Soybean Phenotypic Variation Under High Night Temperature Stress

By

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Abstract

Soybean is the fourth most important commodity crop globally grown on over 83.1 million acres in the United States. Crop yield is required to increase a 50% to satisfy the demand of a growing population that will increase to 10 billion by 2050. However, high day and night temperature could influence plant growth reducing crop yield. High day temperature was proved to negatively affect photosynthetic activity then decrease yield. People have developed soybean cultivars that were tolerant to high day temperature. However, there is no research exploring cultivar tolerance and sensitivity to high night temperature on soybean. Our objectives were: 1) Investigate the effects of different short-term high night temperature applied during the reproductive stage on soybean and study if there is cultivar variation; and 2) Identify physiological effects of long-term high night temperature stress during vegetative stages and determine if there are cultivar variation to high night temperature. To accomplish objective one, four cultivars were exposed to different high night temperatures: extremely high night temperature (EHNT): 32/32 °C; moderate high night temperature (MHNT): 32/26°C; control night temperature (CNT): 32/20°C day/night temperature at full flowering (R2) for 14 days to explore short time HNT effects on reproductive stage. To accomplish objective two, 9 soybean cultivars were exposed to high night temperature from the beginning of germination for 50 days to study HNT long-term effect on vegetative stage (Control: 30°C/20°C, EHNT: 30°C/30°C day/night). In both experiments, measurements included: photosynthesis, respiration, chlorophyll content and fluorescence. We found that short time EHNT (32°C) did not affect photosynthesis and respiration rate in reproductive stage but decreased seed production in one cultivar, therefore finding yield cultivar variation to EHNT. MHNT did not negatively affect plant growth in any of the cultivars. Long-term EHNT (30°C) from vegetative stage decreased photosynthetic rate, stomatal conductance, chlorophyll content and the efficiency of PSII (Fv/Fm) but not changing respiration rate. Some cultivars showed tolerance to EHNT as null effect over the aboveground biomass. In cultivar CM422 and DS25-1, EHNT tolerance may be associated with photochemical efficiency of PSII (Fv/Fm). We concluded from these two experiments that higher light intensity and longer exposure time intensified negative effect of high night temperature stress. These findings open the possibility to breed for high night temperature tolerance, but more research needs to be done to better understand the physiological effects of high night temperature stress and which are the most important traits to acquire tolerance.

Key words: High night temperature, Soybean, phenotypic variation, seed size, photosynthesis, respiration.
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Chapter I: Literature Review

The need for higher yields in a changing climate

Crop yield needs to increase by 50% to meet the food demand of 10 billion people in the world by 2050 (FAO, 2017). In the U.S., the yield of corn, rice, cotton and soybean increased 12.71%, 13.29%, 6.31% and 15.41%, respectively in the last 10 years (USDA, 2019), which demonstrates that increasing yield and production by 50% is possible by 2050. However, negative impacts of land-use modification and abiotic stress such as elevated temperatures, ozone, and drought are reducing the expectation of future crop production and yields (Karl et al. 2009).

Karl et al. (2009) listed an analysis of recent Climate Changes scenarios in the U.S. and projected changes over the next century. Temperature patterns across the U.S. showed a warming tendency (1.5 to 2 °C) for the next 30 years with only a slight increase in precipitation (Tebaldi et al. 2006; Karl et al. 2009). According to IPCC (2019), global mean surface temperature (GMST) for the decade 2006-2015 was 0.87°C higher than the average over the 1850-1900 period. With the current greenhouse emissions rates, the IPCC (2019) has predicted that human induced global warming is currently increasing the global temperature at a rate of 0.2°C per decade (IPCC, 2019). Data in Alabama (NOAA, 2019) showed that the number of very hot days (annual number of days with maximum temperature at or above 35°C) increased from 5 in 1970s to 20 in 2010s. The number of very warm nights (annual number of days with minimum temperature at or above 24°C) increased from 1 in 1970 to 10 in 2010. Karl et al. (2009) noticed that there is an increase frequency of warm nights in the eastern half of the U.S.
It has been reported that the increase of atmospheric temperature above an optimum can affect crop development reducing yield and production. Therefore, the effect of high temperatures in crops needs to be further investigated (Prasad et al. 2008; Ruiz-Vera et al. 2013).

**The effects of high day temperatures on crops**

Elevated temperatures generally cause plants that are below their optimum temperature to grow faster and bigger (Hatfield et al. 2011). However, based on current scenarios, the increase in global temperature is exceeding the optimum temperature for most crops and has the potential to become high temperature stress (Karl et al. 2009). High temperature stress is defined as the increase in temperature beyond a threshold level that leads to irreversible damage to plant growth and development (Wahid & Close, 2007). High temperature stress negatively affects plant growth and development, damages membrane integrity, disturbs metabolic and physiological functions, resulting in lower yields (Prasad et al. 2008; Jahan et al. 2019).

High temperature stress will affect the structure of the leaves of showing damaged membranes at the cell, mitochondria and chloroplast level (Pettigrew et al., 1993; Hays et al., 2007; Djanaguiraman et al., 2011). This membrane damage will be associated with a degradation of the photosynthetic pigments such as chlorophyll and carotenoids in tomato (Jahan et al., 2019) and pigeonpea (Guapta, 2019); and is related with an increase in fluorescence, as the pigments cannot absorb the light that the plant receives due to damages in thylakoid membrane (Govindjee et al., 2017). This changes have been associated with a reduction of photosynthetic rate in soybean, pigeonpea, mungbean, rice, wheat, hemp and tomato (Djanaguiraman et al. 2011; Vera et al. 2013; Bagley et al. 2015; Guapta, 2019; Hanif
and Wahid, 2018; Sailaja et al. 2015; Aiqing et al. 2018; Hansen et al. 2019; Chandra et al. 2011; Jahan et al. 2019). In addition, high temperature stress was found to deactivate photosynthetic enzyme activity. In soybean, the maximum rate of Rubisco carboxylation capacity of RuBP ($V_{\text{cmax}}$) and the maximum rate of electron transport ($J_{\text{max}}$) decreased under high temperature (Bagley et al. 2015). Reactive oxygen species (ROS) was observed to increase under high temperature disturbing photosynthetic activity (Siebers et al., 2015).

To repair the membrane and protein damage produced by high temperatures, the respiration rate will increase to obtain more energy and C skeletons, which implies a high energy cost and biomass penalty (Jumrani et al, 2017). Less photosynthesis results in less energy for the flowers therefore a decreased percent will develop into pods. Yield loss has been observed on various crops under high temperature stress. Corn (Zea mays L.) and soybean from 1982 to 1998 had a 17% yield loss with every 1°C increase (Lobell and Asner, 2003). Yields decreased by 3 to 5% per 1°C increase above 15°C in wheat [Triticum aestivum L.], accompanied with reduced wheat grain yield by 78%, kernel number by 63% and kernel weight by 29% (Gibson and Paulsen, 1999).

A lot of research has investigated how physiological characteristics respond to elevated temperatures in a range of crops, and a lot of cultivars have been screened for traits that are responsible for high temperature stress. The soybean cultivar ‘Maverick’ is tolerant to high day temperatures as it overexpresses a gene that produces an enzyme that reduces the negative effects of reactive oxygen species making the cultivar more tolerant to high temperature stress (Allen et al. 2017). In mungbean, cultivar NM-2006 showed tolerance by displaying a high chlorophyll and carotenoid content, photosynthetic rate and stomatal conductance when grown
at elevated temperatures without showing yield reduction (Hanif and Wahid, 2018). This indicates that tolerant characteristics can be screened in different crops and we can breed for high temperature tolerance if it is heritable.

**The impact of high night temperature on crops**

The effects of high night temperature in crop’s physiology and yield have been understudied in comparison with the effects of high day temperature in the scientific literature. High night temperatures have been reported to affect physiological processes during the night impacting leaf/plant performance over the subsequent diurnal period (Flexas et al. 1999). It has been reported to be associated with the increase in night respiration and thus yield reduction in cotton (Reddy et al. 1997; Loka et al. 2009), rice (Mohammed and Tarpley, 2009; Nagarajan et al. 2010; Singh et al. 2018), and grapevine \([Vitis vinifera L.]\) (Tombesi et al. 2018). Elevated night temperature negatively affects the activity daytime photosynthetic rates such as \(V_{\text{cmax}}\) and \(J_{\text{max}}\), possibly due to a deactivation of photosynthetic enzymes (Singh et al. 2018). Additionally, increased reactive oxygen species accompanied with cellular membrane damage was observed in grapevine and rice (Jing et al. 2016; Mohammed and Tarpley, 2009). Although the effects of high night temperature are known in several crops, there is no evidence of crop tolerance as limited research to test the effect of high night temperature over different cultivars of the same crop.
The history and economic importance of soybean

Soybean [Glycine max (L.) Merr.] originated 5000 years ago from its wild annual progenitor G. soja that was distributed in East Asia; covering China, Korea, and part of Russia (Hymowitz, 2004). Linguistic, geographical and historical evidence suggest that soybean was domesticated during Shang dynasty (1700-1100 B.C) in the eastern half of north China. It was not until 1712, that Europeans realized the potential of soybean as a food and forage plant (Hymowitz, 1990). In the late 1850s, soybean was approved to be a potential forage by farmers in the United States, and by 1898, the USDA started to import different soybean varieties to use with breeding purposes (USSEC, 2017).

In the late 1920’s the USDA realized of the potential of soybean seed as an oil and protein source as it contains high levels of oil (20%) and protein (40%) (Hartman et al. 2011). When soybean is crushed for oils extraction, a solid part containing mainly protein, carbohydrates, and fibers are leftover, dried and called cake or soybean meal. Around 97% soybean meal in the U.S. goes to animal feed (NCSA, 2019). The animal feed industry consumes 80% as soybean meal due to its high content protein (Shahbandeh, 2020). Soybean oil can be produced into biodiesel, industrial lubricants, solvents and cleaners (NCSA, 2019).

Soybean drew more interest in the U.S. as an oil crop during World War II (1939-1945) due to its great potential as a source of fuel, lubricant and other products (Hymowitz, 1990). After the war, soybean meal, the protein and sugar portion of the crushed seed when the oil is separated, became the preferred livestock feed in the US (USSEC, 2017). The primary usage of soybean changed from green forage to animal seed feed, which led to the acreage increase from 1.56 million acres in 1924 to 80 million acres in 2019 (YCHART, 2020).
Currently, soybean is the fourth highest cash crop globally (Hartman et al., 2011). Soybean production was reported to be 358 million tons in the world (USDA, 2019). The top three producing countries are Brazil (123 million metric tons), U.S. (96 million metric tons) and Argentina (53 million metric tons) (USDA, 2019). Within the U.S., the three leading states are Illinois, Iowa and Minnesota (Figure 1) (USDA, NASSA, 2019). Alabama planted 265,000 acres in 2019. In that year, the U.S. exported 52.3 million metric tons valued at over 18.6 billion dollars with China being the highest importer of soybean (USDA, ERS, 2019).

The impact of high day temperature on soybean

As in other crops, high daytime temperatures affect soybean leaf physiology by reducing chlorophyll content (Djanaguiraman et al. 2011; Djanaguiraman et al. 2013; Bagley et al. 2015; Siebers et al. 2015) and midday and diurnal photosynthesis (Ruiz-Vera et al. 2013; Bagley et al. 2015; Siebers et al. 2015; Thomey et al. 2019) (Fig. 2). It deactivates rubisco activase, the enzyme that activates Rubisco, and therefore maximum rate of Rubisco carboxylation capacity of RuBP ($V_{\text{cmax}}$) and maximum rate of electron transport ($J_{\text{max}}$) are reduced (Bagley et al. 2015; Thomey et al. 2019). To repair the damage caused by high temperature in the different tissues, respiration is observed to increase (Ruiz-Vera et al. 2013; Bagley et al. 2015).

Soybean yield is determined by its pod number, seed number per pod and seed size (Allen et al. 2017). These three yield components are negatively affected when high temperatures occur during the flowering (R2) and seed-filling period (R5) (Egli and Bruening, 2000; Bruening and Egli 2006; Djanaguiraman et al. 2010). Repairing damaged by elevated temperatures lead to more energy consumption and therefore less C is leftover for flowers
decreasing the percent of flowers that transition to pod which decrease yield (Ruiz-Vera et al. 2013). Yield loss of soybean under high day temperature stress has been observed before in field and controlled experiments (Dornbos & Mullen, 1990; Gibson & Mullen, 1996; Salem et al. 2007; Djanaguiraman et al. 2011; Siebers et al. 2015; Choi et al. 2016; Allen et al. 2017; Thomey et al. 2019). Decreased seed and pod numbers are possible causes for yield reduction observed under high temperature stress (Gibson & Mullen, 1996; Choi et al. 2016; Allen et al. 2017). Reductions in number of seed per pod have been associated with an increase in the percentage of barren pods and a decrease of in seed filling rate (Gibson & Mullen, 1996).

In addition, other yield characteristics such as seed quality and germination percentage are found negatively affected by high temperature stress (Gibson and Mullen, 1996; Custodio et al. 2012; Thomey et al. 2019). Custodio et al. (2012) indicated that an increase in temperature by 3 °C in warmer regions decreased the seed size of soybean by decreasing the cell number and seed growth rate. Nutritional value of soybean can also be affected by high temperature. Ren et al. (2009) concluded that sugar concentration decreased under high temperature stress (37/30°C day/night temperature). However, high temperature did not change the total content of proteins, but it changed the relative content of specific proteins (Ren et al. 2009).

Research explored if there is cultivar variance on soybean to high day temperature for the purpose of breeding tolerant soybeans. Alsajri et al. (2019) screened 64 soybean cultivars and found cultivar variability existed to high temperature with differences in shoot and physiological parameters. Canopy temperatures and chlorophyll content measurements demonstrated a tolerant response to high temperature in cultivar ‘45A46’ and ‘CZ5242LL’ (Alsajri et al. 2019). Salem et al. (2007) screened 44 soybean cultivars to determine temperature
effects on soybean pollen germination and found that cultivar ‘DG5630RR’ had higher pollen germination rate and pollen tube length. These cultivars demonstrate that there is variability in the response to high day temperature and therefore we can breed for high day temperature tolerance.

The impact of high night temperature on soybean

In the future, nighttime temperatures are projected to increase faster than daytime temperature (Djanaguiraman et al., 2011). In addition, it is predicted that increase in night temperature will be more accentuated in the South East of United States due to the high humidity and the fact that water can keep longer the heat in the atmosphere (Gaffen and Ross, 1999). However, limited research had been done to explore the impact of high night temperatures on soybean. In studies simulating a heat wave during night, Djanaguiraman et al. (2013) increased night temperature from 30/20°C all the way to 30/30°C during 10 days at full bloom in one cultivar of soybean. In this experiment, yields decreased due to higher respiration rates under high night temperature that led to lower pod set and seed weight (Gibson and Mullen, 1996; Djanaguiraman et al., 2013). This decrease was also associated with a reduction in diurnal photosynthetic activity (Djanaguiraman et al., 2013). However, there is not any scientific report showing different phenotypic response to high night temperature between cultivars. To select and breed for high night temperature tolerance, it needs to be demonstrated that phenotypic variation in physiological characteristics and yield exist. Therefore, one of the objectives is to investigate which physiological traits are affected by high night temperatures, and if there is cultivar variation in the response to high night temperatures.
Although the effect of high night temperature has been studied before in soybean, all the literature has been focused on the effect of a heat wave lasting around 2 weeks in the reproductive stage of soybean (Figure 2) (Gibson and Mullen, 1996; Djanaguiraman et al, 2013). In addition, in some field experiments, the increase of temperature 6°C above the normal ambient temperature for 2 weeks did not affect vegetative or reproductive biomass in corn (Siebers et al., 2017). Heat waves will be more common in the future, however, because of the increase in elevated CO₂ to 450-500 ppm for the middle of the century, and its effect as a greenhouse gas, temperatures will increase steadily and during the whole year between 0.8 to 1.5 °C (IPCC, 2007; Hatfield et al. 2011). This will affect crop growth from germination until maturity, therefore studying the effect of high temperature over the whole growth cycle is paramount to select for high temperature tolerance. The effect of high day temperature in crop development is different as studied at vegetative and reproductive stage (Siebers et al., 2017). The negative effect of high temperatures in vegetative stages seems to be milder than in reproductive stages because in vegetative stages has more time to recover from the stress (Hatfield et al. 2011). However, if the stress continues and accumulates during the reproductive stages, the effect on growth and yield can be very negative (Jumrani et al. 2018). In other study high day temperature decreased more photosynthetic activity during vegetative growth reducing more biomass accumulation at this stage (32%) than during the reproductive stages (15%) (Al-khatib and Paulsen. 1990). The effect of high night temperature over vegetative stages, and whether there is cultivar variation to this stress has not been studied in soybean. If we want to breed for high night temperature tolerant cultivars, we need to know which physiological parameters may make cultivars tolerant to high night temperature. Therefore, one
of the aims of this thesis is to identify the physiological effects of long-term high night temperature stress on soybean during vegetative stages and determine if there is cultivar variation for these traits.

**Objectives**

1. Investigate the effects of different short-term high night temperature applied during the reproductive stage on soybean and study if there is cultivar variation.

2. Identify physiological effects of long-term high night temperature stress during vegetative growth and determine if there are cultivar variation for these traits.
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Figures and Tables Legends

Figure 1. Map of soybean acres planted by county in United States in 2019. USDA.

Figure 2. High temperature effect on soybean. Gibson & Mullen, 1996; Ren et al., 2009; Djanaguiraman et al., 2011; 2013; Vera et al., 2013; Bagley et al., 2015; Choi et al., 2016; Jumrani et al., 2017; Allen et al., 2017.
Figure 1.

Figure 2.

High day temperature

1. Photosynthetic rate, photo-pigment content decrease \(^1\).
2. Membrane of chloroplast and mitochondria get damaged \(^2\).
3. Photosynthetic enzyme activity decreased \(^3\).
4. Respiration, transpiration and stomatal conductance increased \(^4\).
5. Flowering is reduced therefore seed yield gets reduced including seed size, seed number, pod number and seed per pod \(^5\).
6. Seed quality such as protein and sugar concentration decrease \(^6\).

High night temperature

1. Decrease photosynthetic rate, while increase respiration \(^7\).
2. Yield, pod set percentage and seed weight decrease \(^8\).

References:
1. Djanaguiraman et al., 2011; Vera et al., 2013; Bagley et al., 2015
2. Djanaguiraman et al., 2011
3. Bagley et al., 2015
4. Junrani et al., 2017
5. Gibson & Mullen, 1996; Choi et al., 2016; Allen et al., 2017
7. Gibson & Mullen, 1996; Djanaguiraman et al., 2011
8. Gibson & Mullen, 1996; Djanaguiraman et al., 2013
Chapter II: Soybean phenotypic variation to high night temperature stress during full bloom.

Abstract

A 50% increase in crop yield is needed to satisfy demand for growing population in 2050. High day temperature was proved to negatively affect photosynthetic activity then decrease yield. High night temperature is projected to increase faster than day temperature in the Southeast US. Previous research studied the effects of high night temperature which is contradictory and only based on the response of one cultivar at a time. The objectives of this research were: 1) Investigate the effect of different heat wave intensities in soybean during full bloom; 2) Identify physiological traits that relate to high night temperature stress and determine if there are genotype variation for these traits. To accomplish these objectives, four cultivars were exposed to different high night temperatures: extremely high night temperature (EHNT): 32/32 °C; moderate high night temperature (MHNT, 32/26°C); control night temperature (CNT, 32/20°C) at full bloom (R2) for 14 days to explore short time HNT effects on reproductive stage. In this experiment, measurements included: photosynthesis, respiration, chlorophyll content, fluorescence and yield. We found that two weeks EHNT (32°C) did not affect photosynthesis, respiration $V_{\text{cmax}}$, $J_{\text{max}}$ and SPAD in reproductive stage, which may be associated with not surpassing 32-34°C maximum limit of soybean and low light intensity (500 μmol mol$^{-1}$) in this research. Decreased seed production and size was observed in cultivar PI360846, therefore finding yield cultivar variation to EHNT. MHNT (26°C) did not negatively
affect plant growth in any of the cultivars.

**Key words:** High night temperature, Soybean, phenotypic variation, seed size, photosynthesis, respiration, $V_{cmax}$, $J_{max}$ and SPAD, reproductive stages.
Introduction

Crop yield needs to increase by 50% to meet the food demand of 10 billion people in the world by 2050 (FAO, 2017). Plant scientist and agronomist are researching new breeding and management technologies to increase production and yield by targeting important crop traits such as photosynthetic conversion efficiency, lower respiration, and higher nitrogen fixation (Ainsworth et al. 2012; Baslam et al., 2020). However, combined, or isolated abiotic stresses such as heat, drought, and flooding among others, will reduce yields up to 50% by 2050 if new management techniques like precision irrigation and new breeding target for abiotic stress tolerance are not investigated and implemented on time (Karl et al. 2009). Abiotic stresses such as drought can be managed by increasing the number of acres under irrigation. In fact, the South-East region of US (Alabama, Georgia, Florida, South and North Carolina) has been one of the few areas in the US that has increased the irrigation area in the last 20 years to prevent future drought prevalence (NASS, 2010). However, high air temperatures are more difficult to fight with management techniques, only allowing for early planting, midday irrigation or displacement of the crop to cooler regions. Therefore, breeding for crops that are tolerant to high temperatures is paramount to develop a climate resilient agriculture (Driedonks et al., 2016).

Global temperature is predicted to increase at a rate of 0.2°C per decade with the current greenhouse emissions scenario increasing temperatures between 1.5 to 2°C in the next 30 years, and between 3 to 5°C by the end of the century (Tebaldi et al. 2006; Karl et al. 2009). It is also expected that heat waves, periods of surface temperature higher than the maximum mean for a specific location that can last from days to weeks, will increase in its frequency and intensity
with global climate change (Seneviratne et al., 2012). Elevated temperatures negatively affect plant growth and development, damages membrane integrity, disturbs metabolic and physiological functions, resulting in lower yields in different crops (Prasad et al., 2008; Hansen et al., 2019). In addition, heat waves have been observed to decrease crop yield on soybean and corn depending on the developmental stages (Siebers et al., 2017).

Soybean \([\text{Glycine max} \,(L.) \, \text{Merr.}]\) acreage in the US has increased from 0.63 million ha. in 1924 to 3.36 million ha. in 2020 due to the increase demands of animal seed feed (YCHART, 2020). Soybean is the fourth highest cash crop globally and is cultivated worldwide for its high levels of seed oil (20%) and protein (40%) (Hartman et al., 2011). High day temperatures affect soybean physiology by 1) damaging cell, mitochondria and chloroplast membrane accelerating leaf senescence (Djanaguiraman et al., 2011; Jahan et al., 2019); 2) reducing leaf photosynthesis (Djanaguiraman et al., 2011; Ruiz-Vera et al., 2013); and 3) increasing leaf respiration aimed to repair the damaged tissue (Jumrani et al., 2017). Less photosynthesis and higher respiration reduce the photoassimilates derived to reproductive structures and reduce yields (Dornbos & Mullen, 1990; Djanaguiraman et al., 2011; Ruiz-Vera et al., 2013; Thomey et al., 2019). It has been estimated that for every 1°C increase in atmospheric temperature, a 17% of soybean yield will be lost in southern US (Hatfield et al., 2011). High temperature stress tolerance to high day temperatures have been studied and tolerant traits have been introgressed in breeding lines by screening cultivars for high antioxidant capacity (Allen et al., 2017), high chlorophyll content (Alsajri et al., 2019), and high pollen availability (Salem et al., 2007). Although great advances have been produced in the understanding of the effects of high temperature in crops, very little attention has been given to the effect of high night temperature
in the crop physiology and growth response.

An increase in the frequency of warm nights has been observed for the south east US (Karl et al., 2009). For example, the number of very warm nights in Alabama (number of days with minimum temperature at or above 24°C) have been increasing from 1.4 in 1970 to 5.8 in 2015 (NOAA, 2019) and the number of nights as well as the maximum night temperature it is expected to increase in the future (Maleski and Martinez, 2016; NOAA, 2019). High night temperatures have been reported to affect physiological processes during the night, such as respiration, impacting leaf/plant performance over the subsequent diurnal period (Flexas et al., 1999). Yield reduction has been observed in cotton, rice, grapevine under high night temperature stress (Reddy et al., 1997; Loka et al., 2009; Mohammed and Tarpley, 2009; Singh et al., 2019; Tombesi et al., 2019). Soybean produces lower yields under high night temperature stress due to decreased pod set and low seed weight as a consequence of high respiration rates (Djanaguiraman et al., 2013) and a reduction in the photosynthetic activity during the day (Djanaguiraman et al., 2013). However, other research showed that yield was not affected by high night temperature after longer exposure time (Gibson & Mullen, 1996; Zheng et al., 2002).

All these soybean research only studied one soybean cultivar at a time under high night temperature stress from reproductive stages for different exposure period. In the existing literature there is no research exploring several soybean cultivars responses to different high night temperature stresses in the same experiment.

Different soybean cultivars need to be screened for high night temperature to select tolerance phenotypes in physiological characteristics. With this goal, the objectives of this research were: 1) investigate the physiological effects of different high night temperatures
treatments on soybean during a simulated heatwave during the flowering period; 2) investigate if there is phenotypic variation to high night temperature due to genotype.
Materials and Methods

This research was conducted in the greenhouse and growth chamber facility at the Plant Science Research Center at Auburn University, AL, USA from May 2019 to October 2019 and from May 2020 to September 2020. Four soybean genotypes PI360846, DS25-1, PI458098, and AG48x9 were selected for this experiment based on the tolerance or sensitiveness to high daytime temperature (Table 1.).

The 3.8-L pots containing 1.75kg potting mix (Pro-Mix BX) were used to grow soybean plants. Soybean seeds were treated with fungicide (Evergol Energy, Bayer Crop Science). This experiment was repeated three times for each night temperature treatment. In each experiment, each cultivar was sowed in ten pots with forty pots in total. Three seeds per pot were sown at the depth of 2.54 cm, after emergence, approximately at V0 developmental stage plants were thinned to 1. The growing medium was fertilized with Osmocote at 3 g pot⁻¹ (a controlled release plant fertilizer (AM Leonard), 14:14:14% N: P₂O₅: K₂O, respectively) at sowing. Systemic insecticide Marathon1% G (imidacloprid, Auburn, AL) was applied once a week after the plant reached V2 developmental stage to control greenhouse pests. Plants were well-watered to avoid water stress. Peat-Lite Special (Liquid fertilizer (Scotts-Sierra), 20:10:20% N: P₂O₅: K₂O, respectively) was applied 190-ML per pot weekly after seedling emergence to supplement nutrition.

Soybean plants were grown in the greenhouse at a temperature regime of approximately 30/20 °C day/night with at least a 14-h photoperiod and a photosynthetic photon flux density (PPFD) of 800-1700 μmol m⁻² s⁻¹ provided from natural solar radiation and supplemental LED
lights. Among the ten pots of each cultivar, the 6 best were chosen for the growth chamber experiment. At full bloom (growth stage R2), the plants were divided into two groups: one group was exposed to control night temperature and the other was exposed to high night temperature in two growth chambers. Two indoor growth chambers (Conviron Adaptis CMP 6010, Canada) were used for temperature treatments. There were 12 pots per growth chamber. The control chamber was maintained at control temperature (32/20°C, day/night), and the other chamber was maintained at moderate high night temperature (MHNT, 32/26°C, day/night) for 14 days. In the other set of experiments the high night temperature chamber was maintained at extremely high night temperature (EHNT, 32/32°C, day/night). The daytime and nighttime temperature regimes were imposed from 07:00 and 21:00 h, respectively, with a 30-min transition period between the daytime maximum and nighttime minimum temperatures and vice versa. The photoperiod was 14 h, and the PPFD was provided by cool fluorescent lamps with 500 μmol m$^{-2}$ s$^{-1}$ at the top of the plant canopy. The relative humidity in the chambers was 60% at day and 70% at night. Pots in the growth chamber were randomly placed and rotated every 3 days. Each temperature treatment had three replicated pots. The plants were placed in the chambers for two weeks for their respective temperature treatments. After the treatments, the plants were returned to the greenhouse and maintained at the greenhouse conditions described above until full maturity (R8). Physiological measurements were taken at 7 and 14 days from the start of temperature treatments. All the plants were destructively harvested at R8 and separated into stems, leaves and pods. All harvested organs were oven dried for at least 72h at 60°C to later determine dry weight. Pod numbers, total seed weight (g plant$^{-1}$), weight of seed (g seed$^{-1}$), aboveground biomass (g plants$^{-1}$), HI (ratio between seed
weight and aboveground biomass) were calculated.

**Midday photosynthesis and respiration**

The gas exchange measurements were conducted with at least two portable gas-exchange system (LI-6400 and LI-6800, LI-COR, Lincoln, NE, USA). Midday photosynthesis was measured at midday between 11:00-13:00h on an attached leaflet of the third trifoliate leaf from the main-stem apex at 7, and 14 days from the start of high temperature treatment. The gas exchange system was set to a [CO$_2$] of 410 μmol mol$^{-1}$, and PPFD of 500 μmol m$^{-2}$ s$^{-1}$ to calculate maximum midday photosynthesis measurement. Respiration was measured the same day and on the same leaf of midday photosynthesis measurements but at night (22:00-24:00h). During the night respiration measurement leaf chamber [CO$_2$] was set at 410 μmol mol$^{-1}$, and PPFD of 0 μmol m$^{-2}$ s$^{-1}$.

**A/Ci curves**

A/Ci curves is defined as the CO$_2$ assimilation rate (A) at several intercellular CO$_2$ concentrations (Ci) (Bernacchi et al., 2001) used to estimate different leaf biochemical parameters such as maximum rate of Rubisco carboxylation capacity ($V_{cmax}$) and the maximum rate of RuBP regeneration capacity ($J_{max}$). Measurements were taken at 7 and 14 days from the start of high temperature stress treatment. The A-Ci curves were taken when the leaf biochemical machinery was still active between 7:00 to 13:00h (Ainsworth et al., 2007) and with a saturating PPFD of 1500 μmol m$^{-2}$ s$^{-1}$. The [CO$_2$] in the leaf chamber concentration was set to 410, 260, 160, 110, 50, 410, 610, 810, 1110, 1310 and 1510 μmol mol$^{-1}$μmol m$^{-2}$ s$^{-1}$. 
Maximum rate of Rubisco carboxylation capacity of RuBP ($V_{cmax}$) and the maximum rate of electron transport ($J_{max}$) was acquired by analyzing A/Ci curves using the fitting models developed by Sharkey et al. (2007).

**Chlorophyll content estimation**

The chlorophyll content was estimated using a self-calibrating chlorophyll meter (SPAD 502, Spectrum Technologies, Plainfield, IL) at 7 and 14 days from the start of high temperature treatments.

**Data Analysis**

Cultivars and temperature treatments were used as main sources of variance and were considered as fixed effects to quantify the influence of temperature treatments on the physiological growth of four soybean cultivars at flowering stage (R2). The three subreplicates from each chamber experiment were pulled together for a total of 9 replicates per treatment. Statistical analysis was performed using post ANOVA means separation that was determined using Fisher’s least significant difference ($\alpha=0.05$) to compare the effect of temperature in each cultivar. Sigma-Plot 14 (Systat Software, Inc., San Jose, CA) was used to generate figures.
Results

Leaf photosynthesis and respiration

Leaf gas exchange measurements were taken two times during high night temperature treatment: at 7 days and 14 days from the start of high night temperature treatment according to similar research performed by Djanaguiraman et al. (2013). Mid-day photosynthesis at 7 days after treatment started (DAT) showed significant cultivar, temperature effect and the interaction of temperature by cultivar (Table 2). In comparison with control night temperature (CNT), moderate high night temperature (MHT) treatment increased midday photosynthesis at 7 DAT by 56.6% and 38.64% on Cultivar PI360846 and AG48x9, respectively (Fig. 1a). However, no differences in 7 DAT midday photosynthesis were found between extreme high night temperature (EHNT) and CNT treatment for all cultivars. Considering the effect of temperature treatment in the means of all cultivars, MHNT showed significant increase in photosynthesis by comparing to EHNT at 7 DAT (Fig. 1). When analyzing the data at 14 DAT, no differences were observed between cultivars and/or temperature treatment, however MHNT treatment showed a slight but not significant increase in photosynthesis in PI360846, DS25-1, and PI450898 (Fig. 1b). When comparing stomatal conductance at 7 DAT, the effects of temperature was significant (Table 2). MHNT increased 65.8%, 85.64% and 57.8% leaf stomatal conductance of cultivar PI360846, PI458098 and AG48x9, respectively by comparing with CNT treatment (Fig. 1c). Considering the overall effect of temperature on the mean of all cultivars, EHNT decreased stomatal conductance in comparison with CNT and MHNT (Fig. 1c). Temperature was observed to have significant effect on 14 DAT stomatal conductance. When the results of all cultivars were averaged by temperature treatment, stomatal conductance under MHNT and EHNT showed significant lower stomatal conductance than CNT. However,
when the effect of temperature was considered by individual cultivar, MHNT increased stomatal conductance by 23.8% and 46.7% respectively in cultivar DS25-1 and PI458098 (Fig. 1d). No differences were found in 7 DAT respiration (Fig. 1e), while MHNT significantly increased respiration at 14 DAT in comparison with CNT (Fig. 1f).

**A/Ci curves and Chlorophyll content**

A/Ci curves and Chlorophyll content (SPAD value) measurements were taken 2 times at 7 and 14 days from the start of treatment. Maximum rate of Rubisco carboxylation capacity ($V_{c_{max}}$) and the maximum rate of RuBP regeneration capacity ($J_{max}$) were acquired by analyzing A/Ci curves (Fig. 2). Significant temperature effect was observed in $V_{c_{max}}$ at 7 DAT but not at 14 DAT (Table 2). MHNT significantly decreased $V_{c_{max}}$ in the mean of all cultivars, but cultivar PI360846 was not affected (Fig. 2a). EHNT did not affect $V_{c_{max}}$ in all cultivars except for PI360846 that showed a 31.0% increase in $V_{c_{max}}$ (Fig. 2a). At 14 DAT measurement the effect of temperature by cultivar interaction was significant in $V_{c_{max}}$. Cultivar PI360846 showed a 34.6% and 85.6% increase in $V_{c_{max}}$ in MHNT and EHNT respectively by comparing with CNT on cultivar PI360846 (Fig. 2b). No temperature effect was observed in the mean of all cultivars at 14 DAT. Only cultivar PI360846 showed increased $V_{c_{max}}$ under EHNT treatment in both 7 and 14 DAT. Significant effect of temperature and cultivar were observed in 7 DAT $J_{max}$ but not at 14 DAT (Table 2). At 7 DAT, MHNT significantly decreased $J_{max}$ in comparison with CNT for the mean of all cultivars (Fig. 2c). No effect of temperature was found in $J_{max}$ at 14 DAT (Table 2). In the other hand, only considering temperature effect within cultivar AG48x9, it showed 18.8% and 33.1% significant decrease in $J_{max}$ at MHNT and EHNT respectively at 14
Temperature was not observed to have effect on SPAD value in both 7 and 14 DAT measurements (Table 2). However, Significant temperature and cultivar interaction was found in 14 DAT (Table 2). PI360856 and PI450846 did not showed to be affected by MHNT or EHNT. However, MHNT and EHNT decreased SPAD values in DS25-1, and MHNT decreased SPAD values in AG48x9. No temperature effect was observed from mean of all cultivars at both 7 and 14 days (Fig. 2ef).

Leaf temperature

Leaf temperature (Tleaf) was measured at 7 and 14 days from the start of treatment. Significant temperature effect was observed in both 7 and 14 DAT for daytime Tleaf (Table 2). In the other hand, there was not cultivar or temperature by cultivar effect on any of the measurements. EHNT during the day increased daytime Tleaf in all cultivars at 7 DAT when comparing with CNT (Fig. 3a) as mean of all cultivars showed significant increase. Effect of EHNT at 14 DAT was not registered because of a logging error due to a LICOR-6400 malfunctioning. Significant effect of temperature was observed at both 7 and 14 DAT in leaf night temperature (Table 2). Mean of all cultivars under MHNT and EHNT showed significant increase in nighttime Tleaf by comparing with CNT at both 7 and 14 DAT. EHNT increased Tleaf by 33.41%, 30.55%, 32.43%, and 34.98% in cultivar PI360846, DS25-1, PI458098 and AG48x9 respectively at 7 DAT by comparing with CNT. EHNT also increased Tleaf in all cultivars at 14 DAT. 17.93%, 16.93%, 16.69%, and 20.44% increase were observed in cultivar PI360846, DS25-1, PI458098 and AG48x9 respectively at nighttime 14 DAT (Fig. 3d).
Yield and Biomass

Biomass sampling was conducted at full maturity. Parameters including pod number, pod weight, stem weight, seed number, total seed weight, weight per seed, aboveground biomass, and Harvest Index (HI) were collected after harvesting. Slight temperature effect was observed in total seed weight (p=0.0787) (Fig. 4a). EHNT decreased total seed weight by 42.9% (significant decrease), 34.5% and 15.3% on cultivar PI360846, DS25-1 and AG48x9, respectively (Fig. 4a) and did not change for PI458098. Considering the effect of temperature over the mean of all cultivars, MHNT showed increased total seed weight in comparison with EHNT but not with CNT (Fig. 4a). Significant effect of temperature, cultivar and temperature by cultivar interaction were observed on individual seed weight. In cultivar PI360846, EHNT decreased weight per seed by 20.5% compared with CNT while MHNT increased 33.3% compared with CNT (Fig. 4b). No differences in individual seed weight were found in MHNT and EHNT compared with CNT on the other three cultivars. No significant temperature or temperature by cultivar interaction effect were observed in pod number, pod weight, seed number, aboveground-biomass and harvest index (Table.2).
Discussion

Crop response to temperature depends on the specific optimum temperature for photosynthesis, growth, respiration and yield (Conroy et al., 1994). If the temperature is below optimum, an increase in temperature can increase photosynthesis and plant growth, but if temperature is close to the maximum, a small increase in temperature can decrease photosynthesis, increase respiration and then reduce yield (Baker & Allen, 1993). The maximum leaf temperature for maintaining photosynthesis in soybean is thought to be around 32-34°C (Thomey et al., 2019), however the maximum to maintain respiration at night is unknown. The effect of high night temperature heat waves on soybean physiological (Photosynthesis, respiration, etc.) and yield parameters are poorly understood. There are contradictory reports that vary between the lack of effect to negative effects of high night temperatures to very negative effects (Gibson & Mullen, 1996; Djanaguiraman et al., 2013; Lin et al., 2020). In addition, none of these studies have been focused on finding cultivar variation to this stress. In the present study we investigated the effect of two high night temperatures, 32/26°C (Moderate high night temperature, MHNT) and 32/32°C (Extremely high night temperature, EHNT), over yield and physiological parameters of 4 soybean cultivars selected for its day tolerance and sensitiveness to high temperature.

In our study, MHNT increased the photosynthesis and stomatal conductance in 2 of 4 cultivars at 7 DAT. Transient increases in photosynthesis at high day temperatures have been reported before in field (Thomey et al., 2019) and controlled environments (Sanz-Saez et al., 2013) when the optimum leaf temperature for photosynthesis is not surpassed. In soybean temperature above 32-34°C of leaf/canopy day temperatures start to decrease day
photosynthesis (Jumrani et al., 2017; Thomey et al., 2019). Our findings agree with them (Fig. 1). Under these conditions, photosynthesis seems to be stimulated by the opening of the stomata, and when the stomatal conductance is reduced after 14 days of treatment, the midday photosynthesis decreased again as it can be observed in Fig. 1cd. Our data is supported by the observations of Jumrani et al. (2017) that found that when water is available high day and night temperate tend to increase transpiration of the plant by increasing the number of stomata and stomatal conductance to cool down the leaf surface and not incur in high temperature damage. However, when the high temperatures are maintained over longer periods of time this positive effect disappears (Fig. 1d), and if the high temperatures continue the photosynthetic system can be affected negatively (Jumrani et al., 2017; Thomey et al., 2019); event that was not observed in our experiment as the stress only lasted 14 days. The positive effect of MHNT over photosynthesis could be observed in a non-significant increase of 17.8%, 114.6% and 44.1% on total seed weight on cultivars PI360846, PI458098, and commercial cultivar AG48x9. Gibson & Mullen (1996) showed a slight increase of photosynthesis when night temperature was increased from 20 to 30℃ from R1 to R5 developmental stage and caused a significant increase in total seed weight per plant. Their data agrees with our study when it is compared with the MHNT (32/26℃) treatment but not with the EHNT (32/26℃) where we observed decreased on yield on one cultivar.

In the EHNT treatment (32/32℃) we were expecting negative effects on physiological traits as other field (Thomey et al., 2019) and growth chamber (Djanaguiraman et al., 2013) experiments have shown before when the night temperature is increased 10 ℃ over the control temperature. In our study, EHNT did not decrease midday photosynthesis or increase
respiration rate at night as it was observed by Djanaguiraman et al. (2013) when growing soybean at night temperature treatments of 30/30 °C. Djanaguiraman et al. (2013) observed an increased respiration rate and decreased photosynthetic rate after 10 days high night temperature treatment due to thylakoid membrane damage combined with decreased rubisco activase induced by high night temperature. In a canopy photosynthesis study performed by Frantz et al. (2004) testing more than 6 high night temperatures, only temperatures above 26 °C increased soybean respiration. However, these decreases did not reduce plant photosynthesis during the day or biomass accumulation. Therefore, it seems that the effect of high night temperature in photosynthesis, respiration and other parameters are very dependent on other environmental conditions.

Effects of elevated temperature over photosynthesis and photosystem activity seems to be more evident when light intensity is higher (Gao et al., 2019). High temperature, even during the night, damages chloroplast membranes where the PSI and PSII are placed (Djanaguiraman et al., 2013). At higher light intensity, plants are going to need the photosystems that are damaged by the high temperature and as the plant is unable to absorb that light is going to suffer more damage from oxygen radical species and is going to reduce photosynthesis (Gao et al., 2019). We think that is what is happening in the research performed by Djanaguiraman et al. (2013) because their growth chambers plants were grown at higher light intensity (720 μmol mol⁻¹ PAR) meanwhile in our research the growth chamber reached 500 μmol mol⁻¹ PAR. This would also explain why Gibson & Mullen (1996) did not observed any decrease in photosynthesis with increasing temperatures as their growth chambers were set to 350 μmol mol⁻¹ PAR. For these reasons we believe that if more research is performed to study the effects
of high night or day temperature under growth chamber conditions, light intensity needs to be increased to reach soybean’s light saturation limit of 1750 µmol mol⁻¹ PAR to see a more significant effect and to be close to what plants are going to receive in the field during a growing season (Sanz-Saez et al., 2017).

In previous research, the maximum rate of Rubisco carboxylation capacity ($V_{cmax}$) and the maximum rate of electron transport ($J_{max}$) decreased under high day temperature when the leaf or canopy temperature surpass the optimal limit (32-34 °C, Bagley et al., 2015; Thomey et al., 2019). In our study, $J_{max}$ was almost unaltered by EHNT, and $V_{cmax}$ was unaffected for all cultivars but PI360846 that increased $V_{cmax}$ at EHNT in both timepoints. As the leaf temperature measurement did not surpass the 32-34 °C limit we did not expect to see any sign of damage in this parameter as it has been observed before in a soybean field study (Thomey et al., 2019). Other authors have noticed that increased temperatures that do not surpass the limit for that species usually result in higher $V_{cmax}$ values (Sanz-Saez et al., 2013). However, short time high temperature (day or night) effect on physiological parameters is also inconsistent in the literature. Lin et al. (2020) did not observe significant differences in photosynthesis during first 2 weeks HNT (28 °C) treatment on soybean. The reason can be that inhibition of leaf growth from HNT was offset by rapid leaf expansion in the early developmental stages such as flowering. Short time high temperature stress was also reported to not affect physiological parameters like photosynthesis and $J_{max}$ on soybean (Siebers et al., 2015). No significant differences in photosynthesis and $J_{max}$ from their research indicated that short time HNT was sufficient to induce strong oxidative stress, and quick recovery was observed during early developmental stages.
Although the effect of high night temperature or cultivar variation was not very evident over physiological parameters; we observed a 40% and a 20% reduction in yield and weight of individual seed respectively in the cultivar PI360846 under EHNT but not for the other cultivars (Fig. 4). This data corroborated the existence of cultivar variation to high nigh temperature and opens the opportunity to breed for increased tolerance to high night temperature. Decreased yields under EHNT (30/30 °C) has been reported by Djanaguiraman et al. (2013) which were accompanied by decreased flower success, pod number, and seed size. Our data showed decreased yield and seed size but was not accompanied by a decrease in pod number. This would suggest that although EHNT was applied during full bloom and beginning of pods, it did not affect pod development, but decreased seed filling even after the EHNT was over, resulting in smaller seeds. Siebers et al., (2015) also reported that the effects of a heat wave can be observed by the decrease in total seed weight and the weight of individual seeds even if the stress was suffered earlier in the growing season. The decrease on seed size but not in pod number is most probably due to a reduce sugar flow to the pods that is not low enough to “abort” that pod but that reduce the size of the seed.

Conclusions

From this experiment, we did not observe negative effects of EHNT (32/32°C) on photosynthesis, respiration $V_{\text{cmax}}$, $J_{\text{max}}$ and SPAD in 14 days treatment, probably because leaf temperature did not surpass the 32-34°C maximum limit. In addition, the low light intensity of our chambers (only 500 μmol mol$^{-1}$) may have reduced the negative effect of EHNT. Therefore, no significant damage which can translate into reduction on photosynthesis was observed in
our study. Due to contradictory result in the literature regarding physiological effect of high night temperature, experiments need to be performed in growth chambers with high light intensity (1500-2000 PAR) as they would intensify the effect of high temperature and are more in accordance with the reality of the field in summer.

In this experiment, we observed a 40% and a 20% reduction in yield and weight of individual seed in the cultivar PI360846 under EHNT. Decreased yield and seed size in the cultivar PI360846 proved the sensitivity of this cultivar to EHNT and the existence of cultivar variation to high night temperature in soybean and can be the first step to screen and explore high night temperature tolerant characteristics in soybean.
References


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**Figures and Tables Legends**

**Figure 1.** Physiological parameters measured at 7 days and 14 days after temperature treatment started: Midday photosynthesis (a,b) (CO\(_2\) µmol m\(^{-2}\) s\(^{-1}\)), Stomatal conductance (c,d) (H\(_2\)O mol m\(^{-2}\) s\(^{-1}\)), Respiration (e,f) (CO\(_2\) µmol m\(^{-2}\) s\(^{-1}\)) in four soybean cultivars under control (32/20°C), moderate high (MHNT) (32/26°C) and extreme high night temperatures (EHNT) (32/32°C). Each data point shows the average nine replicates, three per block. Blue symbols show the mean of the 4 cultivars in each temperature treatment. Different letters in the mean of 4 cultivars indicate significant differences between temperature treatments (p ≤ 0.05).

**Figure 2.** Physiological parameters at 7 and 14 days after temperature treatment started: Maximum rubisco carboxylation (V\(_{\text{cmax}}\): a,b) (µmol m\(^{-2}\) s\(^{-1}\)), maximum electron transport rate (J\(_{\text{max}}\): c,d) (µmol m\(^{-2}\) s\(^{-1}\)), and estimation of chlorophyll content-SPAD (e,f) in four soybean cultivars under control (32/20°C), moderate high (MHNT) (32/26°C) and extreme high night temperatures (EHNT) (32/32°C). Each data point shows the average nine replicates, three per block. Blue symbols show the mean of the 4 cultivars in each temperature treatment. Different letters in the mean of 4 cultivars indicate significant differences between temperature treatments (p ≤ 0.05).

**Figure 3.** Day and night leaf temperature (T\(_{\text{leaf}}\), °C) measured by the LI-6400 at 7 and 14 days after temperature treatment started in four soybean cultivars under control (32/20°C), moderate high (MHNT) (32/26°C) and extreme high night temperatures (EHNT) (32/32°C). Each data point shows the average nine replicates, three per block. Blue symbols show the mean of the 4
cultivars in each temperature treatment. Different letters in the mean of 4 cultivars indicate significant differences between temperature treatments (p ≤ 0.05).

Figure 4. Physiological parameters at maturity (R8): a) Total seed weight per plant (g plant⁻¹), b) Weight per seed (g seed⁻¹) in four soybean cultivars under control (32/20°C), moderate high (MHNT) (32/26°C) and extreme high night temperatures (EHNT) (32/32°C). Each data point shows the average nine replicates, three per block. Blue symbols show the mean of the 4 cultivars in each temperature treatment. Different letters in the mean of 4 cultivars indicate significant differences between temperature treatments (p ≤ 0.05).

Table 1. Information about the four soybean cultivars used in experiment including maturity group, growth habit, high day temperature tolerance or sensitiveness and origin (source) of the seed.

Table 2. ANOVA P-value results (two-way ANOVA, including block as a fixed factor) for midday photosynthesis (CO₂ µmol m⁻² s⁻¹), stomatal conductance (H₂O mol m⁻² s⁻¹), respiration (CO₂ µmol m⁻² s⁻¹), maximum rubisco carboxylation (Vcmax, µmol m⁻² s⁻¹), maximum electron transport rate (Jmax, µmol m⁻² s⁻¹), estimation of chlorophyll content-SPAD, and day and night leaf temperature (°C) all measured at 7 and 14 days after the temperature treatments started.

Table 3. ANOVA P-value results (two-way ANOVA, including block as a fixed factor) for
total seed weight per plant (g plant\(^{-1}\)) and weight per seed (g seed\(^{-1}\)) at 7 and 14 days after temperature treatment started.

**Table 4.** Physiological parameters at maturity (R8): Pod number, Pod weight (g plant\(^{-1}\)), Seed number, Aboveground biomass (g plant\(^{-1}\)), Harvest Index (HI) in four soybean cultivars under control (32/20°C), moderate high (MHNT) (32/26°C) and extreme high night temperatures (EHNT) (32/32°C). Data presented are means of all replicates. Results of statistics are shown (two-way ANOVA, P < 0.05). Letters indicate significant differences between different temperature treatments within each cultivar (Tukey post hoc test P<0.05).
Fig. 1

7 days after treatment

14 days after treatment
Fig. 3

7 days after treatment

14 days after treatment
Fig. 4

Total seed weight

Individual seed weight
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Chapter III: Soybean phenotypic variation to long-term high night temperature stress during vegetative growth

Abstract

Global warming is inducing more frequent abiotic stress impeding the increase of crop yield. Overall mean temperatures are expected to increase in the following decades. In the Southeast US due to the high humidity in the environment in Summer it is expected that night temperature is going to increase at a higher rate than day temperature. Short-time high night temperature has been proved to negatively affect crop yield. Long-term high night temperature effect during vegetative growth in soybean has not been explored. The objectives of this research are: 1) Investigate the physiological effects of high night temperatures on soybean growth from germination to flowering; 2) Identify if phenotypic variation of response to long-term high night temperature on soybean is due to genotype differences. To accomplish these objectives, nine soybean cultivars were exposed to extreme high night temperature (EHNT, 30°C/30°C day/night) from germination to flowering to study the effect on physiological parameters and vegetative growth. In this experiment, measurements included: photosynthesis, respiration, chlorophyll content and fluorescence. Long-term EHNT during vegetative stages under high light intensity (1750 μmol m-2 s-1) decreased photosynthetic rate, stomatal conductance, chlorophyll content and the efficiency of PSII (Fv/Fm) but not changing respiration rate. Cultivar variation was observed from decreased these parameters. Some cultivars showed tolerance to EHNT as null effect over the aboveground biomass. In cultivar S14-15146R and DS25-1, EHNT tolerance may be associated with photochemical efficiency.
of PSII ($F_v/F_m$). More research is needed to investigate more traits that may be related to high
night temperature tolerance.

**Key words:** High night temperature, soybean, phenotypic variation, $F_v/F_m$, photosynthesis,
vegetative growth.
Introduction

Based on current climate change scenarios, temperature patterns across the U.S show a warming tendency of 1.5 to 2 °C for the next 30 years, with only a slight increase in precipitation (Tebaldi et al., 2006; Karl et al., 2009). The IPCC (2019) predicts that human induced global warming is currently increasing the global temperature at a rate of 0.2°C per decade with the current greenhouse emissions. Historically, a number of studies have focused on exploring the short-term effect of high temperatures on crops as a heat wave. Heat wave was primarily imposed on reproductive stages of soybean (Djanaguiraman et al., 2011; Vera et al., 2013; Bagley et al., 2015); pigeon pea (Guapta, 2019); mung bean (Hanif and Wahid, 2018); rice (Sailaja et al., 2015); wheat (Aiqing et al., 2018; Hansen et al., 2019); hemp (Chandra et al., 2011) and tomato (Jahan et al., 2019) for few weeks and observed decreased photosynthetic rate, chlorophyll content, and then yield. Elevated temperature has a negative significant effect on yield when applied during reproductive stages because disrupts biomass accumulation, pollination, and kernel set (Dupuis and Dumas, 1990; Commuri and Jones, 2001). The effects of high temperature on vegetative stages have been less studied, as it is thought to be less sensitive to high temperatures than reproductive stages (Hatfield et al., 2011). For example, a heat wave of +6°C applied during grain filling decreased yield significantly; but when a heat wave was applied during the vegetative stages, the plant recovered and no yield loss was observed (Siebers et al., 2017). However, global warming is increasing the atmospheric temperatures across the crop’s growing season affecting the growth of crops as a whole and not at a specific growth stages (Karl et al., 2009).
Climate change is altering temperature, CO₂ concentration, and precipitation, which will affect plant growth during the whole growing season presenting a potential long-term challenge to crop production (Hatfield et al., 2011). These stresses may affect vegetative and reproductive stages differently, but the effects on plant growth needs to be considered over the whole growing season. For example, elevated CO₂ tends to increase photosynthesis in alfalfa, but when the exposure is prolonged photosynthesis is down-regulated and the effect is not as pronounced as predicted by the short-term CO₂ response (Sanz-Saez et al., 2010). Ruiz-Vera et al. (2013) showed in a long-term experiment that increasing 3.5°C the ambient temperature resulted in a decreased photosynthesis during vegetative and reproductive stages reducing total above ground biomass accumulation and yield.

Soybean [*Glycine max* (L.) Merr.] acreage in the US has increased from 1.56 million acres in 1924 to 83.1 million acres in 2020 to meet increasing demands of animal seed feed (YCHART, 2020). The CROPGRO model predicted the highest grain yield of soybean at 23 to 24°C, with progressive decline in yield, seed size, and harvest index (HI) when temperature increases above this optimum range and dropping to zero at 39 ºC (Boote et al., 1997; 1998). By considering observed and projected data of increased temperature in the southeast US, warming of night-time temperature will be more pronounced than during the day (Alwad et al., 1999). Prior research studying short-time high night temperatures were reported to affect physiological processes during the night impacting leaf/plant performance over the subsequent diurnal period (Flexas et al., 1999). Increased respiration with yield reduction was observed in cotton (Reddy et al., 1997; Loka et al., 2009), rice (Mohammed and Tarpley, 2009; Singh et al., 2018), and grapevine [*Vitis vinifera* L.] (Frioni et al., 2018). Elevated short-term night
temperature negatively affected daytime photosynthetic performance; as $V_{\text{cmax}}$ and $J_{\text{max}}$ was reduced, possibly due to a deactivation of photosynthetic enzymes (Singh et al., 2018). There are few comprehensive experiments researching long-term effect of high night temperature on soybean. Lin et al. (2020) observed decreased biomass accumulation after a long-term high night temperature treatment during reproductive stages, which produced an inhibition of leaf growth by high night temperature. Research exploring possible characteristics responsible for long-term high temperature tolerance are deficient. To select and breed for high night temperature tolerance, phenotypic variation in physiological characteristics and yield needs to be demonstrated. With this goal, the objectives of this research were: 1) Investigate the physiological effects of high night temperatures on soybean growth from germination to flowering; 2) Identify if there is phenotypic variation of response to long-term high night temperature on soybean is due to genotype differences. To accomplish these objectives, we conducted a growth chamber experiment that grew nine soybean cultivars under high night temperature conditions (Control: 30/20 ºC; Extremely high night temperature 30/30 ºC day/night temperature) from sowing to flowering. Physiological measurements such as midday photosynthesis, respiration and fluorescence were taken at 30 and 50 days after planting (DAP) to study long-term high night temperature effect.
Material and Methods

This experiment was conducted at Delta Research and Extension Center, MS, USA from June 2020 to November 2020. Two indoor growth chambers (Biochambers TPRB-73, Canada) were used to apply two different temperature treatments. Nine soybean cultivars were selected for this research of the maturity group IV and V (Table. 1).

Four seeds of each cultivar were sown in 180 PVC pots (6” diameter by 18” height, 8.3 liters) that was filled with 1:1 of soil (silt loam) and sand. The pots were arranged in a completely randomized block design with each cultivar arranged randomly within each chamber for a total of 10 replications per chamber, 5 replications per biomass harvest at 30 and 50 days after planting (DAP). Ninety pots were arranged in each growth chamber before sowing. Beginning at sowing, the pots were irrigated three times a day (08:00, 12:00, and 17:00 h) with standard Hoagland’s nutrient solution through an automated drip irrigation system at the rate of 50 ml min\(^{-1}\) for 90 s per irrigation time. All the pots were then thinned to one plant per pot after emergence. Two temperature treatments were applied from the beginning of planting: Extreme high night temperature (EHNT, 30/30\(^{\circ}\)C day/night); control temperature (CNT, 30/20\(^{\circ}\)C day/night). The daytime and nighttime temperature regimes were imposed from 06:00 and 20:00 h, respectively, with a 30-min transition period between the daytime maximum and nighttime minimum temperatures and vice versa. The photoperiod was 14 h, and the PPFD was provided by cool fluorescent lamps with 1750 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) at the top of the plant canopy to simulate with a plant will receive during the summer growing season and coinciding with light saturating conditions for soybean (Sanz-Saez et al., 2017). The relative humidity in the chambers was 60% at both day and night. Physiological and biomass measurements were taken
at 30 and 50 days after planting (DAP) in 5 replications per cultivar and treatment. All the plants were destructively sampled at 30 and 50 DAP separating the organs in leaves, stems, and pods when they were enough developed (more than 3mm). Leaves were passed through a leaf area meter (LI-2000, LICOR Biosciences) to calculate plant’s total leaf area. All harvested organs were oven dried for at least 72h at 60°C to later determine dry weight. Aboveground biomass was calculated as the accumulated weight of all organs (g plants⁻¹).

_Midday photosynthesis and respiration_

The gas exchange measurements were conducted with 4 sets of portable gas-exchange systems (LI-6800, LI-COR, Lincoln, NE, USA) with a Multiphase Flash™ Fluorometer (6800-01 A). Five pots per cultivar were randomly selected for taking measurements. Midday photosynthesis was measured on the uppermost, fully expanded, mainstem, trifoliate leaf (second unfurled leaf node below the apical meristem) at 30 and 50 days after planting (between 11:00-13:00h). LI-COR leaf chamber [CO₂] was set at 410 μmol mol⁻¹ simulating ambient conditions, and PPFD of 1750 μmol m⁻² s⁻¹ to calculate maximum midday photosynthesis measurement. Respiration was measured the same day and on the same leaf of midday photosynthesis measurements but at night (22:00-24:00h). During the night respiration measurement leaf chamber [CO₂] was set at 400 μmol mol⁻¹, and PPFD of 0 μmol m⁻² s⁻¹.

_Chlorophyll content estimation_
The chlorophyll content was estimated using a self-calibrating chlorophyll meter (SPAD 502, Spectrum Technologies, Plainfield, IL) at 30 and 50 days from the start of high temperature treatments.

Chlorophyll fluorescence

The maximum quantum yield of photosystem II in a dark-adapted state (Fv/Fm), quantum yield of PSII calculated from fluorescence (PhiPS2), and photo synthetic electron transport rate (ETR) were measured at 30 and 50 DAP at night by using LI-COR 6800.

Data Analysis

Considering two growth chambers have the same growth conditions, except night temperature, this experiment was treated as a completely randomized design for statistical analysis purposes. The effect of cultivar, night temperature, and the interaction between cultivar and temperature were considered fix factors and assessed using a full factorial two-way analysis of variance (ANOVA). Observed and derived parameters were analyzed using the ANOVA procedure in SAS Pro 12.0 (SAS Institute, Cary, NC, USA). Post ANOVA means separation was determined using Fisher’s least significant difference ($\alpha=0.05$).
Results

Leaf physiological parameters

Leaf gas exchange measurements (photosynthesis and respiration) were made at 30 and 50 days after planting (DAP). EHNT significantly decreased midday photosynthesis at 30 and 50 DAT for the overall mean of cultivars (Table 2). At 30 DAP, extremely high night temperature (EHNT) significantly decreased photosynthesis by 58.27%, 30.69%, and 45.24% in cultivar S15-17812C, LG01-55875 and DS25-1. At 50 DAP, coinciding with flowering, EHNT significantly decreased photosynthesis in 8 cultivars but not affecting cultivar S15-17812C (Table 2). Significant temperature effect was observed at both 30 and 50 DAP on stomatal conductance (Table 2). EHNT tended to decrease stomatal conductance at 30 DAP in the overall mean of cultivars when compared with the control treatment, and significantly on cultivars LG01-550875, R14-35B and DS25-1, with 84.75%, 62.2% and 71.77% decrease respectively (Table 2). At 50 DAP, EHNT decreased overall stomatal conductance and significantly on LG01-550875, S13-10590c, S13-1955c, S14-15146r and R14-35b. EHNT was found to not affect respiration at 30 DAP in any cultivars (Table 2). However, at 50 DAP, EHNT tended to decrease respiration in the overall treatment when comparing with CNT not including the factor of cultivars (Table 3).

EHNT significantly decreased chlorophyll content (SPAD) only at 50DAP when the overall mean of cultivars was considered (Table 3). This decrease was very significant in cultivar CM422 at 50 DAP (Table 3). Significant temperature effect was observed in Fv/Fm at both 30 and 50 DAP (Table 4). EHNT significantly decreased Fv/Fm in all cultivars except S13-1955c, CM422, and DS25-1 at 30 DAP. At 50 DAP, EHNT significantly decrease Fv/Fm in all
cultivars but not affecting cultivar S14-15146R and DS25-1. Cultivar DS25-1 was the only cultivar that showed slight increase of $F_v/F_m$ (not significant) (Table 4.). Daytime and nighttime leaf temperature were taken from midday photosynthesis or respiration measurements at 30 and 50 DAP. EHNT did not affect daytime $T_{leaf}$ at both 30 and 50 DAP compared with CNT (Table 4). Significant night temperature effect was observed in both 30 and 50 DAP by comparing with CNT.

Other fluorescence and gas exchange traits were obtained from midday photosynthesis and respiration at 30 and 50 DAP including photochemical quantum yield of PSII (PhiPS2), intrinsic water use efficiency ($A/gs$), photosynthetic electron transport rate (ETR), and Intercellular CO$_2$ (ppm) (Table 5). EHNT tended to decrease overall PhiPS2 at 30 DAP ($p=0.0777$). At 50 DAP, significant temperature effect and cultivar effect were observed (Table 5). EHNT decreased overall PhiPS2 mean when compared with control conditions but when studying the effect of EHNT by cultivar the differences were not significant. Significant temperature effect was observed on $A/gs$ at both 30 and 50 DAP. At 30 DAP, EHNT increased overall $A/gs$ in all cultivars but only significantly in cultivar LG01-550875 and DS25-1. At 50 DAP, EHNT increased overall $A/gs$ but only significantly in cultivar S13-10590C. Significant temperature effect were observed at both 30 and 50 DAP. EHNT tended to increase overall Ci when compared with control treatment but only significantly in cultivar LG01-550875, S13-10590C, and DS25-1 at 30 DAP (Table 5.). At 50 DAP, EHNT significantly increased Ci in all cultivars except S15-17812C. EHNT did not affect ETR at 30 DAP, while significant temperature effect and cultivar effect were observed at 50 DAP. EHNT tended to decrease overall ETR in comparison with control temperature but differences cultivar by cultivar were
Plant growth parameters

Aboveground biomass was collected after all measurements were finished at 30 and 50 DAP. At 30 DAP, EHNT did not affect aboveground biomass while cultivar effect was significant (Table 6). Cultivar differences were not analyzed as that is not the objective of this research. At 50 DAP, significant temperature and cultivar effect were observed. EHNT tended to decrease aboveground biomass in cultivar S15-17812C, S13-1955C, CM422, and AG48x9 by 19.61%, 26.69%, 20.24% and 16.8% respectively.

No temperature effect was observed to on total leaf area at 30 DAP. EHNT positively increased overall plant height and node number at 30 DAP. Significant increase in plant height was observed in cultivar LG01-550875, S13-10590C, and AG48x9. Significant increase in node number was observed in cultivar S15-17812C, LG01-550875, S13-1955C, and AG48x9 (Table 6). At 50 DAP, no temperature effect on total leaf area, plant height, and node number was observed.
Discussion

High temperature (day and night) stress on soybean has been found to negatively affect vegetative and reproductive growth (Al-Khatib and Paulsen, 1990). The decrease in growth produced by high night temperature (HNT) has been associated to decrease in photosynthesis, stomatal conductance and yield in soybean (Gibson & Mullen, 1996; Djanaguiraman et al., 2013; Ruiz-Vera et al., 2013; Lin et al., 2020). All these studies focused on HNT effect during reproductive stages because high night temperature has negative effects on pollen viability, fertilization, and grain or fruit formation therefore reducing yield (Hatfield et al., 2011). For this reason, the effects of HNT over vegetative stages were less studied as it was thought that vegetative biomass was less responsive to high night temperature. However, Hatfield et al. (2011) showed how HNT accelerated vegetative development and shorten the period of biomass accumulation reducing growth and then decreasing final yield. Physiological effect of HNT on soybean vegetative growth has not be studied in previous literature. In the present experiment, we investigated the effect of extremely high night temperature (EHNT, 30/30°C) over physiological parameters of 9 soybean cultivars during vegetative growth and identify if there is cultivar variation.

In the present study, many physiological parameters: photosynthesis, stomatal conductance, photochemical quantum yield of PSII (PhiPS2) and maximum efficiency of PSII ($F_v/F_m$) were negatively affected by EHNT in comparison with the lack of effect that was observed in Shu et al., (Chapter 2). This difference in the effect of EHNT was probably due to two reasons: (1) the application of EHNT for a longer period of time and (2) The combination
of EHNT with a higher light intensity during the day. Siebers et al. (2015; 2017) and Thomey et al. (2019) found that longer high temperature stress applied earlier in the growing season affected more negatively the physiological parameters as it has more time to damage chloroplast membranes and other leaf structures affecting yield more significantly. In our experiments, we observed a similar trend. In the current experiment the effects of EHNT are more significant that in Shu et al., (Chapter 2) probably because the time of stress was increased from 14 to a total of 50 days. Other factors that increased the negative effect of high temperature on physiological parameters is the light intensity. Gao et al. (2019) found that high temperature treatments were more damaging for plants when combined with higher light intensities. High temperature damages chloroplast membranes and photosystem I (PSI) and photosystem II (PSII) (Djanaguiraman et al., 2013). These photosystems are in charge of moving the electrons captured by the chlorophylls to the NADP acceptor that is used as an energy intermediary and to fuel photosynthesis. Therefore, if the photosystems are damaged the energy production and the photosynthesis is reduced (Gao et al., 2019). In addition, when photosystems are damaged, the electron transport gets interrupted but the photons coming from the light keep flowing. Under this circumstances those extra electrons must be dissipated by producing oxidative species and that can further damage the leaf structure and function. Mitochondria damage induced by excessive radical oxidative species observed by Djanaguiraman et al. (2013) explained our results of respiration at 50 DAP. EHNT tended to decrease respiration in the overall treatment when in comparison with CNT not including the factor of the cultivars (Table 3).

At higher light intensity, this damage is going to be exacerbated by more electrons flowing
through the damaged photosystems (Gao et al., 2019). In the current experiment the plants were grown at EHNT and with a light intensity of 1750 μmol mol\(^{-1}\) PAR during the day which is enough to saturate the photosynthetic system of soybean (Sanz-Saez et al., 2017) and reduce the physiological parameters related with photosynthesis such as midday photosynthesis, photochemical quantum yield of PSII (\(\Phi_{PSII}\)) and maximum efficiency of PSII (\(F_v/F_m\)). By contrary, in Shu et al., (Chapter 2) light intensity in the chambers was much lower (500 μmol mol\(^{-1}\) PAR) and therefore the effect of EHNT over physiological parameters was less evident.

In our research, chlorophyll content was not affected by EHNT at 30 DAP, while EHNT decreased overall chlorophyll content at 50 DAP and especially in cultivar CM 422 (Table 3). Glaublitz et al. (2014) observed that under HNT (30/28°C), temperature sensitive rice cultivars showed intensified leaf chlorosis when the high temperature treatment was maintained for more than 20 days affecting biomass accumulation. Djanaguiraman et al. (2013) concluded that HNT decreased chlorophyll due to thylakoid dilation and leading to chlorophyll reductions. In our experiment, cultivar CM422 showed significant reduction of 18.56% in chlorophyll content at EHNT (Table 3) probably due to thylakoid damage (Djanaguiraman et al., 2013) that resulted in lower photosynthesis (Table 2) and a reduced biomass accumulation (Table 6.). Although all the cultivars tended to decrease the chlorophyll content, the significant decrease in CM422 could indicate a major sensitiveness to EHNT and therefore some phenotypic variation.

Different parameters related with chlorophyll fluorescence have been used to detect genotypic differences in the response to high temperature stress (Araus et al., 1998; Brestic et al., 2012; Sharma et al., 2012). The ratio \(F_v/F_m\) provides an estimate of the maximum photochemical efficiency of PSII and has been widely used to detect high temperature stress
and tolerance (Baker and Rosenvist, 2004; Haque et al., 2014). In the current research, EHNT decreased overall mean in all measurement dates (Table 4.). At 50 DAP, EHNT decreased $F_v/F_m$ in all cultivars but in S14-15146R and DS 25-1 (Table 4.). Interestingly, these two cultivars did not show a significant decrease of the biomass at 50 DAP. This could indicate that these two cultivars are EHNT tolerant because they are able to maintain a proper flow of electrons in the photosystem. This could be caused for a more resistant thylakoid membranes that are not affected by the high temperatures (Djanaguiraman et al., 2011; 2013). More research needs to be done comparing sensitive and these two tolerant cultivars to understand which are the structural characteristics at leaf and chloroplast level responsible for the observed tolerance. In addition, more research needs to be done to find the causes of tolerance in cultivars LG01-550875 and S13-10590C as they do not show decreases in biomass accumulation, but a clear cause of the tolerance is not found in the measured physiological parameters.

**Conclusions**

From this experiment, we observed significant negative effect of EHNT on photosynthesis, stomatal conductance, $F_v/F_m$, and biomass accumulation. The effect of EHNT in physiological parameters is more significant in this experiment in comparison with the results obtained in Chapter 2, probably because these plants were grown under high light intensity and longer periods of high night temperature stress. Therefore, a significant damage translated into a significant reduction of photosynthesis and other associated parameters. Cultivar variation was observed from aboveground biomass data and several physiological parameters such as photosynthesis, chlorophyll content and fluorescence which indicates that there are cultivars
that are sensitive and tolerant to EHNT. The biomass accumulation of the cultivars S14-15146R and DS 25-1 was not reduced by EHNT for 50 days treatment. This tolerance may be associated with the fact that these two cultivars maintained a high $F_v/F_m$ values at EHNT. More research is needed to detect physiological characteristics that may be responsible for the tolerance of cultivars that did not reduce the biomass accumulation but showed reduced $F_v/F_m$ values.
References


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Haque, M, S., Kjaer, K, H., Rosenqvist, E., Sharma, D, K., Ottosen, C. Heat stress and recovery of photosystem II efficiency in wheat (Triticum aestivum L.) cultivars acclimated to


Tables and Figures Legend

Table 1. Information about the nine soybean cultivars used in experiment including maturity group, growth habit and origin of the seed (breeding program/company).

Table 2. Physiological parameters measured at 30 and 50 days after planting (DAP): Midday photosynthesis (CO₂ μmol m⁻² s⁻¹), and stomatal conductance (H₂O mol m⁻² s⁻¹) in nine soybean cultivars grown under control (30/20°C), and extreme high night temperature (EHNT) (30/30°C). Values show the average of five replicates per treatment (n=5). Statistical p-values derived from a two-way ANOVA are shown in the ANOVA results line. Letters indicate significant differences between different temperature treatments within each cultivar (Tukey post hoc test P<0.05).

Table 3. Physiological parameters measured at 30 and 50 days after planting (DAP): Respiration (CO₂ μmol m⁻² s⁻¹) and estimation of chlorophyll content-SPAD in nine soybean cultivars grown under control (30/20°C), and extreme high night temperature (EHNT) (30/30°C). Values show the average of five replicates per treatment (n=5). Statistical p-values derived from a two-way ANOVA are shown in the ANOVA results line. Letters indicate significant differences between different temperature treatments within each cultivar (Tukey post hoc test P<0.05).

Table 4. Physiological parameters measured at 30 and 50 days after planting (DAP): The maximum quantum yield of photosystem II in a dark-adapted state (Fv/Fm), and day and night
leaf temperature (Tleaf, °C) in nine soybean cultivars grown under control (30/20°C), and extreme high night temperature (EHNT) (30/30°C). Values show the average of five replicates per treatment (n=5). Statistical p-values derived from a two-way ANOVA are shown in the ANOVA results line. Letters indicate significant differences between different temperature treatments within each cultivar (Tukey post hoc test P<0.05).

**Table 5.** Physiological parameters measured at 30 and 50 days after planting (DAP): PSII photochemical quantum yield (PhiPS2), intrinsic water use efficiency (A/gs), intercellular CO₂ (ppm), and photosynthetic electron transport rate (ETR) in nine soybean cultivars grown under control (30/20°C), and extreme high night temperature (EHNT) (30/30°C). Values show the average of five replicates per treatment (n=5). Statistical p-values derived from a two-way ANOVA are shown in the ANOVA results line. Letters indicate significant differences between different temperature treatments within each cultivar (Tukey post hoc test P<0.05).

**Table 6.** Physiological parameters measured at 30 and 50 days after planting (DAP): aboveground biomass (g plant⁻¹), total leaf area (cm² plant⁻¹), plant height (cm plant⁻¹), and node number in nine soybean cultivars grown under control (30/20°C), and extreme high night temperature (EHNT) (30/30°C). Values show the average of five replicates per treatment (n=5). Statistical p-values derived from a two-way ANOVA are shown in the ANOVA results line. Letters indicate significant differences between different temperature treatments within each cultivar (Tukey post hoc test P<0.05).
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**ANOVA Results**

- **Temperature**: <0.0001
- **Cultivars**: 0.136
- **Temp*Cult**: 0.0797

ANOVA Results

<0.0001 | <0.0001 | <0.0001 | <0.0001
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ANOVA Results
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- Cultivars: 0.0001
- Temp*Cult: 0.0007
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**ANOVA Results**

- **Temperature**: <0.0001, 0.7657, <0.0001, 0.1353, <0.0001
- **Cultivars**: 0.6615, 0.3559, 0.625, 0.5313, 0.9487
- **Temp*Cult**: 0.3935, 0.4131, 0.7208, 0.1035, 0.395

*Note: The table provides data for Fv/Fm and temperature readings at 30 and 50 DAP for various cultivars under control and EHNT treatments.*
Table 5.

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ANOVA Results

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ANOVA Results

|                | Temperature     | 0.0777          | 0.5563          | 0.0161          | <0.0001       | 0.0016          | 0.9648          | 0.1001          | 0.5427       |
|                | Cultivars       | 0.724           | 0.2837          | <0.0001         | 0.0008        | 0.002           | 0.0128          | 0.1149          | 0.0251       |
|                | Temp*Cult       | 0.7975          | 0.6392          | 0.1000          | 0.9485        | 0.8731          | 0.3701          | 0.9497          | 0.9964       |