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RESEARCH ARTICLE

Impact of global warming through drought stress simulation on morphological features of *Ananas comosus* L. (pineapple) var. MD2 clonal plantlets

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Abstract

Extreme high temperature has become more common as a result of global warming which acts as a key abiotic stress that inhibits plant development and productivity. In this research, drought-exposed *Ananas comosus* clonal plantlets were triggered to specific physiological responses to overcome the effect of drought stress. The plantlets of *A. comosus* were placed in a greenhouse with recorded temperatures ranging between 32°C to 36°C and treated with three different watering frequency which are once a week (Treatment 1/control), once every two weeks (Treatment 2), and not watered at all (Treatment 3). The first obvious morphological effect is wilting, drying out of the tips or edge of leaves, and yellowing or browning of leaves. Treatment 1 recorded the highest in the mean of leaves length among other samples. In Treatment 2, a lower amount in the mean of leaves length as compared to Treatment 1 was observed. Meanwhile, Treatment 3 recorded the highest wilting record as well as the lowest mean of leaves length. The physiological changes in stomata conductance have greater conductance for Treatment 1 with more open stomata and obvious pores followed by Treatment 2 with stomata opened slightly and Treatment 3 was observed to have sunken stomata and the pores were hardly seen. The physiological changes of stomata have shown the mechanism of adaptation in clonal plantlets to control transpiration and prevent dehydration under thermal and drought stress.

Keywords: Climate change, Water deprivation, Thermal stress, Clonal plants, Plant adaptability

Introduction

Ananas comosus L. or pineapple is one of Malaysia's most popular tropical fruits. Its popularity emanates from the fact that it can be consumed in multiple forms. It is categorized as a major fruit because it has the potential to generate income for farmers as well as countries. Pineapple is a tropical fruit with strong export potential and a high nutritional value that generates money for many smallholders in Malaysia. *Ananas comosus* is the most well-known commercially harvested species (Suhaimi and Fatah, 2021). In the process of pineapple cultivation, a slight increase in temperature acts as a stress impacting the growth of pineapple. Temperature and optimum irrigation are among the important factors in agriculture since they influence seed germination and plant development. Global warming has caused an erratic rise in temperature and prolonged drought hence posing a major danger to global agricultural productivity. As a result, the mechanisms by which plants adapt to high temperatures are of great interest for studies in this area to be intensified. Plants exposed to high temperatures or heat stress suffer serious and often fatal consequences (Zhao et al, 2020). They also evolved to respond to heat stress in dealing with such circumstances.

Previous studies have identified the response of thermal stress at the molecular level for pineapple; however, reports on the effect of thermal stress in terms of morphology and physiology are scarce. Heat stress affected responses at the molecular level as documented in the study by (Zhao et al. 2021) in which several heat shock proteins have been linked to the heat response of pineapple. In fact, most of them exhibited considerably higher expression levels in the high-temperature group than in the normal group. The drawbacks suffered were also reported in the review by (Ortiz, 2021) which includes slower development, an extended flowering forcing period, fruit with a smaller caliper, malformations, lower weight, and inferior quality. It is commonly known that plants may adapt to temperatures that are either higher or lower than those that are best for their growth. Plants become resilient and able to adapt to more heat stress after experiencing mild heat stress.

The studies about the effect of thermal and drought stress on *A. comosus* plants are considerably limited. Therefore, in the present research, we use high temperature and water deficiency as the simulation of drought stress as part of changing climate impact on clonal plantlets of *A. comosus.* This is to assess the plant response in the aspect of morphological changes in the clonal plantlets of the species specifically on how these conditions impact the architectural characteristic of the stomata. Realizing the key role of stomata and cuticle-sealed leaves in the regulation of carbon dioxide influx and water efflux, the evolutionary adaptations involving stomatal structure are crucial to be analysed. This is essential to drive us towards understanding the impact and plant adaptation responses to the changing climate.

Materials and Methods

Plant treatment condition

The study was conducted under the temperature ranges between 32°C to 36°C in the greenhouse. In this study, the *A. comosus* clonal plantlets from the MD2 variety were treated in three different conditions and maintained under the stated treatments for three months. In Treatment 1 or control treatment, every plant was watered once a week, meanwhile in Treatment 2 every plant was watered every fortnight, and in Treatment 3, no water was provided to all of the plantlets. Each treatment was replicated by five plantlets.

Morphological measurement

In terms of morphological observation, the number of plant wilting, leaf number and the leaf length were taken. This is very important to observe changes between the samples after they have been treated with different treatments. The data were recorded in Microsoft Excel for comparison.

Biomass record of sample

In each treatment, both leaf and root parts were taken and cleaned for fresh weight and dry weight measurements before and after drying process. The samples were dried in the drying cabinet at 40°C. The dry weight measurement was taken by using an analytical balance and was measured until the recorded values were constant. Four parameters were observed in the biomass record of samples i.e. the fresh weight of leaves, the fresh weight of roots, the dry weight of leaves, and the dry weight of roots. The data was recorded in Microsoft Excel and the percentage of water content in all samples was calculated as follows:

$$Water\ Content\ Percentage = \frac{Fresh\ Weight(g) - Dry\ Weight(g)}{Fresh\ Weight(g)}$$

Microscopic observation by Scanning Electron Microscope (SEM)

Parts of pineapple leaves were taken from each treatment to conduct microscopic observation by using SEM. The morphological changes in stomata due to drought and thermal stress were the subject of interest in this analysis. The samples were washed and dried before loading. Next, the leaves were cut into small pieces and mounted onto the aluminium stub by using a sticky carbon tab (Carbon Conductive Adhesive 502) to ensure the sample was rigidly fixed to avoid vibrations. Next, the pineapple leaves were viewed under the SEM to observe the changes in stomatal morphology or any other differences among the samples. The results can be viewed in images and were captured for each treatment of the pineapple leaves sample.

Results and Discussion

Morphological observation

As referred to (Tab. 1,) Treatment 1 (control treatment) samples were watered once a week and recorded the low plant wilting number and the highest mean of leaves length among other samples. Meanwhile, for samples that were watered every fortnight and not watered at all, lower amounts in the mean of leaves length were recorded.
In the treatment with deprivation of water where no water was provided throughout the course, the highest value of plant wilting, as well as the lowest value in the mean of leaves length were observed. The comparisons between Treatment 1 and Treatment 3 in terms of their morphological features are shown in (Fig. 1).

Figure 1. ***A. comosus*** **clonal plantlets after three months of observation in (A) control treatment (Treatment 1) and (B) water-deprived treatment in Treatment 3**

This finding showed that water supply plays a significant role in influencing the morphological parameters taken. Other than that, the surrounding temperature provided for the samples also affects the pineapple plant in the greenhouse. Transpiration causes more water to be lost than is brought in in a hot, dry condition which might affect the plant's internal water balance. The plant starts to wilt because of the collapsing, dehydrated cells in the leaves and stems being unable to stand upright.

Table 1. **The morphological record after three months of thermal stress and watering frequency**

Treatment	Number of Leaves (Mean ± SEM)	Number of Plant Wilt (Mean ± SEM)	Length of Leaves (Mean ± SEM) (cm)
Treatment 1 (control)	18.00 ± 0.860	5.80 ± 0.836	20.27 ± 1.293
Treatment 2	16.80 ± 0.812	5.60 ± 1.772	19.59 ± 2.200
Treatment 3	13.00 ± 0.678	6.60 ± 1.000	17.78 ± 1.241

Microscopic observation by SEM

The stomatal conductance after the samples have been experimented under thermal and drought stress was analysed microscopically by SEM. According to (Malézieux et al. 2003), stomata are pores located in the leaves where they can open and close to the atmosphere. Based on the SEM viewing findings, it has been demonstrated that the stomata on the pineapple leaf sample in Treatment 1 (control treatment) were opened with apparent pores, as depicted in (Fig 2).

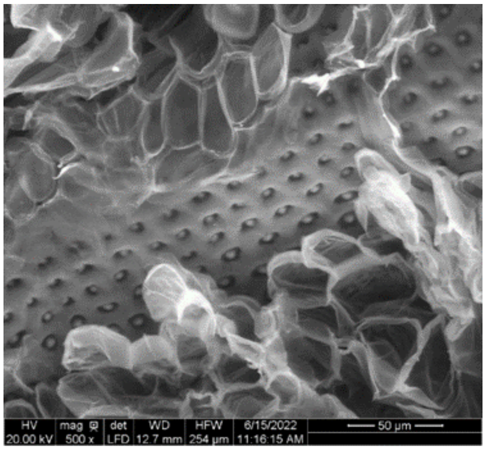

Figure 2. The stomata on the leaf of the pineapple sample in Treatment 1.

In this treatment, the plants were optimally watered once a week as compared to the other treatments. The opening of the stomata can be inferred due to the increase in temperature from surroundings hence leading to an increase in transpiration rates accordingly. This is also in agreement with the report in a previous study by (Kostaki et al. 2020) in which the high temperature induced guard cell expansion. This situation further led to the opening of stomatal pores to facilitate the cooling process in leaves.

Meanwhile, the stomata of pineapple leaf in Treatment 2 were slightly opened and the pores were less apparently observed as compared to those in Treatment 1. These circumstances could be observed under 500x magnificent as depicted in (Fig. 3). Nevertheless, the leaf in Treatment 3 has established different findings where the stomata were identified to change their normal form, specifically into sunken shape under 500x magnificent as showed in (Fig. 4) and the pores were hardly seen. According to (Roth-Nebelsick, 2007), sunken stomata or stomata placed in crypts is identified as a drought adaptation measure in plants. Hence it is generally interpreted as having reduced transpiration.

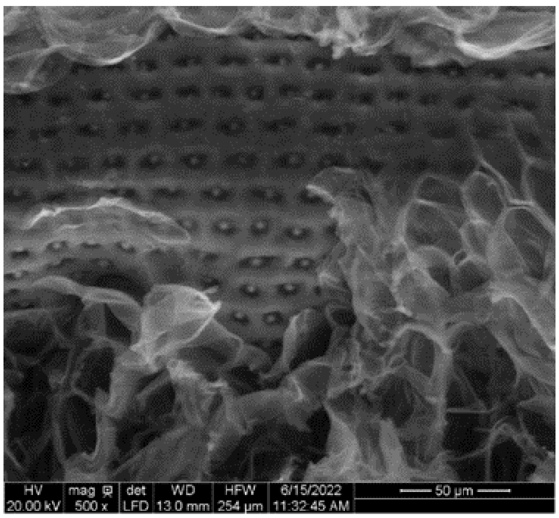

Figure 3. The stomata on the leaf of the pineapple sample in Treatment 2.

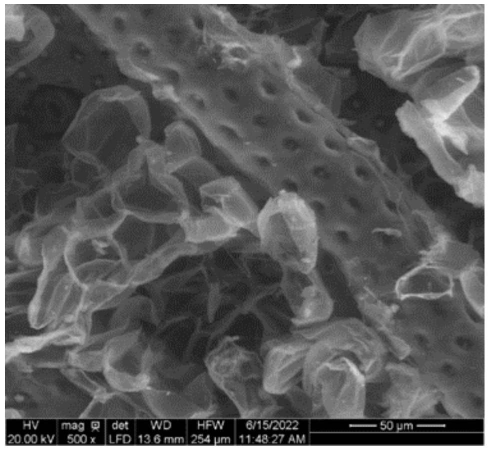

Figure 4. The stomata on the leaf of the pineapple sample in Treatment 3.

The extent of carbon uptake and water loss in plants are regulated by stomata and cuticles on leaves. Stomata, as important anatomical features of plants consist of a pair of guard cells for each stoma and the existence of small pores. The changes in the structure of stomata for instance in terms of its shape, pore aperture as well as density may suggest the mechanism of plant adaptation towards the surrounding environment. As mentioned by (Liang et al. 2023), stomatal conductance is sensitive to various environmental changes, and factors influencing stomatal conductance are essential parameters of earth system models.

Generally, to allow the gas exchange of carbon dioxide and oxygen during photosynthesis, plants must open their stomata pores. Hence, transpiration is inevitable in photosynthesis. As plants are subjected to drought stress, the most important component that occurs initially is a reduction in water-use efficiency, which varies depending on variety and cultivar. Plants reduce stomatal density and leaf size in these conditions, hence minimizing water loss and maintaining internal water balance (Seleiman et al., 2021). Understanding stomatal physiology and gas exchanges between plants and the atmosphere requires elucidating the stomatal response to CO_2 (carbon dioxide) concentrations. Plants must control stomatal growth and behavior to balance CO_2 and water exchange via the leaf epidermis in a changing environment to adjust CO_2 intake for photosynthesis and water release for transpiration (Xu et al., 2016).

According to (Lambers et al. 2014), plants will reduce water efflux when temperatures are too high during the day. In addition, large vapor pressure deficits might induce high transpiration rates as well, in which increasing Vapor Pressure Deficit (VPD) will in turn, increase atmospheric demand for water (Massmann, 2019). The amount to which transpiration causes leaf tissue dryness is determined by multiple factors among which are the availability of water in the soil, the water uptake capacity of roots, and the hydraulic system in roots and stems to transfer water. As discussed by (Lambers et al. 2014), plants with consistent access to soil moisture may maintain their stomata open during the day. In such a situation, plants may rehydrate at night to balance the substantial leaf water loss due to low osmotic potentials.

Balancing the need to prevent water loss while maintaining survival by allowing sufficient carbon gain is crucial in the physiological process of a plant. Under optimal and well-tolerated conditions of water loss, stomata serve as an efficient plant-atmosphere interface to maximize carbon dioxide influx hence a balanced transpiration and photosynthesis process could be attained (Vico et al., 2013; Šantrůček, 2022). Therefore, to maintain a stable water balance in the leaves, plants tend to close or partially close their stomata. This behavior has been demonstrated by pineapple leaves in Treatment 2 and 3 due to the low amount of water provided to the plants.

Therefore, from the current study, it can be inferred that the*A. comosus* plantlets can maximize carbon dioxide intake for photosynthesis while reducing water loss by adjusting the stomatal pore apertures. It is also inferred that stomatal development may also be modified throughout time, with stomatal size and density being specifically tailored to the environment.

Conclusions

Climate change that is experienced globally is indeed imparting significant impacts on lives. Being part of an important ecosystem, plants do respond to the changes to survive by regulating a desirable balance between carbon dioxide influx and water efflux while overcoming excessive transpiration. From the morphological point of view, clonal plantlets of *A. comosus* var. MD2 were observed to accordingly respond to the stress and deprivation by adjusting the stomatal structure and pores aperture. In conclusion, the findings support the fact that plants suffer from environmental stress caused by climate change, and they have specific mechanisms to overcome the detrimental effects that can be manifested by the morphological changes of stomata. However, persistent exposure to stressors caused by climate change through global warming may negatively impact the survival of plants in the long run. Intense awareness should be instilled about the impact of global warming and more studies are indeed needed to intensify research in this area.

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