Original Research Article

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

ABSTRACT

The impact of climate change on agricultural production will be most pronounced in tropical and subtropical regions, with numerous climate modeling studies predicting that more occurrences of heat waves in the future. Elevated temperatures resulting from global warming pose a significant threat 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 optimal daytime temperatures (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 46.86% reduction in net photosynthetic rate. However, prolonged exposure to heat for 28 days caused even more severe effects, with a 72.46% 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.

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

1. INTRODUCTION

Plants are exposed to a variety of environmental conditions that induce stress and one of the major stresses is heat stress which is usually accompanied by other stresses such as 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]. Extreme weather events as a consequence of global climate change will result in the occurrence of heat stress in plants and significant decline in many crop productions including corn. Previous studies indicated that corn is more sensitive to heat stress (one of the most important abiotic stresses) than wheat and rice [4,5]. Corn plants exhibited various effects of heat stress at distinct phenological periods of vegetative and reproductive stages [6].

The optimal temperature for optimal vegetative growth of maize crop varies from 25 to 33 °C for the whole growing season [7]. Heat wave is defined as a period of abnormally hot weather, often defined with reference to a relative temperature threshold, lasting from two days to months [8] ; and another definition is five or more consecutive days during which the daily maximum temperature surpasses the average maximum temperature by 5 °C or more [9]. Malaysian Meteorological Department defined heat wave as a daily maximum temperature exceeding 37 to 40 °C for at least three consecutive days [10]. 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 north of peninsular Malaysia [11], 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 feed industry [12], 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 [13].

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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	Ambiance 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

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2.1.2 Growing Condition inside the Climate-controlled Rooms

The heat stress treatment of 38 °C was conducted from 9:00 to 15:00 (6 h) followed by 30 ± 2 °C for another 6 h inside climate-controlled room 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 9:00 and extended to 21:00 (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 ambiance 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 (Figure 1). For ambiance, the mean temperature was 30.29 °C of the same duration.

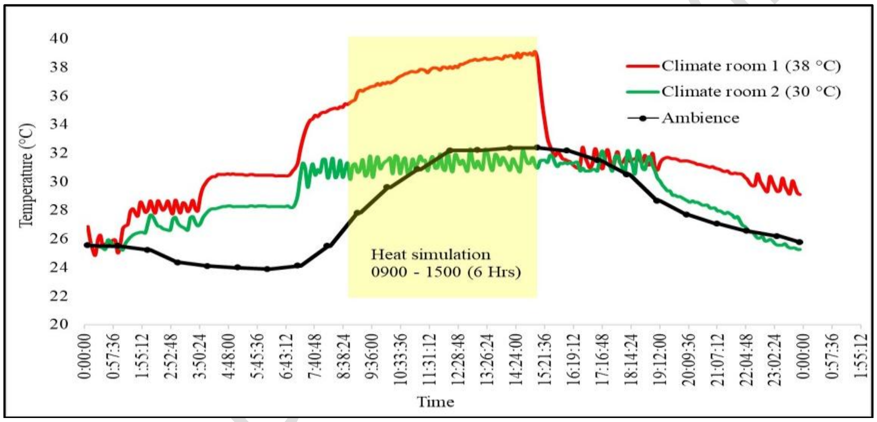

Fig. 1. Mean diurnal temperature variation inside climate-controlled rooms (30 and 38 °C) and ambiance conditions under a rain shelter

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[14]. 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:

$$\text{DLI} = \text{PAR} \times \text{LHD} \times 3600 / 1000000$$

where PAR is photosynthetically active radiation; and LHD isLED light hours in a day.

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 ambiance treatment, 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 ambiance), 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 9:00 and 12:00 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 (a & b) 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 [15].

Fig. 2. The heat stress treatment of 38 °C and non-heated control temperature of 30 °C were conducted from 9:00 to 15:00 (6 h) inside two climate-controlled rooms (left) and ambiance control plants were grown under natural conditions under a rain shelter (right)

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) Ambiance conditions (under a rain shelter), (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[16] 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 ≤ 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 46.86 and 44.61% as compared to ambiance and non-heated treatment. Prolonged heat stress of 28 days caused severe effects with 72.46 and 63.17% reduction in leaf net photosynthetic rate as compared to ambiance and non-heated treatment.

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	Ambiance 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	Ambiance 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*

Heat stress for 7 days caused reduction in stomatal conductance, transpiration rate and chlorophyll fluorescence at 98.89, 15.00 and 5.13% as compared to ambiance; and 90.91, 34.05 and 5.13% 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 72.73, 38.60 and 43.67% as compared to the ambiance and 57.14, 28.57 and 41.18% 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. Many studies have reported that heat stress affected plant physiological performances. A significant reduction in the net photosynthetic rate at 28.6% in heat stress treated plants of maize was reported [17]. Lower net photosynthetic rate and chlorophyll fluorescence (Fv/Fm) after 7 days of heat stress treatment showed that high temperature has damaged Photosystem II and manifestation of stress in a leaf [18] and leaf photosynthetic capacity were affected. The damage worsened as the reduction of chlorophyll fluorescence measurement increased from 5.13 to 43.66% from day 7 to 28, respectively. Chlorophyll fluorescence (Fv/Fm ratio) provides detailed information on the saturation characteristics of electron transport [19], thus its reduction indicates that overall photosynthetic performance of a plant was negatively affected.

Heat stressinduced 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 [20]. The activation state of Rubisco in maize leaves decreased at temperatures exceeding 32.5°C, with nearly complete inactivation at 45°C [21]. 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 affects biological carbon fixation [22], thus restraining the synthesis of glucose and starch in the kernels, and influencing the activities of related enzymes [23]. However, leaf physiological performances of the in non-heated treatment plants were slightly lower compared to ambiance conditions after 28 days of treatment. These results were probably 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).

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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 20.91 and 18.18% as compared to ambiance and non-heated treatment (Table 3 and Figure 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 21.78, 36.27, 51.66, 34.76 and 30.20% reduction in plant height, canopy length, leaf number, leaf width and leaf length respectively as compared to ambiance conditions. Prolonged period of heat stress also decreased 51.50% of photosynthetic pigments of chlorophyll a and b. 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].Plant growth parameters, chlorophyll contents and net photosynthetic rate of maize were severely affected under heat stress in maize [26].

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	Ambiance 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	Ambiance 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 (Figure 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[27]. 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[28].

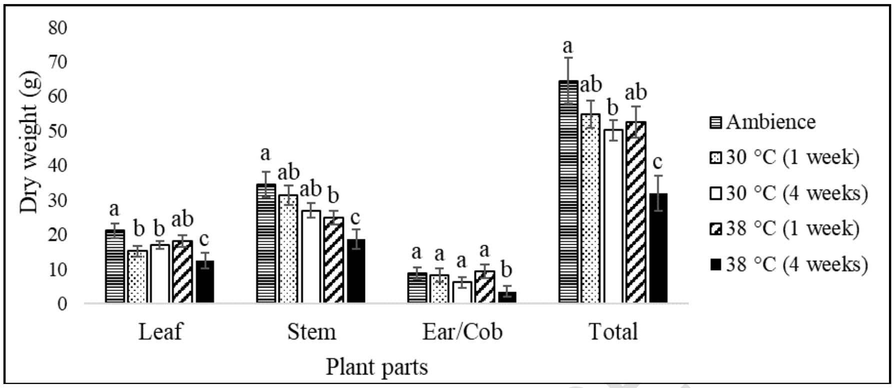

Fig. 4. Leaf, stem, ear and total above-ground biomass of grain corn as affected by 7 and 28 days of heat stress duration. The biomass assay was conducted on 53 DAT after 28 days of heat stress treatment completed (n=8). Error bars represent standard error of the means. Means followed by the same letter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

A short period of heat stress did not affect the biomass and physical appearance of young ears (Figure 5). However, for the prolonged heat stress of 28 days, the young ears were severely affected with 59.07% 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. High temperatures damage the activity of proteins and the fluidity of membrane lipids, thus affecting the activity of mitochondria- and chloroplast-based enzymes and membrane integrity[29]. Both severe heat stress and long-term exposure to moderately high temperatures can result in cellular damage and cell death. 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.

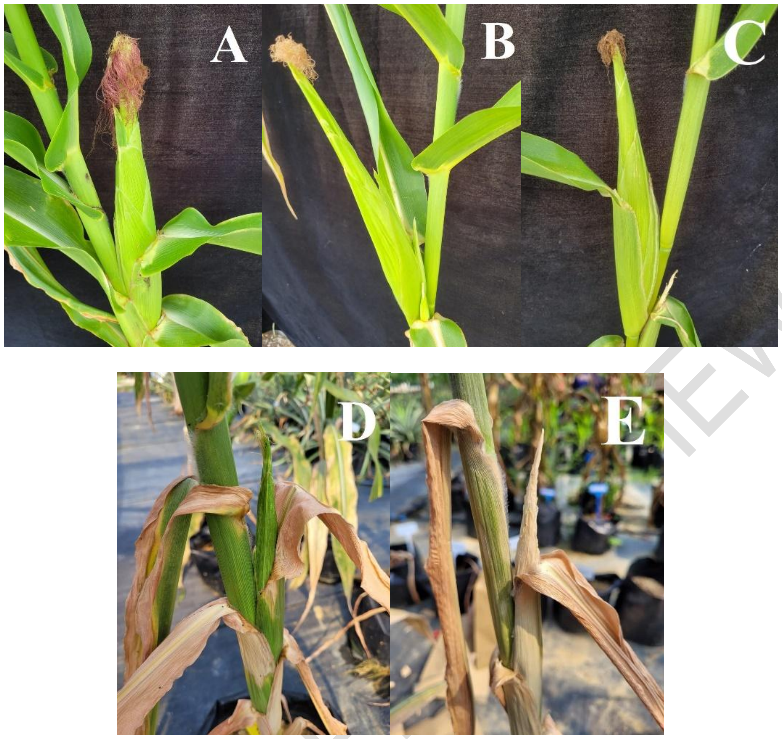

Fig. 5. Cob condition as affected by heat stress treatment and different duration (A) Ambiance conditions for 28 days (B) Non-heated treatment of 30 °C for 28 days (C). Heat stress treatment of 38 °C for 7 days (D and E). Leaf senescence with underdeveloped ear and outer husk turned brown indication of cellular damage and cell death from heat stress treatment of 38 °C for 28 days

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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 50.40% lower but the non-heated treatment (30 °C for 28 days) was significantly lower at 22.18% compared to the ambiance 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[30].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.

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

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 ambiance and non-heated treatments were more than 97%. The kernel set of cobs as affected by heat stress treatment was 46.12 and 45.59% lower as compared to ambiance and non-heated treatment, respectively (Table 5). Both ambiance 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.

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

	Ambiance 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 $P \leq 0.05$.

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 [31]. Heat stress damages pollen morphology, interrupts sugar to starch physiological process, decreases starch granule number and size in the pollen and reduces pollen viability [32]. 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 [17,33]. The short duration of heat stress around anthesis affects pollen shedding, pollen germination ability, and embryo development in maize[31].Additionally, heat stress affected the period anthesis and silking, resulting in reduced kernel number, limited kernel setting and distinctly decreased yield [32]. 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[34]. 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 [35].

Heat stress also caused 73.94, 48.13, 65.08, 66.14 and 39.65% reduction in total kernel number, cob weight, de-husked cob weight, grain weight and cob length compared to ambiance,respectively; and the reduction at 60.87, 24.89, 49.27, 57.44 and 23.49% as compared to non-heated treatment, respectively. Although the size of cobs in the non-heated treatment were smaller than ambiance conditions (Figure 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.[36] that explain the optimum temperature for anthesis was 30.5 ℃ and a temperature above 38 ℃ can hinder maize pollination.

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

Total kernel number, cob weight, de-husked cob weight, grain weight and cob length of non-heated treatment were significantly lower compared to the ambiance conditions (Table 5) and this could be ~~probably~~ 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 66.14 and 20.43% lower as compared to the ambiance, respectively. Therefore, an estimation can be made that heat stress potentially caused 45.71% reduction in grain yield, whereas 20.43% was most probably influenced by the environmental conditions of the climate-controlled room. For the total kernel set, heat stress potentially caused 40.54% reduction, whereas 33.40% was influenced by the environmental conditions of the climate-controlled room.

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 ambiance 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[30].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 initial grain filling period, and any condition that is not optimal for plant requirements will have negative effects on the corn grain yield.

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Comment [KM10]: 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 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 ~~has~~ significantly affected the leaf physiological performances of grain corn plants. A prolonged heat stress duration of 28 days ~~has~~ caused even more severe effects and significant reduction in leaf physiological performances. The effects of heat stress were not manifested in the plant's vegetative growth after a short duration of heat stress treatment but were severely affected after prolonged durations of 28 days. These reductions have the potential to cause substantial effects on grain corn cob quality and yield 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 to reduce the risk of yield production loss due to the heat stress in the future.

Comment [KM11]: Rephrase, we cannot say effects were severely affected. Since you were mentioning manifestation, you could say 'clearly visible'

Suggestion;
...short duration of heat stress treatment but were clearly visible after a prolonged duration of 28 days.

Comment [KM12]: Sentence too long, rephrase, something like this;

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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Comment [KM13]: Italicise species names, do the same for the rest of the references where there is a species name

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