THE IMPACT OF HIGH TEMPERATURE AND DROUGHT ON PHOTOSYNTHESIS RATE AND STIGMA CONDITION DURING ANTHESIS STAGE USING RICE (Oryza sativa MR253) AS A MODEL PLANT

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ABSTRACT

Rice is one of the most important crops in the world, after wheat and corn. This plant is consumed by over 7 billion people worldwide. Until now, rice has dominated the Asian continent because it is a plant that does best in warm and humid climates. The rice industry began to feel the effects of El~Nino after its occurrences in 1997–1998 and 2014–2016 because of prolonged hot and dry conditions. The flowering stage is the most crucial stage for determining rice yield. High temperature and water stress or drought may have an effect on rice flowering. It will indirectly affect the yield of rice production. This research seeks to ascertain how abiotic stress such as water stress and high temperature affects the photosynthesis rate and stigma condition of the rice plant flowering process focusing on the anthesis stage. For this study, the MR 253 variety was used. Plants were subjected to water stress (WS), a high temperature (HT) of 38 °C and a normal (C) temperature 27-34 °C as treatments and controls. The study was conducted under a rain shelter using plant containers and a simulation chamber. The water in the container of WS container was drained out 14 days before flowering to create drought conditions until temporary wilting. The high-temperature simulation was controlled over three days, from 1000h to 1400h with 70% humidity and CO2 at 400ppm, in a phytotron chamber. The results of this observation demonstrated that dry conditions and 3 consecutive days of high temperatures were sufficient to interfere with rice's flowering process, resulting in burned-dry stigma and low photosynthesis rate. Observations revealed that the HT and WS that occurred during the rice flowering phase resulted in a 2.0 to 3.5 t/ha decrease in yield compared to the average national yield (4.5 t/ha).

Keywords: *flowering, high temperature, photosynthesis, water stress, rice, stigma*

INTRODUCTION

In Asia, rice is the most crucial crop. Almost 4 billion people on earth rely on this plant as a primary source of nutrition. Due to continuous population growth, there is never enough rice produced. To address this issue, numerous studies and initiatives have been undertaken, some of which include the development of high-yield rice varieties, advancements in fertiliser technology, improvements in crop and field management, and the implementation of more efficient harvesting techniques (Horie, 2019). Although the ASEAN region's rice productivity has been improving, with further expansion anticipated in the future, there is significant worry about the escalating indicators of climate change that might have a negative impact on rice production. Malaysia experiences this issue with other Southeast Asian countries (Duasa & Mohd-Radzman, 2022). According to recent research by (Firdaus et al., 2020), the minimum and maximum temperatures in Peninsular Malaysia's primary rice-growing regions have increased by 0.3 to 0.5°C and 0.2 to 0.3°C, respectively, during the previous ten years. Rice cultivation is restricted in tropical regions with high temperatures, like Malaysia. For rice, higher temperatures may shorten the time of the grain-filling process, while extremely high temperatures during the flowering stage may result in smaller grains or less grains. (Boon Teck et al., 2021). Spikelets are less fertile when under heat stress during the anthesis stage, and grain weight will be affected by prolonged stress during seed development. There are trade-offs between the overall tiller/panicle number and spikelet number per panicle that determine the total grain number per plant due to reduced photosynthesis under heat (Li et al., 2019). In rice, high day and night temperatures or high day temperature exposure during the early stages of pollen formation substantially impacted anther and stigma development and lowered pollen viability, resulting in lower spikelet fertility (Guo et al., 2018; Xu et al., 2020). This study used the rice variety MR 253 to examine the impact of high temperatures (38 °C) and water stress on the photosynthesis rate of the leaves and the stigma condition during the flowering stage. This stage was chosen because it is the most important in determining the yield.

PROBLEM STATEMENT

Extreme weather events such as El Nino as in (1997-98, 2014-16) have been predicted to occur and will affect the agricultural industry, especially rice crops. High temperatures will cause drought and cause a decrease in yield. The average yield loss due to El Nino in 1998 was around 1.5 tons/ha. Currently, our country lacks solid information on the effects of this extreme weather on rice plants, particularly during the flowering phase. Malaysia currently relies heavily on studies from countries such as the Philippines, Vietnam, and Thailand. Through the simulation conducted by MARDI, a 1°C temperature increase will result in a

10% reduction in rice yield. While 2°C will cause a 13% decrease in rice yield. The high temperature and less water availability at the flowering stage are one of the main factors for abnormal spikelet formation because the anthesis process is affected. However, for local rice plants, it is still not possible to ascertain the specific temperature and availability of water that will affect the growth of rice especially during flowering. Therefore, this study aims to see the effect of high-temperature and drought stress on photosynthesis and stigma conditions that will cause rice plants in Malaysia to be affected during the flowering process focusing on the anthesis stage.

MATERIALS AND METHODS

The research was conducted at MARDI's Serdang in a greenhouse and plant physiology laboratory. The research method used in this study was a Randomised Complete Block Design (RCBD) with seven independent replicates. The seeds came from the MR 253 variety. The different treatments are Control (C), High Temperature (HT) and Water Stress (Drought) (WS). Each plant was planted in containers measuring 45 cm in diameter and 30 cm in height (Fig 1).

The standard methods of plant management, including fertiliser rate and pest and disease management, were applied in all treatments following MARDI Padi Lestari (Hashim et al., 2008.). All plants basically were grown at room temperature (30 - 33°C during the day and 23 °C at night) and relative humidity (RH) of 75 – 85 % in direct sunlight. Drought conditions were created by draining the water from the WS container 14 days before flowering, resulting in temporary wilting (Hussain et al., 2022).

Fig 1. Plants were grown in 45 cm diameter and 30 cm tall containers

Every day, 500 mL of water was reintroduced until the tiller reached anthesis (i.e. the appearance of anthers). On the first day of anthesis, the plants in HT treatment were subjected to HT for 4 h (1000-1400) in a phytotron (ThermoStable GC-1000, Daihan Scientific, Korea) for 3 consecutive days (Fig 2).

Fig 2. A phytotron simulated HT at 38 °C from 1000h to 1400h during flowering (4 h).

Photosynthesis parameters during the flowering stage were monitored daily for three days to see how WS and HT affected them. Net photosynthetic rates were measured with a portable photosynthesis system (LI6400XT, LICOR Inc., Nebraska, USA). To support the results for photosynthesis parameters, there are several analyses need to be done such as chlorophyll fluorescence activity and chlorophyll contents. To determine chlorophyll fluorescence, a portable Plant Efficiency Analyzer (PEA) was used

(FMS 2, Hansatech Instruments Ltd, U.K.). The chlorophyll fluorescence responses in the leaves were calculated using the Fv/Fm ratio. Chlorophyll 'a' and 'b' were analysed using the Arnon method (Pappas et al., 2016) to determine the amount of chlorophyll activity in each treatment. Each treatment had 3 cm^2 of leaves removed and immersed in an acetone solution at 80% concentration for 7-10 days. The wavelengths 647 nm and 664 nm were used on a Spectrophotometer (Genesys 10S UV-VIS) to analyse the solution.

A microscopic examination was performed to determine the condition of the flower stigma. For each treatment, 5 stigma samples were collected. The condition of the stigma was examined under a Nikon ECLIPSE 50i electron microscope at a x40 magnification. The colour and freshness of the flower stigma distinguish the state of the stigma.

The 105-day-old crop was harvested, and its yield was calculated using a digital scale and a grain counter model INDOSAW S6709 (Precisa XB220A, Switzerland).

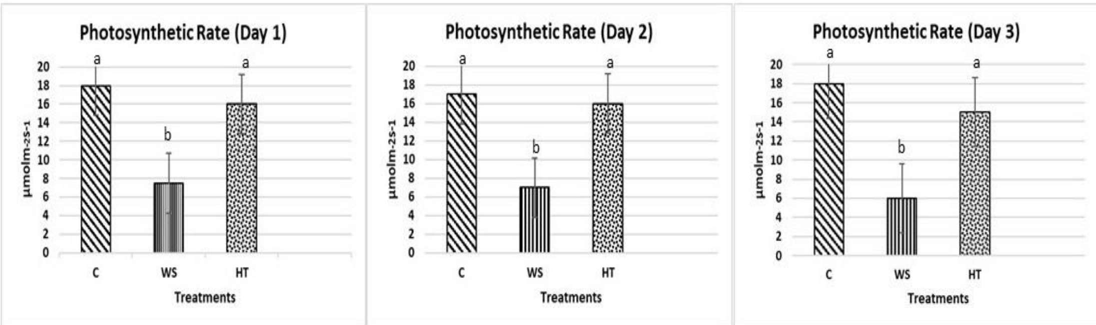
Several study parameters were subjected to analysis of variance (ANOVA) to determine whether or not there were statistically significant differences between treatments. The statistical analysis system (SAS) version 9.4 was used to compare means using factorial Duncan's Multiple Range Test (DMRT) at the 5% level.

RESULT AND DISCUSSION

i) Photosynthetic rate

The photosynthetic rate was significantly lower in WS-treated plants compared to C and HT. When a plant is stressed from a lack of water during the flowering stage, photosynthesis will slow down between 6-8 mmol/m2/s-1 (Fig 3). Stomatal conductance increased despite a decrease in leaf water potential, an increase in transpiration, an increase in intercellular CO_2 concentration, and the fact that it became uncoupled from photosynthesis as the temperature rose. This was because stomatal conductance was positively correlated with temperature (Urban et al., 2017).

Fig 3. Three-day flowering photosynthetic rate



**Error bar represents the standard error of the mean. Means followed by the same letter are not significantly different by DMRT at P ≤ 0.05.*

The increased production of ABA on the leaves causes the stomata to close, which has the effect of reducing the amount of gas that can pass through the leaves. Stomatal closure is caused by water stress, which results in a state known as photoinhibition or light inhibition. This is because water stress causes plants to use a lot of CO_2 intercellularly. According to the findings of a study conducted by Zain et al., (2014), drought stress on rice for 15 days during the reproductive stage resulted in the lowest levels of net photosynthesis (14.28 $mol/m^2/s^{-1}$) and stomatal conductance (0.31 $mmol/m^2/s^{-1}$).

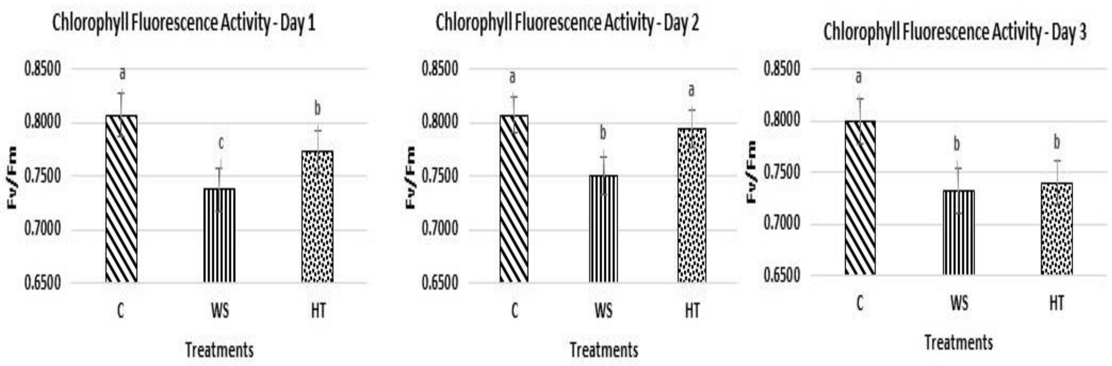
In addition to ABA, various other factors, such as plant hormones (auxins, methyl jasmonate, ethylene, brassinosteroids, and cytokinins), microbial elicitors (salicylic acid, harpin, Flg 22, and chitosan), and polyamines, accumulate during drought and affect the function of the stomata. As a result of the accumulation of these various factors, there is a change in the function of the stomata. Extensive research has been done to investigate the function of the various signaling components and secondary messengers that are present during the opening or closing of stomatal pores. Calcium, cytosolic, pH, reactive oxygen species (ROS) and nitric oxide (NO) are some of the well-documented signaling components that are involved in the process of stomatal closure. These signaling components, such as ROS and NO, as well as cytosolic pH and free Ca^{2+}, have quite complicated interrelationships and interactions with one another, which calls for further investigation on a more in-depth level (Agurla et al., 2018).

ii) Chlorophyll Fluorescence Activity

The ratio of the maximum quantum yield of Photosystem II (PSII) to the maximum quantum yield of Photosystem I (PSI) is denoted by the symbol Fv/Fm. The quantum yield of photosynthesis is related to this ratio. In most cases, it is known as an indicator of photoinhibition or other types of damage to photosystem II. Plants are considered to be in non-stressed conditions if their values fall between 0.78 and 0.84; if their values fall outside of this range, they are considered to be under stress (Maxwell & Johnson, 2000).

The results showed that the Fv/Fm values of WS treatment decreased the most from day 1 to 3, which indicated that the PSII reaction center of rice leaves were damaged by drought stress, resulting in the potential energy conversion efficiency being weakened due to photoinhibition (Fig 4).

Fig 4. Fluorescence of chlorophyll during the flowering stage for all treatments.



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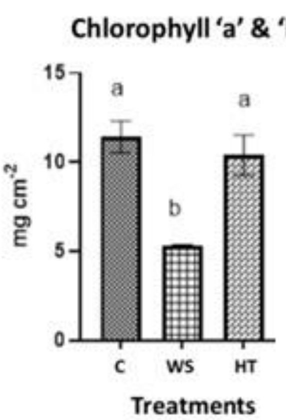
iii) Chlorophyll 'a' and 'b'

The damage done to chloroplasts, which are the primary organelles involved in the process of photosynthesis, is another factor that contributes to the decline in the rate of photosynthesis. When compared to those of the C and HT treatments, the chlorophyll content of the leaves that were subjected to the WS treatment was significantly lower. This demonstrates that inadequate water conditions had a significant influence on the physiological development of the plant. The breakdown of chloroplasts has a negative impact on the process of photosynthesis (Fig 5).

Because of water stress, the capacity of mesophyll cells in the leaf to use the CO_2 that is available for absorption is reduced. As a direct consequence of this, the total amount of chlorophyll exhibits a declining trend (Kadioglu et al., 2012).

Fig 5. Chloroplast content during the flowering stage as influenced by high temperatures and water stress.



**Error bar represents the standard error of the mean. Means followed by the same letter are not significantly different by DMRT at P ≤ 0.05.*

iv) Stigma condition

By analysing and observing the flower stigma under a microscope, the most recent condition of the stigma can be determined (Fig 6). The flower stigmas of each treatment were collected and observed under a microscope at x40 magnification for three days during the treatment. Observations are based on the characteristics of colour, freshness and form.

The stigma was in normal and regular condition on the first day of observation, particularly in the C treatment. In contrast, for the HT and WS treatments, the stigma is slightly brown but retains its regular shape.

Only the C treatment had normal stigmas by the second day of flowering. Stigma on WS treatment begins to show signs of dehydration, wilting, and discoloration. The HT treatment causes stigma to bend and roll as if they were burnt and dark in colour. On the third day of flowering, virtually all stigmas exposed to WS and HT exhibited signs of deterioration. The WS treatment stigma is most adversely affected by discoloration, burning, abnormal shape, and deterioration such as shrinking. The shape of a stigma treated with HT does not change significantly, but the stigma curls and bends due to burning symptoms.

Rice is sensitive to drought stress, especially during the flowering stage, resulting in significant yield losses. The physiological processes that affect spikelet fertility during the sensitive flowering stage under water stress [anther dehiscence, pollen germination] were similar to high-temperature stress. Furthermore, panicle exsertion and peduncle length contributed to increased sterility under water stress.(Rang et al., 2011).

High temperatures (> 35°C) at this stage reduce spikelet sterility in rice and can even result in no harvest, which can be attributed primarily to inhibitions of anther dehiscence, pollen sterility, and failed germination on the stigma (Jagadish et al., 2010).

Fig 6. Stigma conditions based on different treatments from day 1 to 3

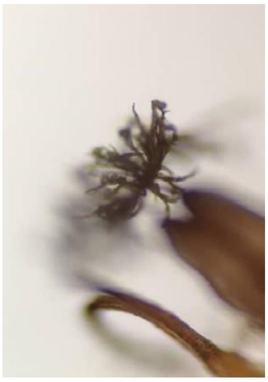
C – Day 1-3

WS – Day 1

WS – Day 2

WS – Day 3

HT – Day 1



HT – Day 2

HT – Day 3

v) Yield

A lower yield is more likely to result from a lack of water than from an increase in temperature. The amount of chlorophyll that is present in the leaves has a physiological reaction with the amount of photosynthetic activity and potential yield that will be harvested. According to Yıldırım et al., (2016), a plant that is experiencing water stress will have its ability to synthesise chlorophyll reduced. However, the decrease in yield caused by HT is not due to a decrease in photosynthesis rate alone. According to the microscopic analysis of the flower stigma, the decrease in yield in the HT treatment was largely due to damage to the flower stigma which inhibited the anthesis process. (Table 1).

Table 1: The yield harvested 105 days after planting, as affected by various treatments.

Treatments	Yield (tonne/ha)
Control	5.3^{a}
Water Stress (Drought)	1.0^{c}
High Temperature (38°C)	2.5^{b}

**Means followed by the same letter are not significantly different by DMRT at P ≤ 0.05.*

Since the treatment was run during the flowering phase, Moonmoon et al (2017) claim that drought can reduce fertility in the panicles and the proportion of full grains. Rice plants under water stress during the flowering stage may experience a disruption in floret initiation, spikelet sterility, poor grain filling, and decreased rice yield.

CONCLUSION

Drought-induced water stress disrupted leaf physiological activity and lowered yield. High temperatures during flowering not only reduce yield but also cause flower stigma to be damaged. Droughts have a larger impact than high temperatures. In reality, high temperatures occur only for a short time, as compared to droughts, which happen as a consequence of an increase in ambient temperature and last for a long time. As a result, plants negatively affected by heat waves may recover and generate a larger yield than plants damaged by drought, which may perish and be damaged. Based on research, it is proven that the flowering stage is the most critical phase for rice plants to determine the yield. Losses to farmers may be reduced if adaptation measures can be taken through future weather projections.

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