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Heat Stress Effects on Leaf Physiological Performances, Vegetative Growth and Grain Yield of Grain Corn (*Zea mays* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The impact of climate change on agricultural production will be most pronounced in tropical and subtropical regions, with numerous climate modeling studies predicting more occurrences of heat waves in the future. Elevated temperatures resulting from global warming pose a significant threat

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to the agricultural sector, as warmer conditions can hinder plant growth and development, leading to reduced crop yields or even crop failure under extreme circumstances. Therefore, this study aimed to determine the effects of ambient, non-heated (30 °C) and heat stress conditions (38 °C) on the plant physiological responses, growth and yield of grain corn during both the vegetative and reproductive stages. The results demonstrated that exposure to heat stress for 7 days significantly impacted the physiological performance of the plants, resulting in a substantial 46.9% reduction in net photosynthetic rate. However, prolonged exposure to heat for 28 days caused even more severe effects, with a 72.5% reduction in net photosynthetic rate. Although the effects of heat stress on vegetative growth were not apparent after 7 days, the plants exhibited severe damage after 28 days of heat stress treatment. During the flowering stage, heat stress led to significant reduction in kernel set, total kernel number, and grain weight of grain corn by 45%, 41%, and 46%, respectively. Poor and scattered kernel set on cobs during the heat stress treatment at the anthesis period indicated damage to pollen grains, failed pollination, and fertilization. These findings highlight the vulnerability of grain corn varieties cultivated in Malaysia to the negative impacts of heat stress, leading to potential losses in production yield.

Keywords: Heat stress; high temperature; grain corn; leaf physiological responses; grain yield; kernel set.

1. INTRODUCTION

Plants are exposed to various environmental conditions that induce stress and one of the major stresses is heat stress, which is usually accompanied by drought or salinity [1]. More and intensified extreme climatic events including heat waves are anticipated in the future [2] and these unprecedented climatic extremes will negatively influence plant physiology, vegetative growth and development [3]. Rising temperatures expected with climate change and the potential for more extreme temperature events will impact plant productivity [4]. Maize is very sensitive to high temperatures during tasseling, flowering, pollination and kernel filling [5]. Simulation results indicated that a 10% reduction in maize yield was shown for each 1 °C increase in global temperature [6]. Corn plants exhibited various effects of heat stress at distinct phenological periods of vegetative and reproductive stages [7].

The optimal temperature for vegetative growth of maize crop varies from 25 to 33°C for the whole growing season [8]. Malaysian Meteorological Department defined heat wave as a daily maximum temperature exceeding 37 to 40°C for at least three consecutive days [9]. The incidents of high temperatures up to 40.1°C were frequently recorded in the northern Peninsular Malaysia at Agro-ecological Zone 1, region with a clear and regular dry season annually. The recommended environment for domestic grain corn production was in the northern Peninsular Malaysia [10], therefore the risk for heat wave incidents to occur is higher compared to other regions. Malaysia imports almost 100% of its grain maize supply for the livestock feed [11], therefore The National Grain Maize Action Plan was developed under the Ministry of Agriculture, Malaysia that targets the local production of grain maize of 1.44 million tonnes by 2023 to reduce such imports [12].

The objectives of this study were to evaluate the impact of heat stress during vegetative and reproductive stages on the plant’s physiological responses, growth and grain yield of grain corn. The grain corn varieties used were the varieties that are currently being tested for cultivation and mass production in Malaysia by Malaysian Agricultural Research and Development Institute (MARDI). The data obtained will be useful for the development of adaptation strategies for heat wave incidents to reduce grain corn yield loss and maximizing yield production.

2. MATERIALS AND METHODS

2.1 Experiment 1: Heat Stress Treatment during the Plant’s Vegetative and Early Reproductive Stage

2.1.1 Treatments and experimental design

In this study, the effects of heat stress on plant growth parameters and physiological performances of grain corn GWG888 during the vegetative stage until the early reproductive stage were investigated. The experiment was laid out in a nested design with 2 factors and 4 replications (Table 1). For each replication, two (2) plants were tagged and used for the determination of leaf physiological responses and plant growth parameters (n=8). The seeds were sown in peat moss media in the plug trays and the seedlings were transferred to 16” x 16” sized polybags containing topsoil:sand:organic matter at 2:2:0.5 under a rain shelter after 14 days. Heat stress treatment started from 25 days after transplanting (DAT) until 53 DAT (28 days) with the seedlings transferred to two climate-controlled rooms (6.0 m length x 3.8 m depth x 3.0 m height) according to temperature treatment and duration.

Table 1. Heat stress experiment with 2 temperature treatment durations

Factor	Treatment
Temperature	Control (Ambient conditions under a rain shelter)
	Non-heated (30 ± 2 °C) 0900-1500 (6h) daily
	Heat stress (38 ± 2 °C) 0900-1500 (6h) daily
Duration	7 days
	28 days

2.1.2 Growing condition inside the climate-controlled rooms

The heat stress treatment of 38 °C was conducted from 0900 to 1500 (6 h) followed by 30 ± 2 °C for another 6 h inside climate-controlled room 1 (Fig. 1). Heat stress treatment was following the definition of the heat wave by the Malaysian Meteorological Department. The non-heated treatment of 30 °C was conducted from 0900 and extended to 2100 (12 h) inside climate-controlled room 2 and served as a control. The temperature during night time was set at 25°C. Both climate-controlled rooms were equipped with heating system, air-conditioner and circulation fans. Mean diurnal temperature variation inside climate-controlled rooms and ambient conditions were determined using temperature sensors installed in the rooms and under the rain shelter. For the non-heated treatment, the average temperature from 0900 to 1500 (6h) was 30.53°C while for heat stress treatment, the temperature increased gradually with the mean temperature of 38.05°C (Fig. 2). For control, the mean temperature was 30.29°C of the same duration.

Plants in both rooms were provided with artificial light-emitting diode (LED) light at 400 µmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) for 15 h and CO_2 was set at 450 µmol $m^{-2}s^{-1}$. PAR was measured using a quantum light meter (FieldScout, Spectrum Technologies, Inc, USA). Daily light integral (DLI) is the cumulative measurement of the photosynthetic light in a day [13]. DLI in the climate-controlled rooms was calculated based on PAR measurement at different distances of LED light for 15 hours per day.

The DLI was calculated using the following formula:

$$DLI = PAR \times LHD \times 3600 / 1000000$$

Where PAR is photosynthetically active radiation; and LHD is LED light hours in a day.

Fig. 1. The heat stress treatment of 38 °C and non-heated control temperature of 30 °C were conducted from 0900 to 1500 (6 h) inside two climate-controlled rooms (left) and ambient control plants were grown under natural conditions under a rain shelter (right)

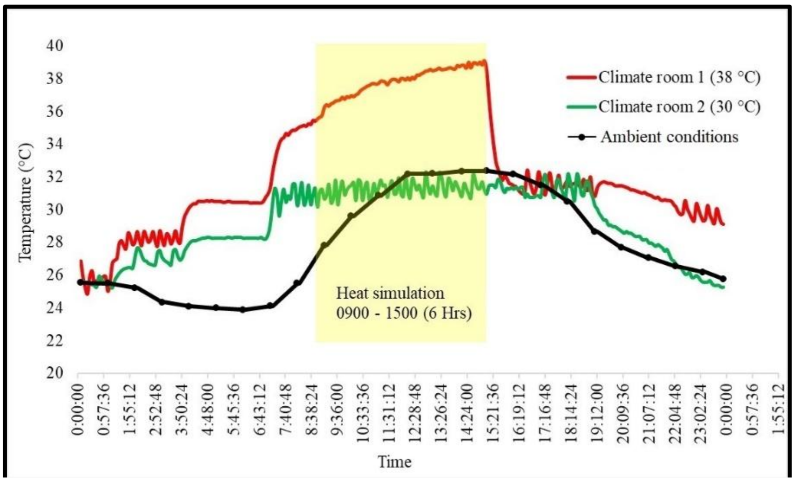

Fig. 2. Mean diurnal temperature variation inside climate-controlled rooms (30 and 38°C) and ambient conditions under a rain shelter

In this study, after the heat stress treatment for 7 days was completed inside the climate-controlled rooms, the plants were transferred to the rain shelter until 53 DAT. For ambient conditions, the control corn plants were grown under natural conditions under a rain shelter throughout the experiment. Irrigation, fertilization and pest control for all experimental plants were following standard practice and were well-controlled.

2.1.3 Leaf physiological responses measurement

The measurements of gas-exchange measurements, including net photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (E) were taken using a portable photosynthesis system (LI-6400XT, LICOR Inc., Nebraska, USA). Fully expanded leaves were clamped in the sensor cuvette, flushed with ambient air (flow rate 500 mol $m^{-2}s^{-1}$) and data were logged after readings reached stable. Measurements were conducted with temperature according to treatment (38 °C for heat stress; and 30 °C for non-heated and ambient treatment), CO_2 reference concentration was maintained at 400 $\mu molmol^{-1}$ and the photosynthetic photon flux density was set at 1200 µmol $m^{-2}s^{-1}$. All measurements were performed between 0900 – 1200 h.

The chlorophyll fluorescence measurements were made using a portable Plant Efficiency Analyzer (PEA) (FMS 2, Hansatech Instruments Ltd, U.K.). The measurements were done between 0900 - 1030 h. The completely expanded leaves were chosen for these measurements. Leaves were darkened for 30 min with standard leaf clips before the fluorescence responses were induced by LED (1500 $\mu molm^{-2}s^{-1}$). Measurements of Fo (initial fluorescence), Fm (maximum fluorescence) and Fv 22 (variable fluorescence) were obtained and Fv was derived as the differences between Fm and Fo. The Fv/Fm ratio was used to determine the leaf chlorophyll fluorescence responses.

2.1.4 Growth parameters measurements

Non-destructive vegetative growth assessments of the plant height, canopy length, leaf number per plant, leaf width, leaf length, stem diameter and total chlorophyll content (a & b) were done on day 7 and 28. Canopy length was measured by the longest canopy length. Leaf width and leaf length were measured from the youngest fully expanded leaf of each plant. Stem diameter was measured 5 cm from the soil level. At 53 DAT, the plants were harvested and plant parts (leaves, stem, tassel and cob) were then dried to constant weight at 80 °C for 72 h in a drying oven (Model 100-800, Memmert, Germany). The dried weight was measured using a semi-micro analytical digital balance (GR-200, A&D Company Limited, Japan) for the determination of above-ground biomass. The tassel and young cob were then combined with the stem weight. For total chlorophyll content, 3 cm^2 of fresh leaves were sampled and soaked in 80% acetone (20 mL) in glass bottles covered with aluminium foil in the dark for 7 days or until all the leaves were decolorized. The spectrophotometer (Genesys 1XX, ThermoFisher Scientific, Madison, USA) was then used to measure the chlorophyll extraction at the wavelengths of 664 and 647 nm [14].

2.2 Experiment 2: Heat Stress Treatment during the Plant's Reproductive Stage

2.2.1 Treatments and experimental design

Experimental plants for the second experiment were grown under a rainshelter following agronomic practices described in Experiment 1 except for the grain corn variety used was the DuPont P4546 hybrid. This experiment was laid out in a nested design with four replications. The treatments were (T1) Control (Ambient conditions), (T2) Non-heated (30 ± 2 °C) and (T3) Heat stress (38 ± 2 °C). Before the experiment started, ear's shoot needs to be securely bagged using paper envelope before any silks emerged to prevent cross-pollination. This study was conducted during the anthesis period from 45 to 59 DAT. Plants with developed tassel and silks that emerged outside the husk (silking or R1 stage) were then selected and transferred to the climate-controlled rooms. Experimental plants in T2 and T3 were exposed to the designated temperature treatment for 6h daily from 0900-1500 (6h) for 14 days inside the climate-controlled rooms. For each replication, two (2) plants were tagged and used for the determination of grain yield assessment (n=8). Pollen shedding may last for 5 or 6 days, but the total pollen shedding period may last up to 14 days depending on the levels of pollen release [15] and silks are viable and receptive to pollen up to 7 to 10 days. During the experiment, pollens from the tassel were collected and sprinkled onto all of the silks on each ear for assisted pollination which was done for 3 times. After 14 days of heat stress treatment completed, experimental plants from climate-controlled rooms were transferred inside a rainshelter until the cobs reached maturity.

2.2.2 Grain yield parameters

The cobs were harvested when the cobs reached maturity and the grain moisture content was less than 30% using a grain moisture meter (Handheld Portable Grain Moisture Tester, Riceter F, Kett, CA USA). The cobs were harvested at 95 DAT and grain yield parameters measured were the percentage of kernel set, total kernel number, cob weight, de-husked cob weight, total grain weight, cob length and diameter.

2.2.3 Statistical analysis

The collected data were subjected to two-way analysis of variance (ANOVA) and one-way ANOVA in the first and second experiment, respectively to evaluate the significant differences among treatments. Means were separated by using the Duncan's multiple range test (DMRT) at P= 0.05 with statistical analysis system (SAS) version 9.4 (SAS Institute Inc. Cary, North Carolina, USA).

3. RESULTS AND DISCUSSION

3.1 Experiment 1: Heat Stress Treatment during the Plant's Vegetative and Early Reproductive Stage

3.1.1 Effects of heat stress on leaf physiological performances

Leaf photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll fluorescence of grain corn plants were significantly affected by the temperature treatment ($P \leq 0.05$) (Table 2). Leaf physiological responses were significantly affected by treatment duration except for stomatal conductance. The heat stress treatment of 38 °C for 7 days significantly decreased leaf net photosynthetic rate with the respective reductions of 46.7 and 44.6% as compared to ambient and non-heated treatment. Prolonged heat stress of 28 days caused severe effects with 72.5 and 63.2% reduction in leaf net photosynthetic rate as compared to ambient and non-heated treatment.

Heat stress for 7 days caused reduction in stomatal conductance, transpiration rate and chlorophyll fluorescence at 95.6, 15.0 and 5.1% as compared to ambient treatment; and 63.6, 34.1 and 5.1% as compared to non-heated treatment, respectively (Table 2). After 28 days, heat stress caused reduction in stomatal conductance, transpiration rate and chlorophyll fluorescence (Fv/Fm) at 72.5, 38.6 and 43.7% as compared to the ambient treatment and 57.1, 28.6 and 41.2% as compared to non-heated treatment, respectively. The results indicated that the plants have been exposed to stress and the leaf photosynthetic capacity were affected. A significant reduction in the net photosynthetic rate at 28.6% in heat stress treated plants of maize was reported [16]. Transpiration through stomata is an important heat-dissipating mechanism, with their closure under heat stress will further increase leaf temperature and resulting in severe loss in net photosynthetic rate [17]. The lower stomatal conductance in maize leaves was beneficial for maintaining water-use efficiency but damages photosynthetic apparatus under heat stress. High temperature caused photoinhibition of photosystem II (PSII) and contributed to declining photosynthetic carbon assimilation in maize [18]. Lower net photosynthetic rate and chlorophyll fluorescence (Fv/Fm) after 7 days of heat stress treatment showed that high temperature has damaged PS II and manifestation of stress in a leaf [19] and leaf photosynthetic capacity was affected. The damage worsened as the reduction of chlorophyll fluorescence measurement increased from 5.1 to 43.7% from day 7 to 28, respectively. Chlorophyll fluorescence (Fv/Fm ratio) provides detailed information on the saturation characteristics of electron transport [20], thus its reduction indicates that overall photosynthetic performance of a plant was negatively affected.

Heat stress induced damage on chloroplasts leads to the inactivation of heat-sensitive proteins such as Rubisco activase (RCA) and the down-regulation of important chloroplast components, thereby leading to decreased photosynthetic efficiency, redox imbalance and possible cell death [21]. The activation state of Rubisco in maize leaves decreased at temperatures exceeding 32.5°C, with nearly complete inactivation at 45°C [22]. Leaf net photosynthesis was inhibited as temperature exceeded 37.5°C, and the relative inhibition was much greater when the leaf temperature was increased rapidly compared with gradually. Impaired photosynthesis restrains the synthesis of glucose and starch in the kernels, and influences the activities of related enzymes [23]. However, leaf physiological performances of the non-heated treatment plants were slightly lower compared to ambient conditions after 28 days of treatment. These results could be due to environmental conditions inside the climate-controlled rooms notably the non-uniform PAR intensity and DLI at different plant heights (further discussed in the next chapter).

3.1.2 Effects of heat stress on plant growth parameters

The heat stress for 7 days significantly decreased plant height and leaf number with the respective reductions of 10.8 and 16.9% as compared to ambient conditions (Table 3 and Fig. 3), however, other growth parameters were not affected. Prolonged heat stress treatment for 28 days caused severe damage to more plant growth parameters of grain corn. Heat stress caused 21.8, 36.3, 51.7, 34.8 and 30.2% reduction in plant height, canopy length, leaf number, leaf width and leaf length, respectively as compared to ambient conditions. Prolonged period of heat stress also decreased 51.5% of photosynthetic pigments of total chlorophyll content. Under normal growth conditions, the synthesis and degradation of chlorophyll reach equilibrium but when plants are subject to environmental stress, including heat, the content of chlorophyll decreases, leading to leaf senescence or chlorosis [24]. Under heat treatment, the activity of chlorophyllase and chlorophyll-degrading peroxidase dramatically increases, resulting in a significant reduction in chlorophyll levels [25].

Table 2. The leaf physiological responses and chlorophyll fluorescence (Fv/Fm ratio) as affected by heat stress treatment (n=8)

Duration	Temperature	Photosythetic rate (μmol $m^{-2}s^{-1}$)	Stomatal conductance (mol $m^{-2}s^{-1}$)	Leaf transpiration rate (mmol $m^{-2}s^{-1}$)	Chlorophyll fluorescence (Fv/Fm)
7 days	Ambient conditions	14.34^{az}	0.90^{a}	1.80^{ab}	0.78^{a}
	Non-heated (30°C)	13.81^{a}	0.11^{a}	2.32^{a}	0.78^{a}
	Heat stress (38°C)	7.65^{b}	0.04^{b}	1.53^{b}	0.74^{b}
28 days	Ambient conditions	9.95^{a}	0.11^{a}	1.71^{a}	0.71^{a}
	Non-heated (30°C)	7.44^{b}	0.07^{b}	1.47^{a}	0.68^{b}
	Heat stress (38°C)	2.74^{c}	0.03^{c}	1.05^{b}	0.40^{c}
Significance level	Temperature	**	**	**	**
	Duration	**	ns	**	**
	Temperature x Duration	ns	**	ns	*

^{z}*Means followed by the same letter in the same column and factor level are not significantly different by DMRT at P ≤ 0.05. ns, non-significant difference at P > 0.05. Significant difference at * P ≤ 0.05 or ** P ≤ 0.01*

Table 3. Plant growth parameters of grain corn as affected by heat stress treatment for the duration of 7 and 28 days (n=8)

Duration	Temperature	Plant height (cm)	Canopy length (cm)	Leaf number	Leaf width (cm)	Leaf length (cm)	Stem diameter (cm)	Total chlorophyll content (mg/cm^2)
7 days	Ambient conditions	85.75^{az}	149.90^{a}	10.83^{a}	8.15^{a}	86.17^{a}	2.08^{a}	5.36^{a}
	Non-heated (30 °C)	96.72^{a}	142.75^{a}	11.00^{a}	7.06^{a}	82.07^{a}	2.03^{a}	6.51^{a}
	Heat stress (38 °C)	76.50^{b}	146.00^{a}	9.00^{b}	7.13^{a}	81.50^{a}	2.11^{a}	6.27^{a}
28 days	Ambient conditions	112.50^{a}	121.60^{a}	11.17^{a}	8.17^{a}	88.83^{a}	2.29^{a}	3.34^{a}
	Non-heated (30 °C)	108.8^{a}	82.00^{b}	10.33^{a}	6.63^{ab}	66.17^{ab}	2.08^{a}	3.05^{a}
	Heat stress (38 °C)	88.00^{b}	77.50^{b}	5.40^{b}	5.33^{b}	62.00^{b}	2.07^{a}	1.62^{b}
Significance level	Temperature	ns	**	**	**	*	ns	ns
	Duration	ns	**	**	ns	ns	ns	**
	Temperature x Duration	ns	*	**	ns	ns	ns	ns

*^{z}Means followed by the same letter in the same column and factor level are not significantly different by DMRT at $P \le 0.05$. ns, non-significant difference at $P > 0.05$. Significant difference at * $P \le 0.05$ or ** $P \le 0.01$*

Fig. 3. Reduction of plant growth as affected by heat stress treatment of 38°C (6 h daily) for 7 days (left) and 28 days (right). Heat stress of 38 °C of 28 days has induced the process of senescence and led to leaf death in most of the leaves

In this study, a short period of 7 days of heat stress did not cause significant reduction in the total above-ground biomass but prolonged heat stress of 28 days significantly reduced 42.80% of total above-ground biomass (Fig. 4). Prolonged heat stress has affected biomass accumulations of leaf, stem and ear parts most probably as the effects of the decrease in leaf physiological performances (Table 2). This indicated that heat stress disturbs the normal physiological processes required for optimal growth and development of grain corn. Heat stress up to 36 °C significantly decreased the light radiation use efficiency, less active nitrogen and carbon metabolisms contribute to a decrease in dry matter accumulation and reduced biomass assimilation required for plant growth [26]. Heat stress was reported to alter the photosynthetic process, causing low photosynthetic rates, damaging the biological membranes, affecting nutrient uptake, limiting the functioning of various enzymes, stunted growth and causing impairment in overall corn plants, resulting in reduced grain yields [27].

A short period of heat stress did not affect the biomass and physical appearance of young ears (Fig. 5). However, for the prolonged heat stress of 28 days, the young ears were severely affected with 59.1% reduction in weight. More leaves senescence, the ears were smaller, underdeveloped and outer sheath with cellular damage and death were observed in the prolonged heat stress treatment. Both severe heat stress and long-term exposure to moderately high temperatures can damage the activity of proteins, enzymes, membrane integrity, cellular damage and cell death [28]. The results showed that the grain corn exposed to a short period of heat stress during the vegetative stage did not affect the development of corn cob, but prolonged heat stress from the vegetative until the early reproductive stage affected the corn cob development and possible irreversible damage to the cobs.

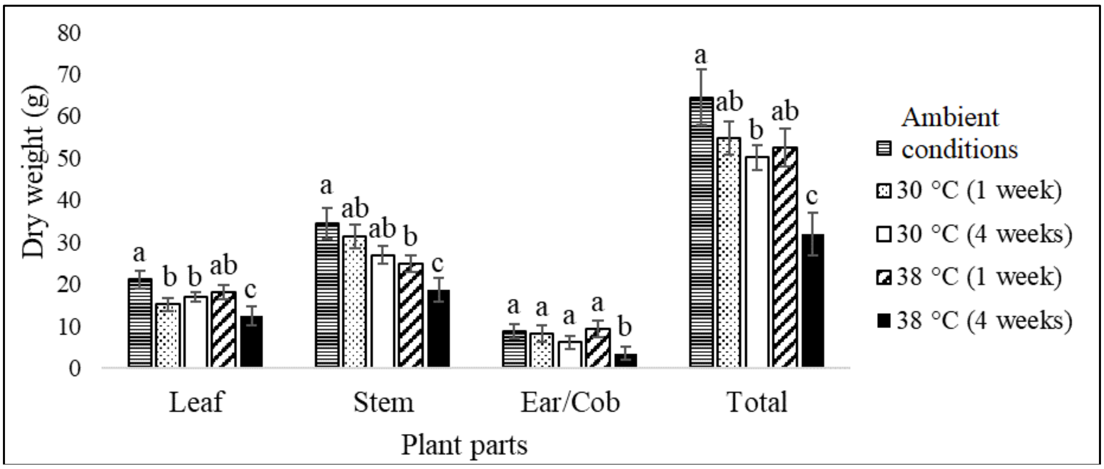

Fig. 4. Leaf, stem, ear and total above-ground biomass of grain corn as affected by 7 and 28 days of heat stress duration. The biomass assay was conducted on 53 DAT after 28 days of heat stress treatment completed (n=8). Error bars represent standard error of the means. Means followed by the same letter in the same week are not significantly different by DMRT at P ≤ 0.05

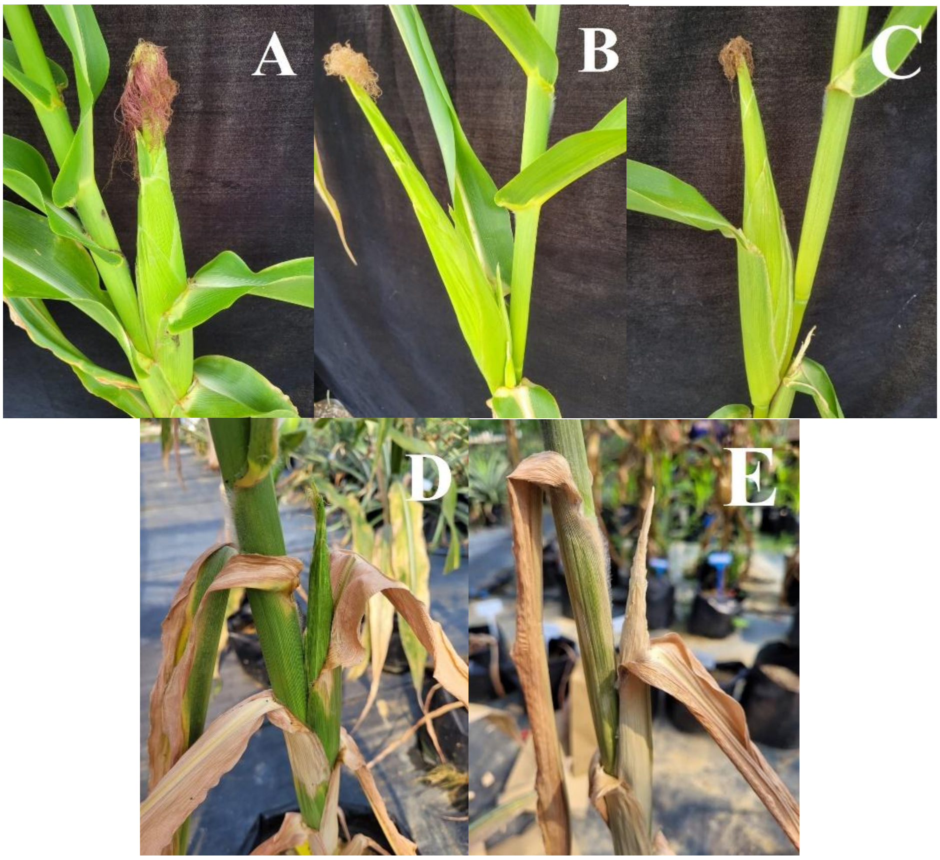

Fig. 5. Cob condition as affected by heat stress treatment and different duration. (A) Ambient conditions for 28 days, (B) non-heated treatment of 30 °C for 28 days, (C) heat stress treatment of 38 °C for 7 days, and (D and E) heat stress treatment of 38 °C for 28 days

Although heat stress has negatively impacted leaf physiological responses and the growth parameters of grain corn, the environmental conditions in the climate-controlled rooms also contributed to the results. The total plant biomass of heat stress treatment (38 °C for 28 days) was

50.4% lower but the non-heated treatment (30 °C for 28 days) was significantly lower at 22.2% compared to the ambient conditions. The reduction of plant biomass accumulation of non-heated treatment was probably due to environmental conditions inside the climate-controlled rooms, notably the non-uniform PAR intensity at different plant heights (Table 4). Leaves at lower plant height received lower PAR and DLI resulted in lower leaf physiological processes, reduced photosynthates production, biomass accumulation and plant growth. The low-light condition decreased the canopy net photosynthetic rate, reduced carbon uptake, decreased dry matter accumulation and affected the reproductive growth in summer maize [29]. Therefore, the effects of environmental conditions inside climate-controlled rooms also need to be put into consideration, especially for a heat stress study inside the facility. This study also can be improved by optimizing climate-controlled room conditions, especially the optimal requirement of light supplemental for grain corn. Until now, information such as optimal artificial light for grain corn inside the climate-controlled facility is not well-documented in the literature.

3.2 Experiment 2: Heat Stress Treatment during the Plant's Reproductive Stage

3.2.1 Kernel set and grain yield parameters

The effects of heat stress on kernel set and grain yield parameters on grain corn were determined during the anthesis and silking period in 14 days. The results showed that the kernel set of ambient and non-heated treatments were more than 97%. The kernel set of cobs as affected by heat stress treatment was 46.1 and 45.6% lower as compared to ambient and non-heated treatment, respectively (Table 5). Both ambient and non-heated treatments with temperature 30 ± 1.0 °C showed complete kernel set whilst heat stress treatment of 38°C showed poor and scattered kernel set on cobs.

Male flowers or tassels that developed at the top of the plant were found to be vulnerable to high temperatures and this affected pollen viability [30]. The short duration of heat stress around anthesis affects pollen shedding, pollen germination ability, and embryo development in maize. Heat stress damages pollen morphology, interrupts sugar to starch physiological process, decreases starch granule number and size in the pollen and reduces pollen viability [31]. Additionally, heat stress affected the period anthesis and silking, resulting in reduced kernel number, limited kernel setting and distinctly decreased yield. At the flowering stage, heat stress inhibited anther dehiscence, induced pollen sterility, reduced pollen viability and pollen germination, which caused kernel abortion and maize yield reduction [16]. Furthermore, plant stress at flowering and early grain filling impairs starch synthesis and results in a limited supply of assimilates to the grain during the seed development [32]. Successful fertilization also does not necessarily translate to a harvestable kernel as for several weeks following fertilization, reduced photosynthate caused by heat stress or any factor reducing photosynthetic activity can cause kernel abortion. The increase in day temperature to 38 °C at the reproductive stage was reported to cause serious damage to pollination and kernel setting that resulted in lower grain yield in spring-planted maize [33].

Heat stress also caused 73.9, 48.1, 65.1, 66.1 and 39.7% reduction in total kernel number, cob weight, de-husked cob weight, grain weight and cob length, respectively as compared to ambient; and the reduction at 60.9, 24.9, 49.3, 57.4 and 23.5%, respectively as compared to non-heated treatment. Although the size of cobs in the non-heated treatment were smaller than ambient conditions (Fig. 6) but the kernel set was complete and without any significant difference, an indication of optimal temperature (30 ± 1.0°C) during anthesis for corn. These results are in agreement with Sanchez et al. [34] that explain the optimum temperature for anthesis was 30.5℃ and a temperature above 38 ℃ can hinder maize pollination.

Total kernel number, cob weight, de-husked cob weight, grain weight and cob length of non-heated treatment were significantly lower compared to the ambient conditions (Table 5) and this could be due to the effects of environmental conditions inside the climate-controlled rooms. For instance, the grain weight of heat stress and non-heated treatments was 66.1 and 20.4% lower as compared to the ambient conditions, respectively. Therefore, an estimation can be made that heat stress potentially caused 45.7% reduction in grain yield, whereas 20.4% was most probably influenced by the environmental conditions of the climate-controlled room. For the total kernel set, heat stress potentially caused 40.5% reduction, whereas 33.4% was influenced by the environmental conditions of the climate-controlled room.

Table 4. Photosynthetically Active Radiation (PAR) dan Daily Light Integral (DLI) from LED light sources at different plant height in the climate-controlled rooms

Condition	Distance from LED light source (cm)	Plant height at 60 DAT (cm)	PAR ($\mu molm^{-2}s^{-1}$)	Daily Light Intergral (DLI) ($molm^{-2}day^{-1}$)
Climate controlled-room	10	160.0	313.7	16.9
	20	140.0	203.5	11.0
	25	135.0	191.8	10.4
	40	120.0	166.2	9.0
	60	100.0	141.8	7.7
	80	80.0	111.5	6.0
	100	60.0	96.0	5.2
	120	40.0	83.5	4.5

Table 5. Effect of heat stress on the kernel set and grain yield parameters of grain corn (n=8)

	Ambient conditions	Non-heated (30 °C)	Heat stress (38 °C)
Kernel set (%)	98.59^{az}	97.62^{a}	53.12^{b}
Total kernel number	373.00^{a}	248.40^{b}	97.20^{c}
Cob weight (g)	162.90^{a}	112.50^{b}	84.50^{c}
De-husked cob weight (g)	128.58^{a}	88.50^{b}	44.90^{c}
Grain weight (g)	73.73^{a}	58.67^{b}	24.97^{c}
Cob length	14.25^{a}	11.24^{b}	8.60^{c}
Cob diameter	3.74^{a}	3.54^{a}	2.85^{a}

^{z}*Means followed by the same letter in the same row are not significantly different by DMRT at P ≤ 0.05*

Fig. 6. Kernel set and cob size without husk for comparison as affected by heat stress treatment on grain corn

As discussed in experiment 1, the non-uniform PAR intensity at different plant heights in the climate-controlled rooms could also potentially be the reason for the lower kernel number, grain weight and cob size. Lower PAR and DLI compared to ambient conditions decreased photosynthetic activities that resulted in low photosynthates production. Previous studies indicated that shading (low-light condition) decreased carbon fixation, canopy net photosynthetic rate and grain yield of summer maize [29]. Therefore, optimizing growing conditions by providing optimal PAR and DLI to the leaves at different plant heights is crucial for the study inside an enclosed controlled-environment structure. Nevertheless, this study provides important information such as the period of anthesis for 14 days is critical for the process of pollen shedding, pollination, fertilization and the initial grain filling period. Additionally, any condition that is not optimal for plant requirements will have negative effects on the corn grain yield.

4. CONCLUSION

The results from the first study showed that heat stress treatment in a shorter duration of 7 days during the vegetative stage significantly affected the leaf physiological performances of grain corn plants. A prolonged heat stress duration of 28 days caused even more severe effects and significant reduction in leaf physiological performances. The effects of heat stress on the plant's vegetative growth were not apparent after a short duration, but the plants exhibited severe damage after prolonged durations of 28 days. These reductions have the potential to cause substantial effects on grain corn yield and cob quality especially when the prolonged high stress caused underdeveloped corn cob with irreversible damage. As the reproductive stage is the most sensitive stage to elevated temperature as reported in many studies, the results from this study also confirmed the detrimental effects of heat stress on kernel development and grain yield. The heat stress caused significant reductions in the kernel set, total kernel number, grain weight and other grain yield parameters. The findings of this study will be utilized to assist in the development of adaptation strategies for elevated temperature and climate change in the agriculture sector. Sustainable and holistic approaches need to be taken into account with different management options such as the selection of heat stress tolerant grain corn varieties and the use of micronutrients to increase pollen viability and kernel set. These will assist in reducing the risk of yield production loss due to the heat stress in the future.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prasad P, Bheemanahalli R, Jagadish SVK. Field crops and the fear of heat stress-Opportunities, challenges and future directions. Field Crop Research. 2017;200:114-121.
2. Ummenhofer CC, Meehl GA. Extreme weather and climate events with ecological relevance: A review. Philosophical Transactions of the Royal Society B: Biological Sciences. 2017;372:20160135.
3. Zafar SA, Hameed A, Nawaz MA, Wei M, Noor MA, Hussain M. Mechanisms and molecular approaches for heat tolerance in rice (*Oryza sativa* L.) under climate change scenario. Journal of Integrative Agriculture. 2018;17:726-738.
4. Hatfield JL, Prueger H. Temperature extremes : Effect on plant growth and development. Weather and Climate Extremes. 2015;10:4-10.
5. Zhang H, Li G, Fu C, Duan S, Hu D, Guo X. Genome-wide identification, transcriptome analysis and alternative splicing events of Hsf family genes in maize. Scientific Reports. 2020;10:8073. Available:https://doi.org/10.1038/s41598-020-65068-z
6. Dong X, Guan L, Zhang P, Liu X, Li S, Fu Z, Tang L, Qi Z, Qiu Z, Jin C, Huang S, Yang H. Responses of maize with different growth periods to heat stress around flowering and early grain filling. Agricultural and Forest Meteorology.2021;303:108378.
7. Lizaso JI, Ruiz-Ramos M, Rodriguez L, Gabaldon-Leal C, Oliveira JA, Lorite IJ. Impact of High Temperatures in Maize: Phenology and Yield Components. Field Crops Research. 2018;216:129-140.
8. Neild RE, Newman JE. Growing Season Characteristics and Requirements in the Corn Belt. In Dale R.F., Hanway, D.G. and Carlson R.E. (Eds.), National Corn Handbook. 1987. Cooperative Extension Service, Purdue University, IN, USA. Available:https://www.extension.purdue.edu/extmedia/NCH/NCH-40.html
9. Aziz MR, Hariz MAR, Hidayah NA, Nadiah MA, Najib MOG. The effects of elevated temperature on plant growth parameters and physiological performances of grain corn under controlled environment system. In Transactions of The Malaysian Society of Plant Physiology. 31st Malaysian Society of Plant Physiology Conference. 2022; 29:7-11.

Available:http://mspp.org.my/files/Transactions%20Vol.%2029%20(2021).pdf

10. Adham A, Ghaffar MBA, Ikmal AM, Shamsudin, NAA. Genotype × environment interaction and stability analysis of commercial hybrid grain corn genotypes in Different Environments. Life. 2022:12:1773.
11. Amna NAMN, Rashid, MR, Alif MA, Hifzan MR. An overview of the grain corn industry in Malaysia FFTC Agricultural Policy Platform. FFTC-AP. 2019. Available:https://ap.fftc.org.tw/article/1377#:~:text=Grain%20corn%20is%20one%20of,the%20ruminants%2C%20broilers%20and%20swine
12. Effendi MMN, Zaulia O, Ismawaty NN, Mirfat AHS, Dzahirah AZ, Faidhi MT, Safuraa NS, et al. Effect of detopping at different stages of maturity on post-harvest quality of P4546 hybrid grain corn. In Transactions of the Malaysian Society of Plant Physiology 31st Malaysian Society of Plant Physiology Conference. 2022;29: 214-217. Available:http://mspp.org.my/files/Transactions%20Vol.%2029%20(2021).pdf
13. Korczynski PC, Logan J, Faust JE. Mapping monthly distribution of daily light integrals across the contiguous United States. HortTechnology. 2002:12:12-16.
14. Coombs J, Hind G, Leegood RC. Analytical techniques. In Coombs J, Hall DO, Long SP, Scurlock JMO. (Eds.), Techniques in Bioproductivity and Photosynthesis (2nd ed.) Pergamon Press.1986:223-240.
15. Nieh SC, Lin WS, Hsu YH, Shieh GJ, Kuo BJ. The effect of flowering time and distance between pollen source and recipient on maize. GM Crops & Food. 2014;5(4):287-295.
16. Niu S, Du X, Wei D, Liu S, Tang Q, Bian D, Zhang Y, Cui Y, Gao Z. Heat stress after pollination reduces kernel number in maize by insufficient assimilates. Frontiers in Genetics. 2021;12:728166.
17. Caine RS, Yin X, Sloan J, Harrison EL, Mohammed U, Fulton T. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. New Phytologist. 2019; 221:371-384. Available:https://doi.org/10.1111/nph.15344
18. Li YT, Xu WW, Ren BZ, Zhao B, Zhang J, Liu P. High temperature reduces photosynthesis in maize leaves by damaging chloroplast ultrastructure and photosystem II. Journal of Agronomy and Crop Science. 2020;206:548-564. Available:https://doi.org/10.1111/jac.12401
19. Maxwell K, Johnson GN. Chlorophyll fluorescence-a practical guide. Journal of Experimental Botany. 2000;51(345):659-668.
20. Ralph PJ, Gademan R. Rapid light curves: A powerful tool to assess photosynthetic activity. Aquatic Botany. 2005;82:222-237.
21. Hu S, Ding Y, Zhu C. Sensitivity and responses of chloroplasts to heat stress in plants. Frontiers in Plant Science. 2020; 11:1-11.
22. Crafts-Brandner SJ, Salvucci ME. Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. Plant Physiology. 2002;129(4):1773-1780.
23. Basu PS, Pratap A, Gupta S, Sharma K, Tomar R, Singh NP. Physiological traits for shortening crop duration and improving productivity of greengram (*Vigna radiata* L. Wilczek) under high temperature. Frontiers in Plant Science. 2019;10:1-18. Available:https://doi.org/10.3389/fpls.2019.01508
24. Rossi S, Burgess P, Jespersen D, Huang B. Heat-induced leaf senescence associated with chlorophyll metabolism in bentgrass lines differing in heat tolerance. Crop Science. 2017;57:169-178. DOI: 10.2135/cropsci2016.06.0542
25. Wang QL, Chen JH, He NY, Guo FQ. Metabolic reprogramming in chloroplasts under heat stress in plants. International Journal of Molecular Sciences. 2018;19:849-871. Available:https://doi.org/10.3390/ijms19030849
26. Cicchino M, Edreira J, Uribelarrea M, Otegui M. Heat stress in field-grown maize: Response of physiological determinants of grain yield. Crop Science. 2010;50:1438-1448.
27. Hussain HA, Men S, Hussain S, Chen Y, Ali S, Zhang S, Zhang K, Li Y, Xu Q, Liao C. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake, and oxidative status in maize hybrids. Scientific Reports. 2019;9:3890.
28. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: an overview. Environmental and Experimental Botany. 2007;61:199-223.

Available:https://doi.org/10.1016/j.envexpbot.2007.05.011.

29. Gao J, Zhao B, Dong S, Liu P, Ren B, Zhang J. Response of summer maize photosynthate accumulation and distribution to shading stress assessed by using $^{13}CO_2$ stable isotope tracer in the field. Frontiers in Plant Science. 2017;8:1-12.
Available:https://doi.org/10.3389/fpls.2017.01821

30. Wang Y, Sheng D, Zhang P, Dong X, et al. High temperature sensitivity of kernel formation in different short periods around silking in maize. Environmental and Experimental Botany. 2021;183:104343.

31. Wang Y, Tao H, Tian B, Sheng D, Xu C, Zhou H. Flowering dynamics, pollen, and pistil contribution to grain yield in response to high temperature during maize flowering. Environmental and Experimental Botany. 2019;158:80-88.

32. Liu X, Yu Y, Huang S, Xu C, Wang X, Gao J, Meng Q, Wang P. The impact of drought and heat stress at flowering on maize kernel filling: Insights from the field and laboratory. Agricultural and Forest Meteorology. 2022;312:108733.

33. Afzal I, Basra S, Shahid M, Saleem M. Physiological enhancements of spring maize (*Zea mays* L.) under cool conditions. Seed Science and Technology. 2008;36:497-503.

34. Sanchez B, Rasmussen A, Porter JR. Temperatures and the growth and development of maize and rice: A review. Global Change Biology. 2014;20:408-417.

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