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Effect of Waterlogging on the Morphological Characters of Mutant Sugarcane (*Saccharum officinarum* L.) in the Early Stage

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ABSTRACT

Waterlogging stress is a limiting factor in sugarcane growth due to global climate change. In Indonesia, Waterlogging stress in sugarcane areas is caused by high rainfall and the increasing number of sugarcane plants cultivated on land with less than optimal drainage systems. In this study, four sugarcane genotypes, 1 Bululawang commercial genotype (M1), and three mutation breeding genotypes using EMS mutagens (M2, M3 & M4) were subjected to waterlogging stress for 90 days. They observed data at 30 DAT, 60 DAT, and 90 DAT. The study results showed that waterlogging stress had an impact on decreasing leaf area, leaf relative water content, chlorophyll content, photosynthetic efficiency, and inhibition of plant height growth. Sugarcane will respond to waterlogging stress conditions; a series of response forms given by plants include anatomical, morphological, physiological, and biochemical responses as a form of resistance in dealing with waterlogging stress. The sugarcane M4 genotype gave the best growth response under waterlogging stress through indicators of leaf area, RWC, chlorophyll content, photochemical efficiency, and plant height compared to other genotypes. All sugarcane genotypes showed changes in root morphology through adventitious root formation in response to waterlogging conditions.

Key words*: Sugarcane, Waterlogging, Morphological characters, Stress response, Adventitious root*

Introduction

Climate change results in an increase in global temperature, which impacts high evaporation rates and increased flooding disasters. Waterlogging of agricultural land due to flooding will significantly reduce sugarcane production (Martínez-Alcantaraeta, 2012). Sugarcane is quite tolerant of excess water conditions. However, this tolerance depends on the duration of waterlogging, water quality, water flow, type of variety, soil structure, and available drainage conditions. The most susceptible phase of flooded sugarcane plants is in the early stages at the age of 3-4 months, relatively tolerant at the age of 5-9 months, and very tolerant at more than nine months (Gomathi *et al.*, 2015).

Waterlogging stress on sugarcane impacts productivity decline by 30-60%. The level of damage due to waterlogging stress can be influenced by several factors, such as water quality, duration, and water level. In addition, each sugarcane variety will provide a different response and level of damage when under waterlogging stress conditions. In line with climate change conditions that increase the risk of flooding, sugarcane varieties are needed that are resistant to waterlogging conditions (Gilbert et al., 2008).

Sugarcane varieties resistant to waterlogging stress are needed to provide high yields in sub-optimal growing conditions. Mutation breeding in sugarcane can be a faster solution to produce sugarcane varieties resistant to waterlogging than conventional breeding. Conventional sugarcane breeding has several difficulties, including a high level of polyploidy, asynchronous maturity of male and female flowers, long flowering times, and tiny flower sizes (Manners *et al.,* 2004). In addition, the limited availability of sugarcane germplasm is also an obstacle for sugarcane breeding efforts.

The best mutant with resistance to waterlogging stress will respond better to morphological changes than the sensitive mutant. Changes in plant morphological characters due to waterlogging stress include decreased plant height, leaf area, chlorophyll content, relative water content, and photochemical efficiency.

Observation of changes in morphological characters in response to waterlogging stress is one of the fastest methods to analyze the level of plant resistance to waterlogging. So it is necessary to study changes in the morphological character of mutant sugarcane due to waterlogging stress in the early stages of growth.

Materials and Methods

Plant Material

This research was conducted in July – October 2021 at the Agronomy Laboratory, Faculty of Agriculture, Jember University using four sugarcane genotypes, one commercial genotype M1 (Bululawang), and three sugarcane genotypes resulting from mutation breeding using EMS mutagens: M2, M3, and M4. Planting material is obtained from the 3rd generation of sugar cane plant, formed into a single bud chip. The seedling media used polybags measuring 10x10 cm in soil and compost media with a ratio of 1:1. Seedlings are maintained until they are 30 days old and then transferred to the treatment media.

Waterlogging Treatment

Waterlogging tolerance test for four sugarcane genotypes was carried out according to the modified Gomathi *et al.* (2015) method. Sugarcane seedlings aged 30 days were transferred to pots containing 5 kg of soil and compost (1:1). The treatment plants will be flooded with a water level of 5 cm above the soil surface.

The waterlogging treatment will be given for 90 days and made three observations at 30, 60, and 90 DAT.

Experimental design

This study used four genotypes and two types of treatment using three replications. Two types of treatment were waterlogging and without flooding as a positive control. Morphological parameters observed were plant height, adventitious roots, and leaf area. The physiological parameters will be monitored for total chlorophyll, relative water, and photochemical efficiency using the MINIPAM WALZ tool.

Leaf Area

The data was obtained by cutting all the leaves on one plant and taking pictures using a digital camera. The shooting process must include a measuring scale. The image results were analyzed using ImageJ software to determine the leaf area.

Total Chlorophyll

The leaves were taken 100 mg and lysed using 5ml of 95% ethanol. The lysate formed was centrifuged at 12,000 rpm for 10 minutes. The supernatant formed was measured using a spectrophotometer with wavelengths 664 and 649.

$$Ch.A = (13.36*Abs.664) - (5.19*Abs.649)$$
$$Ch.B = (27.43*Abs.649) - (8.12*Abs.664)$$

Relative Water Content (RWC)

The third perfect leaf was cut and weighed as fresh weight (FW). After that, the leaves were soaked in distilled water for 6 hours and weighed as turgid weight (TW). The leaves were oven-dried at 70°C for 24 hours and weighed as dry weight (DW). The formula calculates relative water content.

$$RWC = \frac{(FW-DW)}{(TW-DW)} \times 100$$

Data manipulation and analysis

Data analysis used Two-Way Annova to find out two independent variables combined with the expected value of the dependent variable. If the independent factor has a significant effect, it will be continued with the DMRT test.

Results

Plant High

At 30 days, all sugarcane genotypes experienced a decrease in plant height by an average of 24.97% ± 1.26 compared to controls during the waterlogging period. The M1 genotypes experienced inhibition of plant height gain by 14.86%, M2 16.77%, M3 10.99%, and M4 6.19% compared to controls in the 30-day waterlogging period. This condition continued in the waterlogging period of 60 days, M1 20.84%, M2 28.17%, M3 28.07%, and M4 19.65% compared to control.

On the other hand, the inhibition of increasing plant height improved over 90 days; the genotypes of M1 only decreased by 18.99%, M2 20.26%, M3 20.24%, and M4 17.94% compared to control (Figure 1). waterlogging conditions on sugarcane plants significantly inhibited the increase in plant height in all sugarcane genotypes.

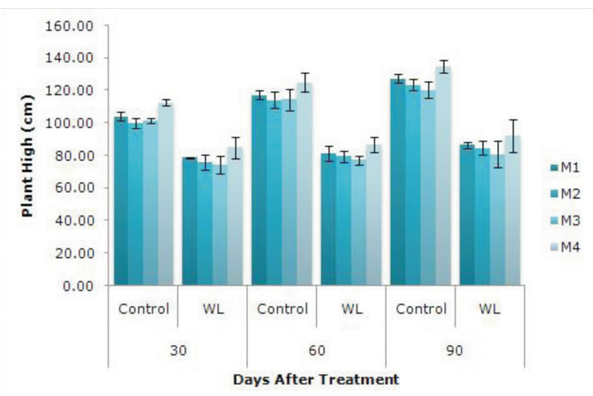

Fig. 1. Decrease in plant height after receiving waterlogging treatment compared to control at 30, 60, and 90 days after treatment. Values are presented as the mean ± SD.

Leaf Area Surface

All sugarcane genotypes experienced a decrease in leaf area during waterlogging by an average of 53.37%±0.94 compared to control. The lowest reduction in leaf area occurred during the 60-day waterlogging period : M1 57.38%, M2 57.85%, M3 58.78%, and M4 57.05% compared to controls. In the 90-day waterlogging period, all sugarcane genotypes responded better to waterlogging stress : M1 54.85%, M2 56.28%, M3 51.55%, and M4 53.45% compared to controls. The average value for 90 days of waterlogging stress, the M4 genotype, gave the best response, which only experienced a decrease in leaf area by an average of 52.19%, compared to M1 of 53.63%, M2 of 54.46%, and M3 of 53.21% (Figure 2).

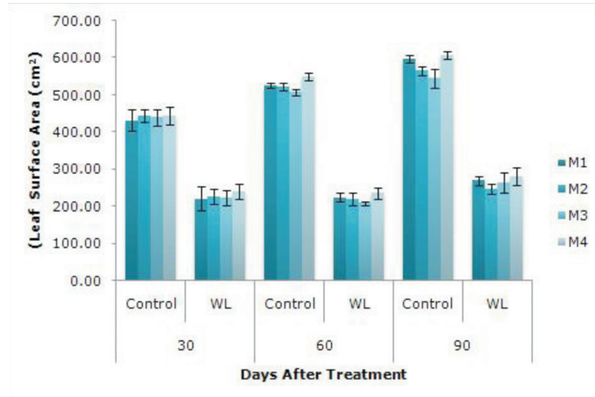

Fig. 2. Decrease in leaf area of plants under waterlogging stress. Values are presented as the mean ± SD.

Total Chlorophyll

The decrease in total chlorophyll content occurred in all sugarcane genotypes, and the most significant reduction was in the 60-day waterlogging period : M1 20.84%, M2 28.17%, M3 28.07%, and M4 19.65% compared to the control. In the 90-day waterlogging period, all sugarcane genotypes that received waterlogging stress experienced an increase in the total chlorophyll content : M1 18.99%, M2 20.26%, M3 20.24%, and M4 17.94% compared to the control. The overall average data for 90 days of waterlogging, the M4 genotype gave the best response for the total chlorophyll content, which only decreased by 14.59% compared to the control (Figure 3).

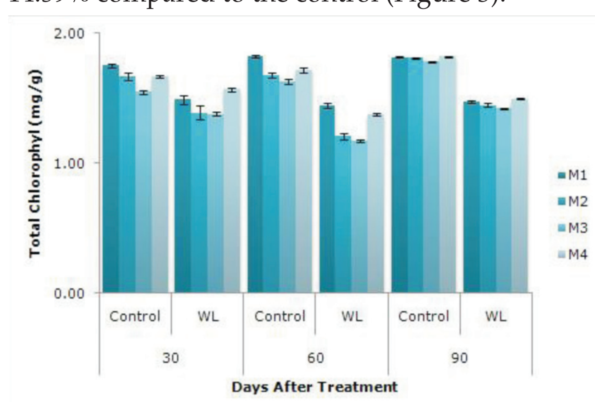

Fig. 3. Total chlorophyll during waterlogging treatment. Values are presented as the mean ± SD.

Relative Water Content (RWC)

The decrease in relative water content occurred in the 30 days of waterlogging treatment. All sugarcane genotypes showed a reduction in relative water content, M1 8.88%, M2 8.69%, M3 7.86%, and M4 7.64% compared to controls. This decreasing value continued in the 60 days of waterlogging treatment: M1 13.65%, M2 12.54%, M3 11.98%, and M4 10.89% compared to control. Sugarcane genotypes with waterlogging treatment experienced an increase in the relative value of water content in the 90-day waterlogging period : M1 7.31%, M2 7.23%, M3 7.81%, and M4 6.60% compared to the control. On the other hand, the relative water content value was relatively stable for all genotypes without waterlogging treatment (Figure 4).

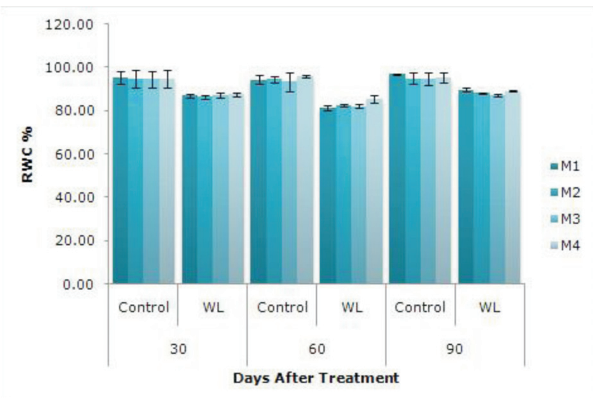

Fig. 4. Leaf Relative Water Content during waterlogging treatment. Values are presented as the mean ± SD.

Photochemical Efficiency

The waterlogging stress treatment also impacted the photochemical efficiency of= all sugarcane genotypes. The decrease in the value of photochemical efficiency decreased in the 30 days of waterlogging treatment and began to increase again in the 60 and 90 day period of waterlogging treatment. The period of 30 days of waterlogging treatment gave a decrease in all genotypes that received waterlogging treatment. Decrease in M1 54.05%, M2 54.51%, M3 59.54% and M4 41.00% compared to the control. Furthermore, 60 days after the waterlogging treatment, each genotype's Fv/Fm value increased : M1 50.43%, M2 50.79, M3 51.67%, and M4 38.64% compared to control. This increase continued through the 90-day waterlogging period, M1 50.41%, 48.69%, and M4 34.88% compared to control. However, for the M3 genotype, the Fv/Fm value in the 90-day waterlogging period decreased to 54.49% compared to 51.67% in the 60-day waterlogging treatment period (Figure 5).

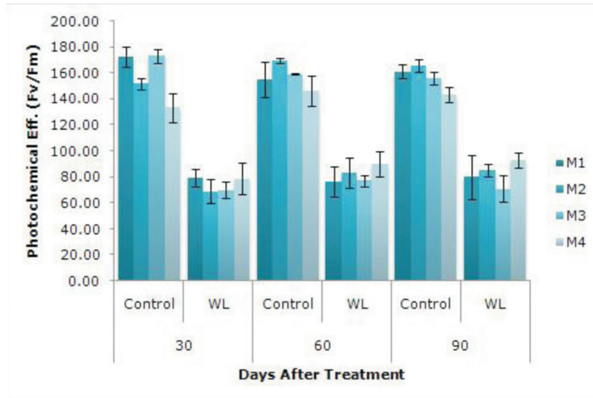

Fig. 5. Effect of waterlogging period on the value of Photochemical Efficiency (Fv/Fm). Values are presented as the mean ± SD.

Discussion

Waterlogging conditions on sugarcane roots impact the inhibition of plant height growth. The value of plant height can reflect the metabolic processes in plants that experience waterlogging. Flooded plant roots will experience disruption of the transport of nutrients from the soil to be distributed to all parts of the plant. This condition will impact nutrient deficiency, which causes metabolism disorder in plants and has an impact on inhibiting plant height growth (Chi Yang *et al.,* 2020). When the soil is flooded, only a tiny amount of gas diffuses in the plant's root area in the soil shaft. So that it impacts the concentration of mineral elements in the soil. Besides that, toxic products from the activity of microorganisms will accumulate in the root area. These changes cause losses to plants due to inhibition of growth and development.

Despite being in a flooded condition, the plants were still able to show the ability to continue to grow and develop through the acclimation process. Acclimation involves physiological, anatomical, or morphological adjustments within an organism that enhance performance or survival in response to environmental changes (Jorgensen, 2008).

The difference in leaf area between plants affected by inundation and control plants is also the fastest way to determine the response of plants to inundation. In general, plants will take two main steps in response to inundation stress, namely the quiescence strategy and the deep-water escape strategy (Chen *et al.,* 2008). In Quiescence, the system aims to save energy consumption used for growth so that plants can prolong life in flooded conditions. This strategy will impact the inhibition of several growth phases, one of which is the inhibition of growth on the leaves of the plant so that the leaf area of the plant affected by inundation will be narrower than the control plant.

The minimal availability of oxygen in flooded conditions is an inhibiting factor in transporting nutrients from the roots to the leaves. This condition can cause a decrease in leaf chlorophyll levels because it cannot fulfill the plant's need for nitrogen as a constituent of chlorophyll. In this study, the reduction value of total chlorophyll content is also correlated with plant height and leaf area value. It will also have an impact on the importance of photochemical efficiency. The formation of more adventitious roots at the age of 90 days after flooding was able to increase the ability of the roots to transport nutrients and oxygen in flooded conditions. This condition can encourage an increase in the arrangement of chlorophyll and photosynthesis in the leaves.

Changes in morphological characters that are easy to observe when plants are in a state of waterlogging stress are the curved leaf shape (flaccidity). Flooded conditions cause the solute concentration to be relatively the same as the solute concentration in the cell. This means that there will be no net movement of water molecules between them. The relative water content value is strongly influenced by turgidity conditions; the leaves of plants with turgidity will look sturdy due to the turgor pressure applied to the cell wall. Whereas in plants that are stressed by waterlogging, the turgidity condition decreases, causing the plant leaves to look limp or mushy.

Waterlogging stress also has an impact on decreasing Photochemical Efficiency (Fv/Fm) values in all sugarcane genotypes. This value is a representative ratio of the maximum potential of photosystem II if all reactions are able to open (optimum conditions). Fv/Fm values fluctuated in each sugarcane genotype that received waterlogging stress. Measurement of the value of Fv/Fm was carried out as a sensitive indicator of the value of damage due to stress in photosystem II (Maxwell and Johnson, 2000). This Fv/Fm is estimated to indicate the level of damage due to the effects of environmental stress on the photosynthetic process, such as waterlogging stress conditions. In our study, the decrease in Fv/Fm has a correlation with the decrease in the value of the total chlorophyll content, and this condition is in accordance with the results of the study of Zheng and Guan. (2006).

The ability of all sugarcane genotypes to survive in waterlogging stress was supported by the presence of adventitious roots and root aerenchyma tissue. In the deep-water escape strategy, plants carry out internal oxygen diffusion by forming aerenchyma so that oxygen can diffuse to the deficient plant parts. At the same time, plants can cope with waterlogging stress by increasing root elongation, forming adventitious roots to obtain sufficient oxygen. The formation of adventitious roots can improve nutrient and oxygen transport, complement or even replace primary roots that die due to lack of oxygen, and increase plant tolerance to waterlogging stress.

Conclusion

Waterlogging stress has an impact on changes in morphological characters in all sugarcane genotypes. Sugarcane plants under waterlogging stress try to acclimatize to be able to continue their life cycle through two main strategies: quiescence strategy and deepwater escape strategy. Quiescence strategy: decreasing leaf area and leaf relative water content; and deepwater escape strategy: formation of adventitious roots and root aerenchyma tissue. The M4 genotype (mutant sugarcane) gave the best response to changes in morphological characters compared to other mutant genotypes. Based on the results of this study, sugarcane can be used as a model for the analysis of plant resistance to inundation stress.

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