizal treatment can influence the pressure in the guard cells of the stomata. Stomata function as a mechanism for plant adaptation to drought stress, which causes them to close as a means of reducing transpiration rates (Mashmud and Oktavia, 2015). The width of the stomatal openings indicates that an increase in transpiration rate is occurring (Taluta, Rampe & Rumondor, 2017). This is consistent with the transpiration rate parameter, as treatments without mycorrhiza also resulted in faster transpiration rates; this is because, without mycorrhiza, the stomata open wider compared to those with mycorrhiza added. An increase in transpiration rate can be achieved by enlarging the stomatal gap or increasing the number of stomata to an adequate level to compensate for evapotranspiration under drought stress conditions (Mangoensoekarjo, 2007). According to Sasli (1999), water stress leads to a decrease in turgor pressure in plant cells, resulting in a decline in physiological processes. Turgor affects cell enlargement and division, the opening and closing of stomata, leaf development, flower formation and development, as well as the movement of various other parts of the plant (Subantoro, 2014)

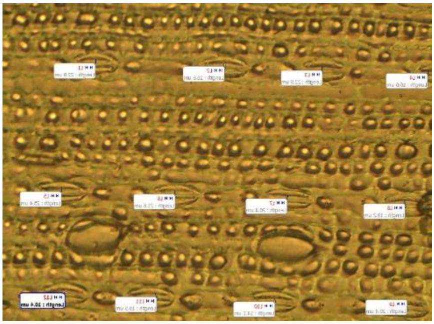

Figure 2. Stomatal Opening Width for Treatment P3M3

d. Stomatal Density (µm)

The measurement of stomatal density is an important parameter as it relates to plant photosynthesis. Stomata play a crucial role as a mechanism for plant adaptation to drought stress. A higher stomatal density accelerates the transpiration rate because stomata are the central pathway for water loss and CO_2 absorption during the photosynthesis process (Kristanto et al., 2022).

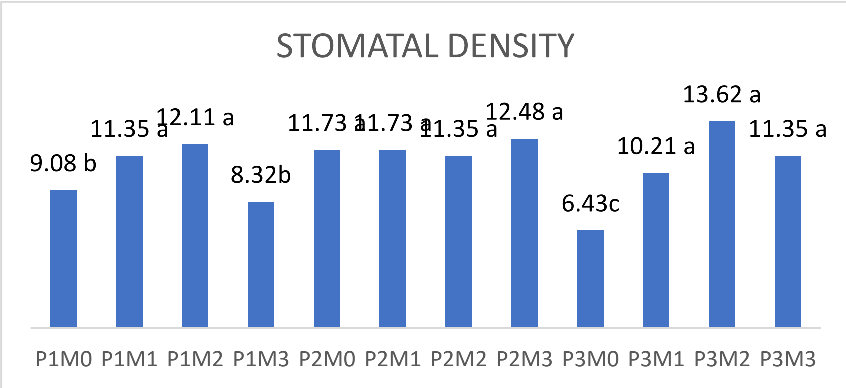

Figure 2. Average Stomatal Density of Sugarcane Plants with Mycorrhizal Application and Irrigation Intervals
Note : M0 = control, M1 = 10grams, M2 = 30grams, M3 = 50 grams and P1 = once every day , P2 = once every three days, P3 = once every five days.

Based on the results of the analysis of variance, there is a significant effect of the combination of arbuscular mycorrhizal dosage and watering intervals on the physiological responses of sugarcane plants. A dosage of 30 g/polybag applied to sugarcane plants with watering every five days (P3M2) resulted in closely spaced stomata, which showed no significant difference from other treatments except for P3M0 (watering every five days, without mycorrhiza) and P1M0 (watering every day, without mycorrhiza) (Figure 2). The density and size of stomata in a plant are related to the plant's resistance to drought (Lestari, 2005). As stomata become more densely packed, the processes of opening and closing become increasingly restricted. According to Dama et al. (2020), in drought stress conditions, the stomatal density of plants will increase. One mechanism by which plants tolerate drought is by optimizing the role of stomata in their activity to prevent water loss through the leaves.

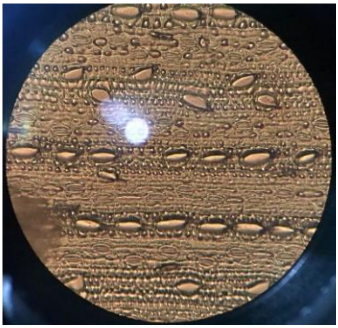

Figure 3. Stomatal Density for Treatment P3M2

5. Conclusion

The application of mycorrhizal dosage and watering intervals significantly affects the physiology of sugarcane seedlings in terms of stomatal density. There is an interaction between the combination of watering intervals and mycorrhizal dosage on stomatal density. The treatment with a mycorrhizal dosage of 30 grams and a watering interval of every five days demonstrates physiological activity indicative of drought-tolerant plants.

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