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# The Effect of Different Day and Night Temperatures on the Growth and Physiology of *Theobroma cacao* under Controlled Environment Condition

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors TSN, MHI, PH and AD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MHI managed the analyses of the study. Authors TSN and MHI managed the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** An experiment was conducted to investigate the effect of a wide range of temperatures on the growth and physiology of *Theobroma cacao*, to study the differences between night and day temperatures and to determine the optimum temperature for the cocoa growth.

**Study Design:** The experiment used five combinations of night and day temperatures (18°C and 30°C [18N30N], 18°C and 36°C [18N 36D], 24°C and 24°C [24N24D], 24°C and 30°C [24N30D] and 24°C and 36°C [24N36D]) using complete randomized design (CRD).

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**Place and Duration of Study:** Crops and Environment Laboratory, University of Reading and International Cocoa Quarantine Centre, between 23<sup>rd</sup> May 2016 and 25<sup>th</sup> July 2016.

**Methodology:** The cocoa seedlings were put into five growth cabinets with five different night and day temperatures combinations (18°C and 30°C, 18°C and 36°C, 24°C and 24°C, 24°C and 30°C, 24°C and 36°C) for two months (63 days) under controlled environment condition where the relative humidity and vapor pressure deficit were controlled. Destructive harvest data was taken at end of the experiment which included fresh weight, dry weight, leaf area and root weight. Non-destructive measurements were height of the plant, photosynthetic rate, chlorophyll fluorescence and total chlorophyll content.

**Results:** Treatment 24N30D have the best growth and treatment 24N36D had the lowest growth performances compared to other treatments.

**Conclusion:** The growth was not only dependent on the day temperature, but also on the night temperature. A large gap between night and day temperatures (DIF) reduced the cocoa growth. The result also showed the optimum temperature amongst those studied for cocoa growth is the combination of 24°C night temperature and 30°C day temperature.

**Keywords:** Cocoa seedlings; day and night temperatures; climate change; photosynthesis attributes; cocoa physiology

## 1. INTRODUCTION

Cocoa is a tropical tree species of the genus *Theobroma* and belongs to the family of Malvaceae. There are more than twenty species of cocoa from this genus but *Theobroma cacao* is the most cultivated due to the value of its seeds. Around 40-50 million people in the world rely on cocoa for their livelihood. There are more than five million small-scale farmers which contribute to 90% of the world's cocoa. It is estimated that the annual production of cocoa is more than 3 million tonnes equivalent to \$ 5.1 billion globally where Malaysia contributed about 1% to this number [1,2]. However, the production of this crop has declined in Malaysia due to several factors and one of them is climate change. Climate change is not a new issue to be discussed. It is an indisputable fact and its causes and effects have been studied for years. Root et al. [3] state that the world temperature is expected to keep rising at a rapid rate and has already increased by about 0.6°C in over the past 100 years. In the worst case is it is expected that the temperature will rise by around 2-3°C in next 30-50 years [4]. In addition, according to Hall and Ziska [5], the greenhouse gas CO<sub>2</sub> is projected to reach a concentration of at least 600 µmol /mol by the end of the 21st century as a result of human activities.

The latest data from Anonymous [6] shows that the current global temperature has warmed by 0.87°C in January 2015 where it reached the peak during 1951–1980 base period. Furthermore, they agree that the reason for this global warming trend is because of human

activities which contributed to increases in greenhouse gases such as carbon dioxide, methane, nitrous oxide and chlorofluorocarbons. The increase in these gases comes from the human activities such as fuel combustion, wastes decompositions, deforestation and agriculture activities which offer major contribution to the greenhouse gases. In Malaysia Tangang et al. [7] reported that the surface temperatures in most areas showed significant warming trends during the 42 years from 1961 to 2002. This study also shows a different warming trend between each region of the country, the south-western region of Borneo where cocoa is mainly planted experienced lower warming rates compared to Peninsular Malaysia [8]. However, a recent study by Tangang et al. [9] stated that by the 21st century, it is estimated the mean surface temperature in this country will increase by 3-5°C.

Even though some of the temperate regions will benefit with the rising of the global temperature as it lifts agricultural production (this is crop dependent), yet it is still not really know as to what extend cocoa yields will be impacted by temperature due to climate change because the physiological and developmental processes of this crop are very sensitive to temperature [10] [11]. In addition, Omolaja et al. [12] showed that temperature can affect the flowering intensity of *T. cacao*, as well as the intensity of rainfall in his study. According to him, both temperature and rainfall control the amount of cocoa flowers produced yet it is also depend on the cocoa clones. Whilst the maximum temperature for the growth of cocoa has not been identified. Sale

[13] observed that temperature has an influence on the extension growth of cocoa where it increases up to optimum in day temperature. The effect of temperature is not only limited to the plant's rate of development, but Daymond and Hadley [14] also reported that it can affect the final size of cocoa fruits and beans as well as increasing wilting of the crop. According to Anonymous [15] increasing temperature will increase the evapotranspiration in cocoa crops. An experiment conducted in Trinidad showed that cocoa plants tend to lose its apical dominance, the axillary buds producing numerous flushes with small leaves in a constant temperature of 31°C whereas, in another experiment, it was shown that high temperature of 51°C was damaging to the leaves [16]. Moreover, Lee as cited in Wood and Lass [16] also proved that a low temperature of 10°C was damaging the crop. On the other a study conducted by Hardy, cited in Carr and Lockwood [1] showed that the mean annual temperature for cocoa production should not be less than 22°C while the mean daily minimum should not be less than 15°C, and the absolute minimum not less than 10°C.

Different day and night temperature also may influence the development of cocoa tree. A study conducted on the rice plant in China showed that the different between day and night temperatures (DIF) effect their rice grain weight [17]. However, the other study in Japan which examined the effect of various night temperatures to the same day temperature on rice, showed high night temperature gave the highest final biomass of the plants [18]. Nakasini et al. [19] in his recent studies also stated that DIF seems to affect the flower setting habit of tomato in greenhouse and also delay flower differentiation while Davies [20] proved that larger DIF gap deteriorate the Chinese lantern lily's stem elongation. Thus, it is believe that there is an effect of different day and night temperature to the development of the crop as well as to the cocoa plant.

To summarize, it can be concluded that temperature plays an important role in determine the growth and yield of the cocoa plant. A report [15] hypothesized that some areas in cocoa producer countries might be too hot for cocoa cultivation, however more physiological research and information about this issue is needed. This basic information on cocoa physiology is important if growers are to adapt to climate change. In order to gain a better understanding of predicted changes in climate, it is important to

investigate what is the optimum temperature for the *T. cacao* growth and to study if there a different effect on day and night temperature on this crop because insufficient information is available about temperature optima in cocoa. Thus the objective of this experiment are to investigate the effects of wide range of temperatures on the growth of cocoa plant and to determine the optimum temperature for cocoa growth. This information could be useful for the cocoa planters in identifying geographical areas that have suitable temperatures for cocoa cultivation and in regards to future planning management, design of new cropping systems and a basis for agronomic practices.

## 2. MATERIAL AND METHODS

### 2.1 Experimental Location and Treatments

Cocoa seedlings of Amelonado variety were raised at the University of Reading's International Cocoa Quarantine Centre before being transferred into controlled environment cabinets in the Crops and Environment Laboratory University of Reading. The age of the cocoa seedlings selected for the study was five months old and sown in a mixture of sand, gravel, and vermiculite at a ratio of 1:2:2. All of these seedlings were divided equally and placed in 6 growth cabinets. The growth cabinet used was Weiss Technik HGC 1514 with a growth volume of 2000 litre. Different combinations of night and day temperatures regimes were set for eight weeks (63 days) from 23<sup>rd</sup> May 2016 to 25<sup>th</sup> July 2016. Temperature and relative humidity (RH) were all controlled from a computer. Table 1 shows the details of treatments that were used in the study. Different values for RH were chosen to maintain a constant VPD across treatments. The plants were watered once a day every morning using "cocoa nutrient solution". This solution (a modified form of Long Aston Solution) contained essential macro and micro nutrients needed for the growth of cocoa in the appropriate proportion. The stock solution contained Potassium Nitrate (KNO<sub>3</sub>) (6048 g) and Ammonium Nitrate (NH<sub>4</sub>NO<sub>3</sub>) (5.5 ltr) in one tank dissolved in 120L water. A second stock tank contained the following chemicals dissolved in 120 L water: Potassium Sulphate (K<sub>2</sub>SO<sub>4</sub>) (1680g), Magnesium Sulphate (MgSO<sub>4</sub>) (3312g), Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) (2112 g), EDTA (480 g) and Nitric acid (500 ml). The micronutrients were dissolved in the same tank and consisted of: Boric acid (H<sub>3</sub>BO<sub>3</sub>) (120

g), Manganous sulphate ( $MnSO_4$ ) (68 g), Zinc sulphate ( $ZnSO_4$ ) (324 g), Ammonium molybdate ( $NH_4(2MoO_4)$ ) (10.4 g), Copper sulphate ( $CuSO_4$ ) (9.6g). There was a separate acid stock solution which contained 2.5-litre Nitric acid, 1.25-litre Orthophosphoric acid was mixed with 80 litres of water. The stock solutions were injected into the mixing tank such that the electrical conductivity was maintained around 2.0 m/S as it suits the cocoa growth well. To maintain the pH of a solution of 5.8 which is optimal for cocoa growth, acid solution was added to the mixing tank. All the seedlings were watered until water drained out of the pot at the bottom.

**Table 1. Different combinations of night and day temperature treatments used in the study**

Treatments	Temperature (°C)		
	Night	Day	Mean
18N30D	18	30	24
18N36D	18	36	27
24N24D	24	24	24
24N30D	24	30	27
24N36D	24	36	30

## 2.2 Non-destructive Methods

### 2.2.1 Height of plants

The height was measured and recorded from the border of the container to the top of the main plant stem by using a measuring tape once a week. At the end of the experiment, the exact increase in height under the experimental condition was calculated by deducting plant height at the beginning of the experiment (week 1) from plant height at the end of the experiment (week 8).

### 2.2.2 Photosynthesis rate

The data for light saturated photosynthesis measurements were taken at the end of the experiment. Measurements were made using an LCpro+ portable infrared gas analyser (ADC) fitted with a light attachment on the youngest fully matured and hardened leaf on each plant. The time when data was taken was around 9.00 to 14.00. Before the measurements, this machine was warmed up. After that, the leaf chamber was clamped onto the leaf to measure gas exchange from which it calculates photosynthetic rate, transpiration and stomatal conductance.

### 2.2.3 Chlorophyll fluorescence

Chlorophyll fluorescence was measured every once a week by using a chlorophyll fluorometer (Handy PEA, Hansatech instruments). Before the measurement was taken, the youngest fully matured and hardened leaf on each plant had a leaf-clip placed on it for at least 20 minutes. Then, the fluorescence meter was placed on the clip, which was opened when the reading was taken.

### 2.2.4 Total Chlorophyll content

Chlorophyll content was measured using a Hansatech instrument chlorophyll meter which uses dual wavelength optical absorbance (620 and 940 nm wave length). The measurements were taken from the youngest fully matured and hardened leaf on each plant. Because of the cocoa leaf was quite large, measurements were taken 3-4 times on different spots of each leaf and the average reading was recorded.

## 2.3 Destructive Methods

Before the experiment started that was on 25th Mays 2016, the initial baseline harvest data was measured on a subset of five seedlings before the remaining 40 seedlings are placed in the growth cabinet. The following data were taken:

### 2.3.1 Fresh weight

The cocoa seedlings were removed from the soil by cutting at the base of the stem using a pair of secateurs. After that, the plants were separated into leaves, roots and stems. The stem was chopped into the section before being weighed and the leaves were weighed straight away after being removed from the stem. The roots of the seedlings were washed to remove any loose soil before being blotted with a soft paper towel gently to remove any free surface moisture and then weighed.

### 2.3.2 Dry weight

After all the measurements of fresh weight were taken, leaves, roots and stems of the seedlings were transferred into the oven at 70°C for at least 48 hours. After two days, the plants were weighed to determine dry weight. The relative growth rates was calculated by the formula  $(LnW2 - LnW1)/t2 - t1$ , whereby W2 is the weight at time 2 (t 2) and W1 is the weight at time 1 (t 1).

### 2.3.3 Leaf area

The leaf area of each cocoa seedlings was measured by using WD3 WinDIAS leaf area meter (Delta-T Devices Ltd., Cambridge, UK) before they were transferred to the oven.

### 2.4 Data Analyses

The data were statistically analysed by using Analysis of Variance in GENSTAT the 16th edition.

## 3. RESULTS

### 3.1 Growth Parameters and Biomass Measurements

#### 3.1.1 Leaf fresh and dry weight

The results were compared to the initial baseline data which was taken right before the cocoa seedlings were put into the growth cabinet. Fig. 1

shows the leaf fresh weight under different temperatures regimes. The mean leaf fresh weight before the experiment started was 23.15 g, however after 63 days of the experiment the mean leaf fresh weight for treatment 24N30D was 88.83 g which had the highest increased in weight (284.2%) whereas treatment 18N36D showed the lowest increment with a decrease in weight of 76.3% (Fig. 1). The differences between the treatments were highly significant ( $P < .001$ ). Fig. 2 indicates the leaf dry weight under different treatments. The mean leaf dry weight for leaf before the experiment started was 7.35 g. The differences between treatments in leaf dry weight at the end of the experiment was statistically significant ( $P < .001$ ) where a very large difference was observed between treatment 18N30D and 24N36D. Treatment 18N36D was drastically decrease to 2.05 g after the treatment and treatment E was increased by 22.3 g compared to dry weight before the treatment initiated.

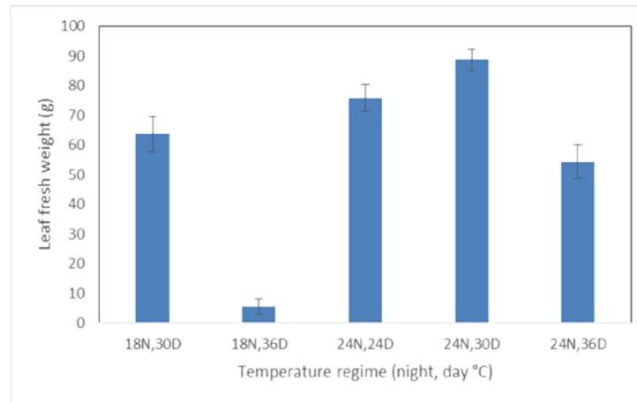


Fig. 1. Impact of different day and night temperatures on leaf fresh weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates

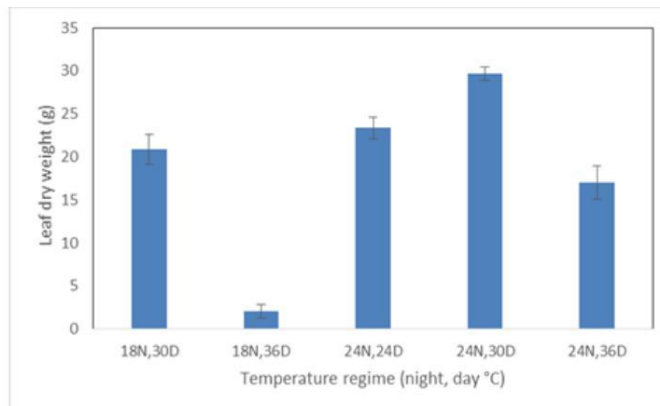


Fig. 2. Impact of different day and night temperatures on leaf dry weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates

### 3.1.2 Stem fresh and dry weight

Fig. 3 illustrates the effect of different temperatures on the cocoa's stem fresh weight. Before the experiment started, the initial baseline weight was 20.68 g. However, after 63 days of the experiment, the mean stem fresh weight for treatment 18N30D was significantly lower than the other treatments followed by treatment 18N36D ( $P=0.001$ ) as shown in Fig. 3. Fig. 4 shows the stem dry weight under the treatments. The mean stem dry weight at the initial baseline was 4.31 g. After 63 days being treated with different temperatures, the results showed treatment 24N30D had the highest weight (a 15.70 g increase from the baseline), and treatment 18N, 36D had the lowest weight (4.71 g increase from the baseline) ( $P<.001$ ; Fig. 4).

### 3.1.3 Root fresh and dry weight

Fig. 5 indicates the root fresh weight under the treatments. There was a very highly significant difference ( $P<.001$ ) between the mean fresh weight of each treatment. Treatment 18N36D had the lowest root fresh weight and the fresh weight of cocoa's root was gradually decrease as the night temperature was decreasing. Fig. 6 shows the root dry weight under different temperatures regimes. At the initial baseline, the root dry weight was 1.53 g. At the end of the experiment, there was a very highly significant difference ( $P<.001$ ) between treatments with the lowest weight observed for Treatment 18N, 36D and the highest weight for treatment 24N24D.

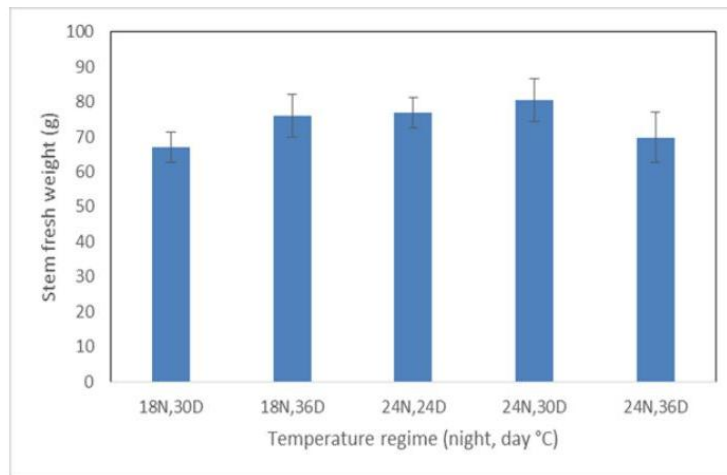


Fig. 3. Impact of different day and night temperatures on stem fresh weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates

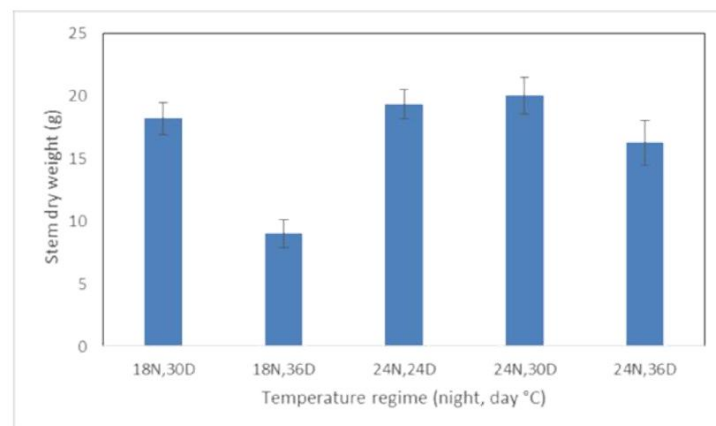
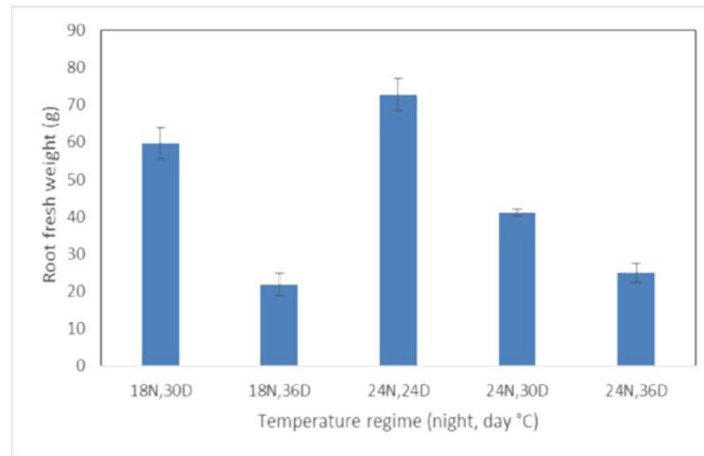
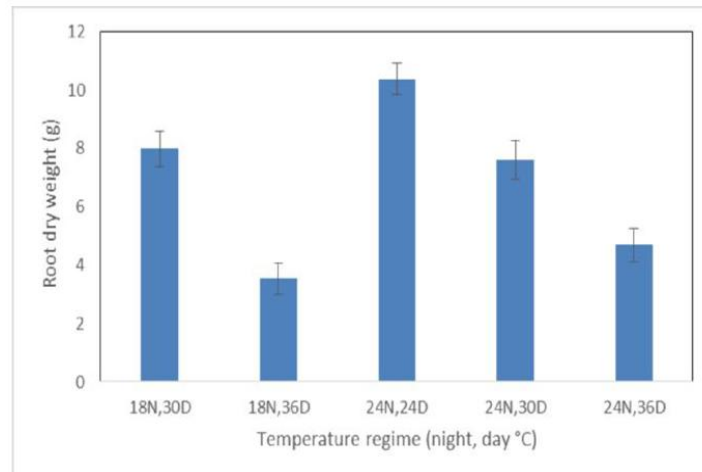


Fig. 4. Impact of different day and night temperatures on stem dry weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates



**Fig. 5. Impact of different day and night temperatures on root fresh weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 6. Impact of different day and night temperatures on root dry weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates**

### 3.1.4 Total fresh weight

Fig. 7 indicates the total fresh weight under different temperatures. The total fresh weight before the experiment started was 52.94 g, however after 63 days of the experiment, there was a very highly significant difference ( $P < .001$ ) where the total fresh weight for the 24°C night temperature treatments decreased with an increase of day temperature and the lowest fresh weight was treatment 18N36D.

### 3.1.5 Total dry weight

Fig. 8 illustrates the total dry weight after being treated with different temperatures regimes. The

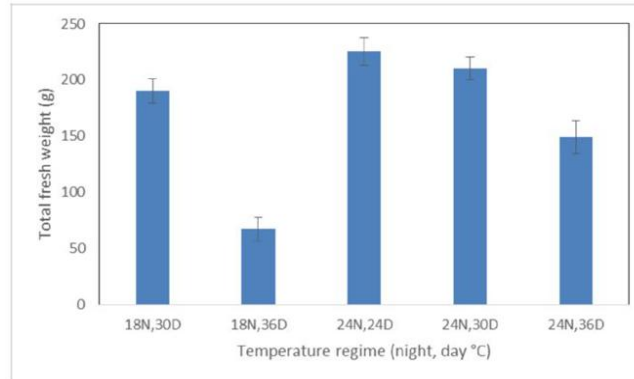
mean total dry weight at the initial baseline was 13.19 g. After 63 days of experiment, there was a very highly significant difference ( $P < .001$ ) between each treatment. Fig. 8 shows that treatment 18N30D had lowest total dry weight and treatment 24N36D had the highest dry weight compared to all of the treatment.

### 3.1.6 Shoot: root ratio

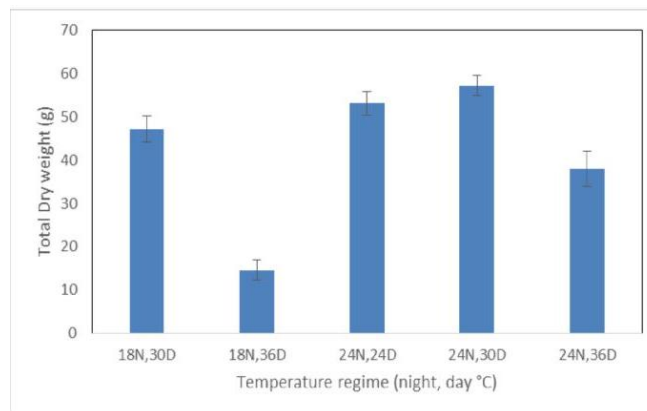
Fig. 9 shows the shoot: root ratio under different temperatures regimes. The result from the harvest indicated different patterned of shoot: root ratio under different temperature treatments. The initial shoot: root ratio before the experiment started was 4.81. At the end of experiment a very

large difference was observed between 18N36D and treatment 24N36D (Fig. 9;  $P < .001$ ). Although they shared the same day temperature,

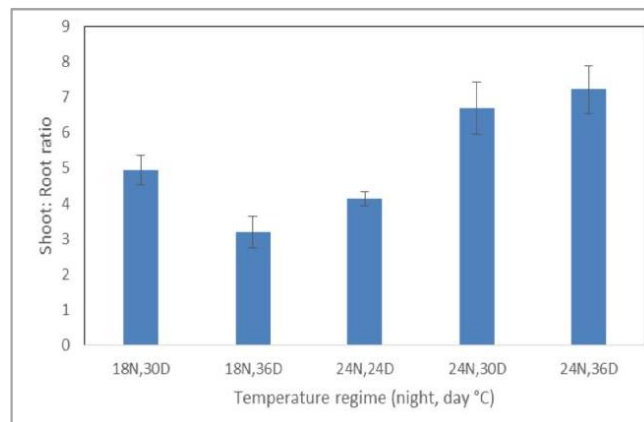
treatment 18N36D showed the lowest shoot: root increment while treatment 24N36D showed the highest compared to other treatments.



**Fig. 7. Impact of different day and night temperatures on total fresh weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 8. Impact of different day and night temperatures on total dry weight of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 9. Impact of different day and night temperatures on shoot to root of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



### 3.1.7 Relative growth rate for total biomass

Figure 10 illustrates the relative growth rate for total biomass under treatments. There was a very highly significant difference ( $P < .001$ ) between the mean relative growth rate of each treatment at the end of the experiment as shown in Fig. 10. Treatment 18N36D had a much smaller relative growth rate compared to other treatments ( $0.002 \text{ g g}^{-1} \text{ day}^{-1}$ ) even though its day temperature was the same as treatment 24N36D (relative growth rate of  $0.03 \text{ g g}^{-1} \text{ day}^{-1}$ ) and its night temperature was the same as treatment 18N30D (relative growth rate of  $0.038 \text{ g g}^{-1} \text{ day}^{-1}$ ).

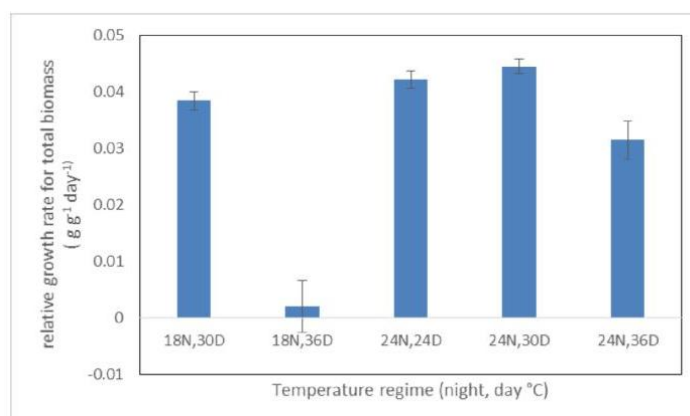
### 3.1.8 Leaf total area

Fig. 10 shows the total leaf area under different temperatures regimes. Initially, before the experiment started the average total leaf area

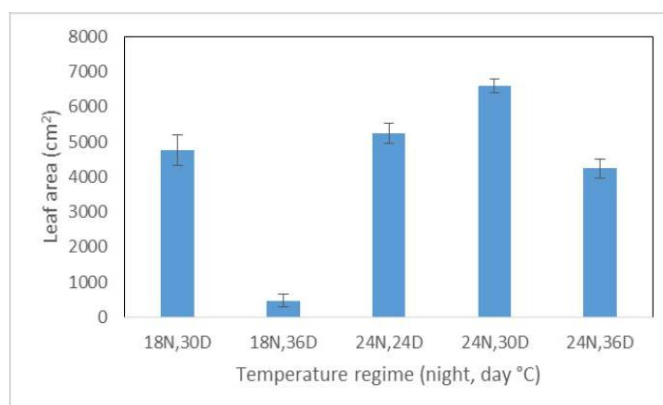
was  $1737 \text{ cm}^2$ . The leaf area of treatment 18N36D decreased until the 63<sup>rd</sup> day of the experiment due to leaf fall when it was  $471.78 \text{ cm}^2$  as shown in Fig. 11. However, treatment 24N30D showed largest total leaf area ( $6598 \text{ cm}^2$ ) at the end of experiment and the differences between treatments being highly significantly ( $P < .001$ ).

### 3.1.9 Leaf number

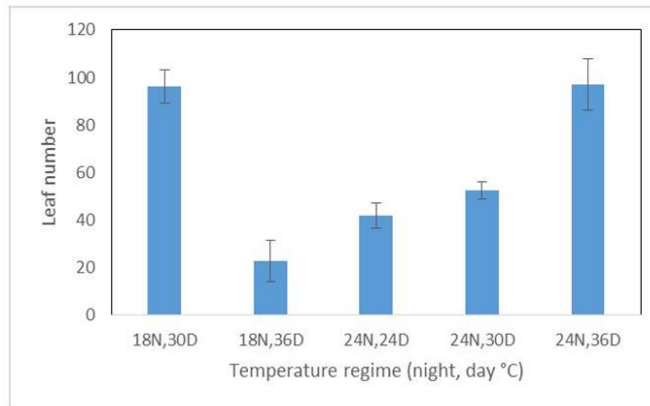
The result from the total leaf number on the 63<sup>rd</sup> day as shown in Fig. 12. The differences between treatments were highly significant ( $P < .001$ ). The total leaf number for the  $24^\circ\text{C}$  night temperature treatments increased with an increase in day temperature. However, there was a huge difference between the  $36^\circ\text{C}$  day temperature with different night temperatures. Treatment 24N36D had 97 leaves whereas treatment 18N36D an average of 23 leaves.



**Fig. 10. Impact of different day and night temperatures on Relative growth rate of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 11. Impact of different day and night temperatures on total leaf area of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 12. Impact of different day and night temperatures on leaf numbers of *Theobroma cacao*. Data are means and standard error of differences of four replicates**

**3.1.10 Plant height**

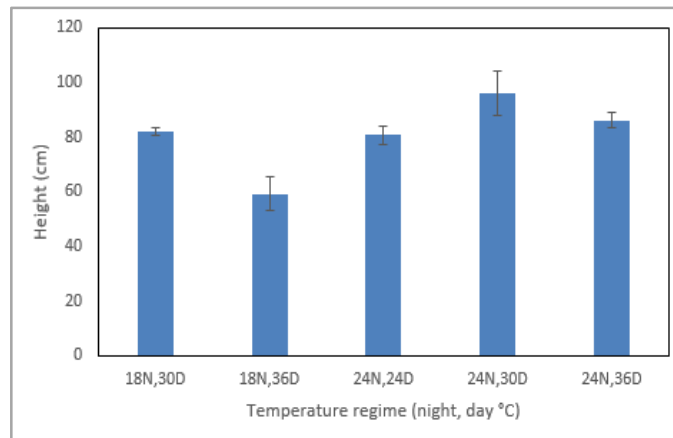
Fig. 13 shows the mean height of cocoa seedlings at the last week of the experiment under different temperature regimes. The height of the cocoa seedlings increasing gradually from day 1 to day 63 and the temperature regimes had a highly significant impact on the height of the seedlings ( $P < .001$ : Fig. 13). Whilst the height of seedlings increased during 63 days, the rate of increase was different between treatments. For example, treatment 18N36D increased by just 5.4% which made this treatment the lowest growth rate. Furthermore, treatment 24N36D higher rate of increase of compared to treatment 24N30D, which increased by 65.57%. 24N36D which shared the same day temperature as treatment 18N36D had a 39.26%.

**3.2 Chlorophyll Content**

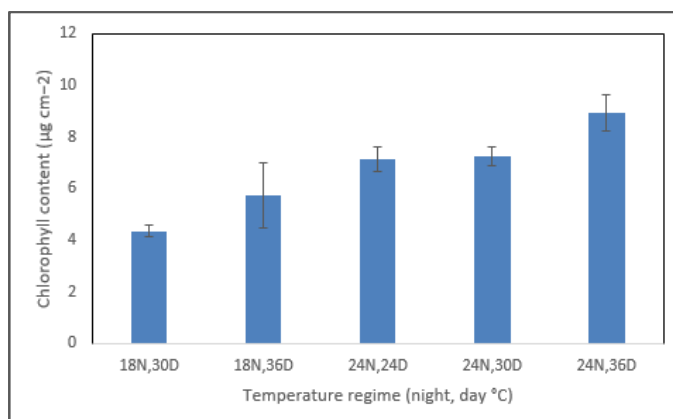
Fig. 14 illustrates the mean chlorophyll content for the 9<sup>th</sup> week under different temperatures. There was a highly significant difference ( $P < .001$ ) between treatments on the chlorophyll content. According to the Fig. 14, the chlorophyll content of each treatment tended to fluctuate from week to week. However, at the end of the experiment, treatment E 24N, 30D and 24N36D showed higher chlorophyll content compared to others while treatment 18N36D maintained the lowest on average of the period.

**3.3 Chlorophyll Fluorescence**

Fig. 15 shows the mean chlorophyll fluorescence for the 9<sup>th</sup> week of the experiment under different temperature regimes. Analysis of variance



**Fig. 13. Impact of different day and night temperatures on plant height of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 14. Impact of different day and night temperatures on total chlorophyll content of *Theobroma cacao*. Data are means and standard error of differences of four replicates**

showed a highly significant difference ( $P < .001$ ) in the effect of temperature on chlorophyll fluorescence. The majority of the treatments showed similar values of Fv/Fm which were around 0.6-0.7, but treatment C 18N36D showed a drastic decline throughout the experiment (Fig. 15).

### 3.4 Photosynthesis Rate

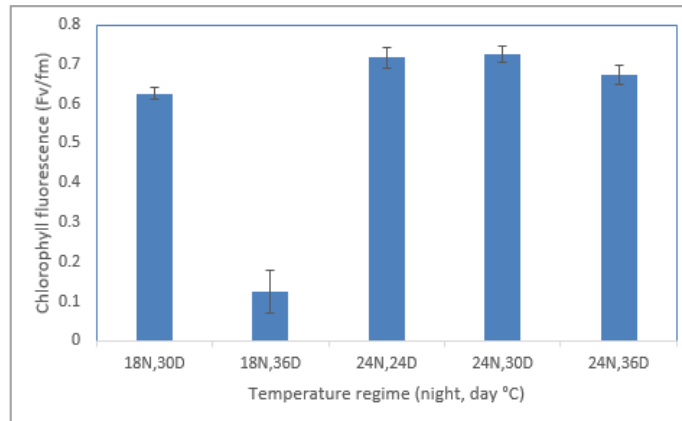
Fig. 16 illustrates the photosynthesis rate under different treatments. The rates of photosynthesis after 63 days in the treatments showing a highly significant difference ( $P < .001$ ) between treatments ( $P = 0.008$ ; Fig. 16). Obviously, treatment 18N36D showed the lowest photosynthesis rate with only 0.845 while treatment 24N24D and 24N30D were higher among others with 5.9025 and 6.1825 respectively.

## 4. DISCUSSION

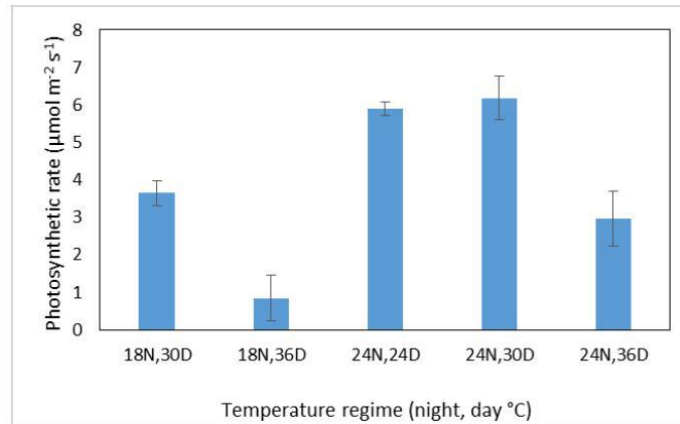
In relation to the objective one of the study, it was proved that wide range of temperatures affect the growth and biomass measurements of cocoa seedlings differently. The highest leaf fresh and dry weight were obtained under the 24°C night and 30°C day temperature regime at the end of the experiment. However, the leaf fresh and dry weight were considerably reduced at high day temperature (36°C) with low night temperature (18°C). In the same way, treatment 24N36D which also experienced high day temperature, showed significant negative effects on some parameters. Both high-temperature treatments resulted in a direct negative impact on the cocoa seedlings' stem and root development,

relative growth rate, leaf total area, as well as the total dry weight at the end of the experiment although the negative effect of 36°C day temperature was much greater when the night temperature was 18°C. According to Wood and Lass [16] high temperature tends to reduce the plant apical dominance. The negative impact of high temperature on the plant dry weight might be due to increasing water and respiration rates in plant, thus decrease the growth parameters measured in the present study [21]. Additionally, RH in this experiment was maintained to keep a similar vapour pressure deficit in all chambers. Yet, Gomes and Kozłowski [22] claimed that high vapour pressure gradient between the leaves and air can dehydrate cocoa leaves at high temperatures which reduced basipetal translocation of carbohydrates and hormonal growth regulators. As the conclusion, the results were in line with the findings of Daymond and Hadley [10] where according to them, the physiological and developmental processes of this crop are very sensitive to temperature.

From the result of the experiment, it can be concluded that there is an effect of day and night temperature different (DIF) on total biomass of the cocoa seedlings, shoot: root ratio, relative growth rate, total leaf area, leaf number, the height of the seedlings, chlorophyll content and chlorophyll fluorescence. Even though treatment 18N36D shared the same day temperature as treatment 24N36D, the result indicated lower performance for most of the parameter of treatment 18N36D than treatment 24N36D. A large gap between night and day temperatures appears to be deleterious to growth and physiology. Furthermore, treatment 18N30D had



**Fig. 15. Impact of different day and night temperatures on maximum efficiency of photosystem II (Fv/Fm) of *Theobroma cacao*. Data are means and standard error of differences of four replicates**



**Fig. 16. Impact of different day and night temperatures on net photosynthesis of *Theobroma cacao*. Data are means and standard error of differences of four replicates**

the same night temperature as treatment 18N36D but the growth performance of the latter was lower. Overall, treatment 24N30D performed better than treatment 18N30D even though they experienced the same day temperature. Thus, this study suggests that cocoa growth is not primarily a response to day temperature only but also to the night temperature. Treatment 18N30D, 24N24D, 24N30D and 24N36D experienced 12, 0, 6, and 12 DIF respectively. However, treatment 18N36D had a greater DIF that was 18. DIF from 0 to 12 seemed not reducing the total biomass of the cocoa seedlings, shoot: root ratio, relative growth rate, total leaf area, leaf number, the height of the seedlings, chlorophyll content and chlorophyll fluorescence as much as DIF 18. Wardlaw [23] come to the agreement in the present study, where

increasing the amplitude of day/night temperature different by 10°C to 20°C can reduce the plant growth. Also, Erwin et al. [24] and Myster and Moe [25] both come to the conclusion that the negative effect of DIF is related to the response of the plant tissue to the endogenous gibberellin content (GAs). This have been confirmed by study conducted by Davies [20] on Chinese lantern lily that demonstrated that the stem of this plant elongated by 55% for DIF between -6 to +12, yet the length reduced for further DIF to +18. Another experiment on tomato also showed the same result as DIF 5 to 7 was suitable for tomato cultivation but reduce flower setting habit as DIF increases from 7 onward [19]. Hence, it can be concluded that DIF affects the growth of cocoa seedlings and DIF 18 gave the most deleterious effect on this crop.

The high temperature decreased the RGR of cocoa seedlings in this experiment, but this was much more marked when combined with a low night temperature. This negative effect of high temperature regimes on the growth of plants was also studied in other plants such as tomatoes and cat grass. According to Eagles [26], RGR of cat grass increased with an increase in temperature up to 20°C but RGR was reduced above this temperature as it was related to the net assimilation rate, not leaf area ratio. However, this view contrasted to the study conducted by Heuvelink [27] on tomatoes. He claimed that the deleterious effect of high temperature on this crop's RGR was caused by lowering in LAR and not by changes in NAR. He also gave the same example on the previous study of sweet peppers and cucumber plants. The result of leaf total area on the 63<sup>rd</sup> day showed highly significance difference between each other where the both treatments which been treated with 36°C have the smaller leaf expansion (18°C night temperature was the smallest). The observation of a decrease in total leaf area with an increase in temperature is consistent with a previous study in tomato [27]. The stem height and shoot: root ratio of treatment 24N36D was not affected by the high-temperature as treatment 18N36D which were extremely affected by the high-temperature treatment. Gomes and Kozłowski [22] demonstrated that the rate of growth decreased with increasing temperature under 18.7°C to 33.3°C regimes. They concluded that this was due to respiration increases as high temperature promotes carbohydrate depletion and shoot desiccation.

Different temperature regimes also had different impacts on the chlorophyll fluorescence, chlorophyll content and photosynthetic activities on the cocoa leaves. Chlorophyll fluorescence is widely used by plant physiologists to measure the energy conversion and efficacy in photosynthesis [28]. In this experiment, treatment 18N36D showed lowest chlorophyll fluorescence result compared to other treatments followed by treatment 24N36D. According to a previous cocoa study conducted by Daymond and Hadley [10],  $F_v/F_m$  value can be vary depending on the temperature regimes. The result is similar to the study by Yamada et al. [29] on the leaves of tropical fruit crops where the  $F_v/F_m$  was decreased with an increased in temperature. According to them, this declination was due to the increase in basal fluorescence which destructs the PSII reaction centres and a

decrease in variable fluorescence, which interrupt the electron donation to PSII reaction centres. Thus, chlorophyll fluorescence emitted by green plants reflects photosynthetic activities in the leaves and this activities provide carbohydrate for cocoa tree which is important for the cocoa growth [30].

Photosynthesis is highly sensitive to high-temperature stress. In the present study, the photosynthesis rate of both treatments C and F (36°C day temperature) showed a significant declination though out 63 days of the experiment. Normally, in most plant the photosynthetic activity is reversible in 10° to 35°C range (this depends a lot on the species), yet apart from it, it will cause irreversible injury to the photosynthetic system and it was reported that the optimum range for cocoa was 31-33°C [31,32]. The study on other cocoa variety by Gomes and Kozłowski [22] proved that high temperature (above 33.3°C) lowered stomatal conductance and photosynthesis rate in cocoa leaves. The decline in photosynthesis rate of both treatments (particularly 18D36D) explained the significant reduced in total dry weight and RGR of seedlings in the last harvest.

The gain in total plant dry weight was greatest at a night temperature of 24°C, smallest at a night temperature of 18°C while highest at a day temperature of 30°C, lowest at a day temperature of 36°C. Most of the results represented the same trend as the total dry weight. According to Carr and Lockwood [1], the mean annual temperature for cocoa production should not be less than 22°C while the mean daily minimum not be less than 15°C, and the absolute minimum not be less than 10°C. However, the Anonymous [33] stated that the maximum annual average temperature should be 30-32°C and a minimum average of 18-21°C. Treatment 24N30D had the highest leaf fresh and dry weight, total dry weight, the relative growth rate, total leaf area, height increment, chlorophyll fluorescence and photosynthesis rate on the last harvest. Similar cases reported in Gomes and Kozłowski [22] in their 60-days study of cocoa seedlings showed that dry weight and relative growth rate of cocoa increased to an optimal temperature of near 33.3°C and 30.5°C improved the leaf area and high of the crop. Of the treatment combinations studied here, it can be concluded that a combination of a night temperature of 24°C and a day temperature of 30°C (24N30D) is the optimal combination for cocoa growth.

## 5. CONCLUSION

As a conclusion, this experiment has confirmed that differences in temperature regimes gave a different effect on cocoa physiology. High day temperature treatment with low night temperature gave a deleterious effect on the growth of this plant. The extension growth rate of this crop was not mostly depended on the day temperature, but also depended on the night temperature. Large differences between day and night temperatures were damaging to the plant growth. From the result of this experiment, it can conclude that the optimum temperature for cocoa growth is the combination of 24°C night temperature and 30°C day temperature.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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