# EVALUATING PHOTOSYNTHETIC RESPONSES OF SORGHUM (GRAIN & FORAGE) AND SOYBEAN TO TEMPERATURE STRESS

By

JHANSY REDDY KATTA

Bachelor of Science in Agriculture

Acharya N.G. Ranga Agricultural University

Hyderabad, Telangana, India

2016

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2019

EVALUATING PHOTOSYNTHETIC RESPONSES OF SORGHUM (GRAIN & FORAGE) AND SOYBEAN TO TEMPERATURE STRESS

Thesis Approved:

Dr. Vijaya Gopal Kakani

Thesis Adviser

Dr. Beatrix Haggard

Dr. Prasanna Gowda

# ACKNOWLEDGEMENTS

I would like to acknowledge my indebtedness and render my warmest thanks to my advisor, Dr. Vijaya Gopal Kakani for his tremendous academic support, patience and motivation. His advice on both research through all stages of work as well as on my career has been invaluable.

Similar earnest gratitude goes to my Committee members Dr. Prasanna Gowda and Dr. Beatrix Haggard for their encouragement, insightful comments and discussions which have contributed greatly to the improvement of the thesis. I take this opportunity to thank senior agriculturist, Jay Prater and Val Gladden, who were always there to fix any issues in the controlled growth chambers.

Special thanks to the Department of Plant and Soil Sciences at the Oklahoma State University for providing me an opportunity to pursue my Master's degree. It definitely stands out in terms of research collaborations and technical committees.

Finally, I must express my gratitude to my family, for their wise counsel, unfailing support and constant encouragement throughout my Masters study.

Name: JHANSY REDDY KATTA

Date of Degree: JULY, 2019

Title of Study: EVALUATING PHOTOSYNTHETIC RESPONSES OF SORGHUM (GRAIN & FORAGE) AND SOYBEAN TO TEMPERATURE STRESS

Major Field: PLANT AND SOIL SCIENCE

Abstract: Physiology, growth and development of crop plants are driven by temperature and temperatures either side of the optimum lead to temperature stress. The objective of this research was to quantify the effect of temperature stress on gas exchange and chlorophyll fluorescence parameters of grain sorghum, forage sorghum, and soybean. An experiment was conducted using six walk-in growth chambers in Controlled Environment Research Facility at Oklahoma State University. Plants in growth chambers were subjected to six different temperatures (20/12, 24/16, 28/20, 32/24, 36/28, and 40/32 °C). Plants of grain sorghum hybrid *Midland Genetics 4772,* forage sorghum *var. Ross Elite* or *Surpass BMR* and soybean *MG 3926 NRS2* were used respectively for grain sorghum, forage sorghum, and soybean respectively. Leaf level parameters were recorded with the help of *LI-6400* instrument fitted with leaf chamber fluorometer (*LCF*), that provides LED-based fluorescence and irradiation, beginning at 45 days after planting. Response to temperature of photosynthesis, transpiration, electron transport rate and chlorophyll fluorescence were studied. Both light and $CO_2$ response curves were measured using the automated software in the instrument. The parameters derived from the curves are further evaluated with response to temperature. The rates of photosynthesis declined as the growing temperatures increased in soybean while the transpiration rates were increased. The responses of photosynthesis to both light and internal $CO_2$ suggested that photosynthetic rates of grain and forage sorghum acclimate to high temperature through an increased rate of electron transport. The photosystem 2 (PSII) values also remained unaffected at high temperatures suggesting there is no damage to photosynthetic machinery. In conclusion, the measured photosynthetic parameters demonstrated that sorghum and soybean tolerate high temperatures under irrigated conditions. The temperature functions can be used to improve leaf level functions in the mechanistic models of sorghum and soybean.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

Figure Page

# CHAPTER I

## GENERAL INTRODUCTION

Global environmental change, including land degradation, loss of biodiversity, change in hydrology, and climate change patterns resulting from enhanced anthropogenic emission of greenhouse gas emissions, will have serious consequences for agricultural productivity. Boyer indicated that environmental factors may limit crop production by as much as 70 % (Boyer, 1982). Agricultural productivity is determined by environmental factors that can be biotic or abiotic. Biotic factors include living organisms, such as fungi and insects, while abiotic includes nonliving factors, such as drought, extreme temperatures, salinity, and pollutants (e.g. heavy metals).

Abiotic stress is defined as an environmental condition deviating from optimum levels that reduces plant growth and yield. Plant responses to abiotic stresses are dynamic and complex (Skirycz, 2010; Cramer, 2010) and are also either elastic (reversible) or plastic (irreversible). It is evident that abiotic stress continues to have a significant impact on plants based upon the percentage of land area affected and the number of scientific publications directed at various abiotic stresses (Table 1) (Cramer, 2011). As summarized in Table 1, the growing concern for impact of abiotic stresses on crop plants is reflected in the increasing number of publications

focused on abiotic stresses. For example, since the pivotal review of systems biology by Kitano (2002), the number of papers published on abiotic stress in plants using a systems biology approach has increased exponentially (Figure 1) (Cramer, 2011).

Recent progress summarizing plant responses to abiotic stress to include transcriptomics, metabolomics, proteomics, and other integrated approaches has been elucidated by Cramer (2011). Crop yields are reduced and vary greatly as a result of abiotic stresses such as drought, excess water (submergence), mineral deficiencies and toxicities and abnormal temperatures. Inhibition of protein synthesis (Good, 1994; Dhindsa, 1975) is one of the earliest metabolic response to abiotic stresses. The energy metabolism is affected as the stress becomes more severe (e.g. sugars, lipids and photosynthesis) (Pinheiro, 2010; Cramer, 2007). Hence, there are gradual and complex changes in metabolism in response to stress mechanisms.

Several independent studies have demonstrated the effects of stress due to temperature and water on crop yields. For example in Canada, the extreme events that occurred during 2001 and 2002 and the droughts and floods during 2010 and 2011 had a devastating impact on crop yield reducing as much as 50% (Wheaton et al., 2008). Leaf structure is affected by higher temperature often causing development of thinner leaves with higher leaf area (Loveys et al., 2002; Poorter et al., 2009). Leaves which develop under water deficit generally have smaller cells and higher stomatal density (Tisne et al., 2010; Shahinnia et al., 2016). Abiotic stress significantly reduces plant productivity and damages plant ecosystems. Roncel et al. (2016) reported the negative effects of nutrition deficiency on photosynthesis. Drought, salinity, nutrition, high-light, UV-radiation, increasing concentration of atmospheric $CO_2$ and $CH_4$ affect photosynthesis and plant productivity. Abiotic stresses results in over-reduction of the electron transport chain, which in turn leads to photo oxidation (Foyer and Noctor, 2005; Nishiyama and Murata, 2014; Takahashi and Murata, 2008; Rochaix, 2011).

Temperature is the most important environmental factor that affects plant distribution and productivity. It is one of the primary environmental factor affecting the rate of plant development. Responses to temperature differs among crop species throughout their life cycle. For each species, there is a range of maximum and minimum temperatures for the growth to occur and optimum temperature at which the plant growth is maximum. Temperature stress can be caused due to shifts in temperature either above or below the optimum, hence giving rise to heat and cold stress. Heat stress and cold stress have independent modes of action on the physiology and metabolism of plant cells. The susceptibility to high and cold temperatures varies with the stage of plant development. The observed effects depend on species and genotype, with abundant inter- and intra-specific variations (Sakata and Higashitani, 2008).

As most environmental stresses affect photosynthesis, measuring photosynthetic parameters is the easiest and fastest way to assess the type and degree of stressful conditions. Stress responses such as rapid hormone signals that affects gas exchange under drought; changes in pigments, lipids, proteins and thylakoid structure under other stresses (high light, UV-radiation, nutrition, drought, salinity and heat) induce remarkable changes in plant growth and development. Stressful environments considerably hamper the process of photosynthesis in most plants by altering the concentration of various pigments and metabolites including enzymes, and ultrastructure of the organelles as well as stomatal regulation.

Heat stress induces changes in respiration and photosynthesis, shortening of life cycle and diminished plant productivity (Barnabás et al., 2008). The heat stress also changes membrane permeability and alters cell differentiation, elongation and expansion (Smertenko et al., 1997; Rasheed, 2009). The photochemical functions of the thylakoid membrane system are the primary site of heat injury (Schrader et al., 2004). In addition, the enzymes of the Calvin-Benson cycle, including ribulose 1,5-bisphosphate carboxylase (Rubisco) and Rubisco activase are very sensitive to increased temperatures (Demirevska-Kepova et al., 2005). In many species, the

effects of heat stress are more prominent on reproductive development than on vegetative growth and the sudden decline in yield with temperature is mainly associated with pollen infertility (Young et al., 2004). Various physiological injuries have been observed under elevated temperatures, such as leaf abscission and senescence, shoot and root growth inhibition, which consequently lead to a decreased plant productivity (Vollenweider and Gunthardt-Goerg, 2005). High temperatures reduce the plant growth by affecting the shoot net assimilation rates (Wahid et al., 2007).

Under cold stress, reduced chlorophyll formation and chlorophyll destruction are the major factors. Reduced chlorophyll formation has been found to limit the growth of maize at low temperature (Alberda., 1969) and chlorophyll development was shown to be light and temperature dependent (McWilliam and Naylor., 1967). Reports have suggested that the rates of $CO_2$ fixation are insufficient to utilize the phosphorylative and reducing capacity of the chloroplast under low temperature conditions, thereby reducing the carbon assimilation efficiency. Taylor and Rowley (1971) showed that severe inhibition of photosynthesis in maize leaves occurs when the leaves are given prolonged exposures to high light at low temperatures. This resulted in permanent photo oxidation. Bulk leaf chlorophyll concentration also declined in maize leaves when grown at low temperatures (Hardacre and Turnbull., 1986). In addition, species such as *Phaseolus vulgaris*, *Lycopersicum esculentum* and *Gossypium hirsutum* suffer severe photoinhibition at low temperature conditions (Powles et al., 1983).

In the process of photosynthesis, two key events; light reactions, in which light energy is converted into ATP and NADPH and oxygen is released, and dark reactions, in which $CO_2$ is fixed into carbohydrates by utilizing the products of light reactions, ATP and NADPH (Lawlor, 2002; Taiz and Zeiger, 2010). There are two main pathways of $CO_2$ fixation, $C_3$ and $C_4$. Plants have been categorized into $C_3$, $C_4$, or $C_3$-$C_4$ intermediate plants depending on the spatial

distribution of these two pathways within leaf tissues. Another special pathway in minority of the plants is the crassulacean acid metabolism (CAM) (Ashraf et al., 2013).

Photosynthesis machinery in plants is comprised of various components, including the photosystems (I and II) and the electron transport system. A stress-induced negative effect on any component in the system may lead to a reduction in the overall photosynthetic performance. Studies have shown that photosynthetic efficiency and transpiration rates decrease under water, salt, and heat stress when applied individually or in combination (Arbona et al., 2013; Zandalinas et al., 2016). Plants have several mechanisms to overcome this problem, e.g. reducing the rate of electron transport by converting the excessively absorbed light into thermal energy (Gururani, 2015). The dissipation of excess excitation energy as heat is known as non-photochemical quenching (NPQ) of chlorophyll fluorescence (Rochaix, 2011; Tikkanen et al, 2011; Spetea et al, 2014).

The specific inhibition of carbon dioxide fixation has been studied in response to combination of temperature, water and salt stress in sorghum. For instance, Yan (2012) reported that the high temperatures significantly decreased photosynthesis but the decrease was lower in the leaves of salt-treated sorghum.

Review of literature identified existing knowledge gaps in the photosynthetic temperature response mechanisms in sorghum and soybean. These crops have significant production potential in the United States under future climates and as biofuel crops. For instance, Taylor and Rowley (1971) reported that there is a slight recovery followed by a rapid decline in photosynthetic rate when temperature was lowered immediately and then recovered back to normal optimum (25 °C). Likewise, limited studies were reported using chlorophyll fluorescence parameters to assess the photosynthetic components in a chloroplast subjected to temperature stress. The chlorophyll fluorescence techniques are useful for eco-physiological studies in assessing plant responses to

environmental abiotic conditions such as water and temperature stress (Srinivasan et al., 1996). However, the behavior of photosynthesis on season long exposure to low temperatures in sorghum was not studied. Hence, sorghum and soybean were selected for this study owing to their sensitivity to sensitive to abrupt changes in temperature.

Hence, we hypothesize that temperature stress reduces the measured photosynthetic parameters leading to decreased photosynthesis. Therefore, the objective of the current study is to evaluate the chlorophyll fluorescence and photosynthetic parameter responses to temperature of grain sorghum, soybean and forage sorghum.

REFERENCES:

Alberda, T. (1969). "The effect of low temperature on dry matter production, chlorophyll concentration and photosynthesis of maize plants of different ages." *Acta Botanica Neerlandica 18(1)*: 39-49.

Arbona, V., et al. (2013). "Metabolomics as a tool to investigate abiotic stress tolerance in plants." *International journal of molecular sciences 14(3)*: 4885-4911.

Ashraf, M. and P. J. Harris (2013). "Photosynthesis under stressful environments: an overview." *Photosynthetica 51(2)*: 163-190.

Barnabás, B., et al. (2008). "The effect of drought and heat stress on reproductive processes in cereals." *Plant, cell & environment 31(1)*: 11-38.

Boyer, J. S. (1982). "Plant productivity and environment." *Science 218(4571)*: 443-448.

Cramer, G. R. (2010). "Abiotic stress and plant responses from the whole vine to the genes." *Australian Journal of Grape and Wine Research 16*: 86-93.

Cramer, G. R., et al. (2007). "Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles." *Functional & integrative genomics 7(2)*: 111-134.

Cramer, G. R., et al. (2011). "Effects of abiotic stress on plants: a systems biology perspective." *BMC plant biology 11(1)*: 163.

Demirevska-Kepova, K., et al. (2005). "Heat stress effects on ribulose-1, 5-bisphosphate carboxylase/oxygenase, Rubisco binding protein and Rubisco activase in wheat leaves." *Biologia Plantarum 49(4)*: 521-525.

Dhindsa, R. and R. Cleland (1975). "Water stress and protein synthesis: I. Differential inhibition of protein synthesis." *Plant Physiology 55(4)*: 778-781.

Foyer, C. H. and G. Noctor (2005). "Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses." *The Plant Cell 17(7)*: 1866-1875.

Good, A. G. and S. T. Zaplachinski (1994). "The effects of drought stress on free amino acid accumulation and protein synthesis in Brassica napus." *Physiologia Plantarum 90(1)*: 9-14.

Gururani, M. A., et al. (2015). "Regulation of photosynthesis during abiotic stress-induced photoinhibition." *Molecular plant 8(9)*: 1304-1320.

Hardacre, A. and H. Turnbull (1986). "The growth and development of maize (Zea mays L.) at five temperatures." *Annals of botany 58(6)*: 779-787.

Kitano, H. (2002). "Systems biology: a brief overview." *Science 295(5560)*: 1662-1664.

Lawlor, D. W. (2002). "Limitation to photosynthesis in water□stressed leaves: stomata vs. metabolism and the role of ATP." *Annals of botany 89(7)*: 871-885.

Lichtenthaler, H. K. (1996). "Vegetation stress: an introduction to the stress concept in plants." *Journal of plant physiology 148(1-2)*: 4-14.

Link, G. (2003). "Redox regulation of chloroplast transcription." *Antioxidants and Redox Signaling 5(1)*: 79-87.

Loveys, B., et al. (2002). "Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast□and slow□growing plant species." *Plant, cell & environment 25(8)*: 975-988.

McWilliam, J. and A. Naylor (1967). "Temperature and plant adaptation. I. Interaction of temperature and light in the synthesis of chlorophyll in corn." *Plant Physiology 42(12)*: 1711-1715.

Nishiyama, Y. and N. Murata (2014). "Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery." *Applied microbiology and biotechnology 98(21)*: 8777-8796.

Pinheiro, C. and M. Chaves (2010). "Photosynthesis and drought: can we make metabolic connections from available data?" *Journal of experimental botany 62(3)*: 869-882.

Poorter, H., et al. (2009). "Causes and consequences of variation in leaf mass per area (LMA): a meta□analysis." *New phytologist 182(3)*: 565-588.

Powles, S. B., et al. (1983). "Interaction between light and chilling temperature on the inhibition of photosynthesis in chilling□sensitive plants." *Plant, cell & environment 6(2)*: 117-123.

Rasheed, R. (2009). Salinity and extreme temperature effects on sprouting buds of sugarcane (Saccharum officinarum L.): some histological and biochemical studies, *University of Agriculture, Faisalabad Pakistan*.

Rochaix, J.-D. (2011). "Regulation of photosynthetic electron transport." *Biochimica et Biophysica Acta (BBA)-Bioenergetics 1807(3)*: 375-383.

Roncel, M., et al. (2016). "Iron deficiency induces a partial inhibition of the photosynthetic electron transport and a high sensitivity to light in the diatom Phaeodactylum tricornutum." *Frontiers in plant science 7*: 1050.

Sakata, T. and A. Higashitani (2008). "Male sterility accompanied with abnormal anther development in plants–genes and environmental stresses with special reference to high temperature injury." *Int. J. Plant Dev. Biol 2(4)*.

Schrader, S., et al. (2004). "Thylakoid membrane responses to moderately high leaf temperature in Pima cotton." *Plant, cell & environment 27(6)*: 725-735.

Shahinnia, F., et al. (2016). "Genetic association of stomatal traits and yield in wheat grown in low rainfall environments." *BMC plant biology 16(1)*: 150.

Skirycz, A. and D. Inzé (2010). "More from less: plant growth under limited water." *Current Opinion in Biotechnology 21(2)*: 197-203.

Smertenko, A., et al. (1997). "Heat stress affects the organization of microtubules and cell division in Nicotiana tabacum cells." *Plant, cell & environment 20(12)*: 1534-1542.

Spetea, C., et al. (2014). Changing the light environment: chloroplast signalling and response mechanisms, *The Royal Society*.

Srinivasan, A., et al. (1996). "Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques." *Euphytica 88(1)*: 35-45.

Taiz, L. and E. Zeiger (2010). "Plant physiology fifth edition." *Sunderland, Massachusetts, USA*: Sinauer Assosiates inc., puplishers.

Takahashi, S. and N. Murata (2008). "How do environmental stresses accelerate photoinhibition?" *Trends in plant science 13(4)*: 178-182.

Taylor, A. and J. Rowley (1971). "Plants under climatic stress: I. Low temperature, high light effects on photosynthesis." *Plant Physiology 47(5)*: 713-718.

Tikkanen, M., et al. (2011). "Novel insights into plant light-harvesting complex II phosphorylation and ‘state transitions’." *Trends in plant science 16(3)*: 126-131.

Tisne, S., et al. (2010). "Keep on growing under drought: genetic and developmental bases of the response of rosette area using a recombinant inbred line population." *Plant, cell & environment 33(11)*: 1875-1887.

Vollenweider, P. and M. S. Günthardt-Goerg (2005). "Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage." *Environmental Pollution 137(3)*: 455-465.

Wahid, A., et al. (2007). "Heat tolerance in plants: an overview." *Environmental and experimental botany 61(3)*: 199-223.

Wheaton, E., et al. (2008). "Dry times: hard lessons from the Canadian drought of 2001 and 2002." *The Canadian Geographer/Le Géographe canadien 52(2)*: 241-262.

Yan, K., et al. (2012). "Responses of photosynthesis and photosystem II to higher temperature and salt stress in Sorghum." *Journal of Agronomy and Crop Science 198(3)*: 218-225.

Young, L. W., et al. (2004). "High temperature stress of Brassica napus during flowering reduces micro□and megagametophyte fertility, induces fruit abortion, and disrupts seed production." *Journal of experimental botany 55(396)*: 485-495.

Zandalinas, S. I., et al. (2016). "Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels." *BMC plant biology 16(1)*: 105.

Table 1: Estimates of the impacts of abiotic stresses on crop production and published research

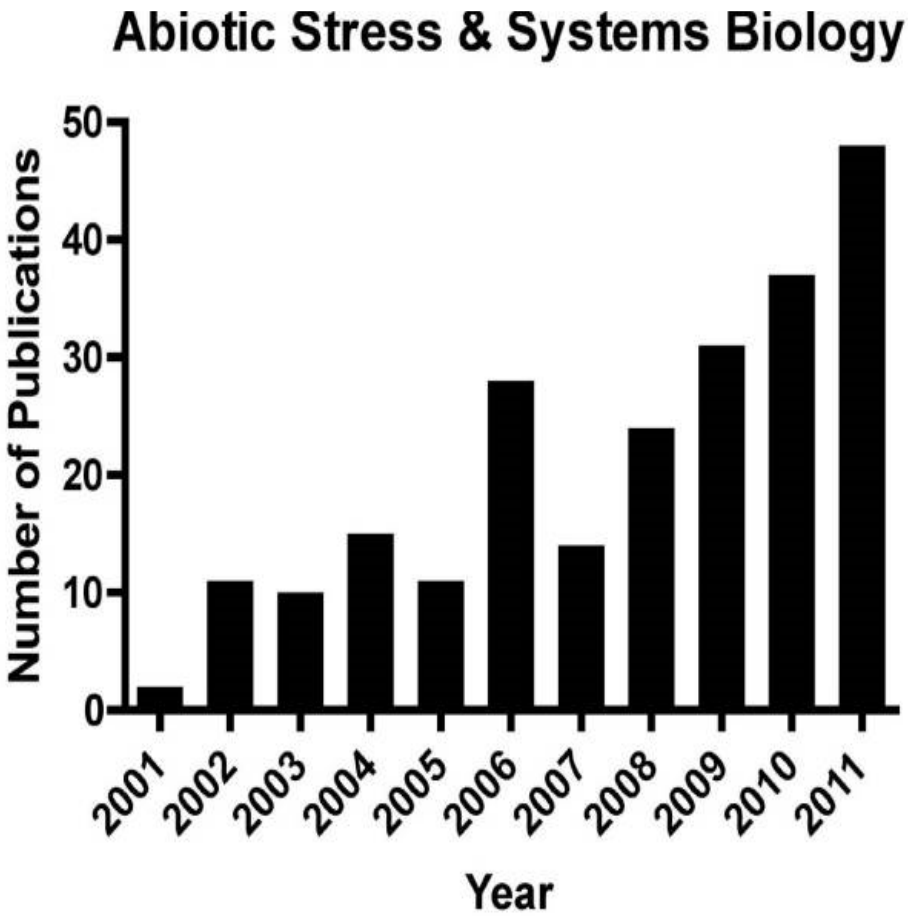
| Stress Type | % of global land area affected* | % of global rural land area affected* | Number of publications*** |
|---|---|---|---|
| **Abiotic Stress** | | 96.5 | **36,363** |
| **Water** | | | **4819** |
| Deficit or Drought | 64 | 16 | 4137 |
| Flooding or Anoxia | 13 | 10 | 682 |
| **Temperature** | | | **9715** |
| Cold | 57 | 26 | 3798 |
| Chilling | | | 187 |
| Freezing | | | 350 |
| High or heat | | | 5380 |
| **Light** | | | **7659** |
| Low | | | 3081 |
| High | | | 4578 |
| **Chemical/Soil** | | 50 | **12391** |
| Salt or salinity | 6 | 6 | 3498 |
| Mineral deficiency or low fertility | 9 | 39 | 222<br>437 |
| Mineral toxicity | 15 | | 3646 |
| Acid Soil | | | |
| Air pollutants | | | 1369 |
| Ozone | | | 378 |
| Sulfur dioxide | | | 2001 |
| $NO_x$ oxide | | | 840 |
| Elevated $CO_2$ | | | |
| Miscellaneous (e.g. wind, mechanical, etc.) | | | **779** |

*based on FAO World Soil Resources Report 2000

** based on Tables three point six and three point seven of 2007 FAO Report

*** data based on simple searches in PubMed between 2001 and July 7, 2011.

Figure 1: The number of publications per year related to systems biology and abiotic stress. Key words used in the search of PubMed included: plant, systems biology, and abiotic stress. *The number for the year 2011 was estimated by doubling the 6-month value. (Cramer, 2011).



# CHAPTER II

## PHOTOSYNTHETIC RESPONSES OF GRAIN SORGHUM TO TEMPERATURE


### Abstract

Sorghum (*Sorghum bicolor* L. Moench) being a major cultivated species in the world due to its multipurpose nature and its potential use as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper) and fertilizer (utilization of organic by-products) is often exposed to temperature stress during growth and development. Plants of hybrid Midland Genetics 4772 were grown in six controlled plant growth chambers at daytime maximum/nighttime minimum optimum temperature of 28/20 °C until seedling emergence and establishment. Thereafter, plants were exposed to different temperature treatments including cold (20/12, 24/16 °C), optimum (28/20 °C) and heat (32/24, 36/28, and 40/32 °C) till the end of the growing season. The photosynthetic mechanism of sorghum with response to temperature was studied with the help of LI-6400 photosynthetic systems (*LICOR*, Lincoln, NE). The response curves were quantified with 6400-40 leaf chamber fluorometer (*LCF*) fitted with the instrument. The results showed that grain sorghum acclimates to prolonged temperature stress, through an increased electron transport rate at higher temperatures. The parameters derived from the response curves are further elucidated with temperature to further justify the acclimation of grain sorghum to temperature stress. The

apparent quantum efficiency remains unaffected at high temperatures because of lack of photorespiration.

1. Introduction

Studies enumerating climate extremities globally and the consequent effects on food production had been widely discussed. Agricultural productivity can be affected by climate change directly due to changes in temperature in the atmosphere (Kalra, Naveen et al., 2019). Crops such as sorghum which is the predominant crop in the United States are sensitive to abrupt temperature changes, leading to decrease in grain yield. Hence, responses of sorghum to temperature by evaluating the effects on photosynthesis with use of response curves is a much needed study.

The increase in global temperature of approximately 4 °C or higher may represent great risks for agricultural food production at the global level (IPCC, 2014b). In this paper, we are going to study the physiological responses of sorghum in response to temperature stress. The mean optimum temperature range for sorghum has been reported to be 21 to 35 °C for seed germination, 26 to 34 °C for vegetative growth and development and 25 to 28 °C for reproductive growth (Maiti, 1996). Any temperature outside this range can be considered a stress for crop growth.

Temperature stress can be caused due to shifts in temperature either away from the normal optimum or below the optimum (or stress can be caused either due to high or low temperature leading to subsequent changes which result in reduction in the photosynthetic rate; in the environment in which the plants have been growing). Photosynthesis is a key phenomenon which gets affected in either case thus leading to substantial reduction in crop yields. Temperatures above the normal optimum are defined as heat stress and can cause retardation in growth and development. Photosynthesis is highly sensitive to high temperatures (Wang et al. 2010, Centritto et al. 2011) and heat stress can impair electron transport systems and $CO_2$ reduction pathways which can inhibit overall photosynthetic mechanism of a plant (Ashraf et al.2013). Expanding to this, Sonal Mathur and Divya Agarwal (2014) reported the primary target sites of HT stress are Rubisco and Photosystem (PSII). Stress due to temperature can also be caused due to low

temperatures in the environment at which the crop plants are exposed. Taylor and Rowley (1971) reported that there is a slight recovery followed by a rapid decline in photosynthetic rate when temperature was lowered immediately and then recovered back to normal optimum (25C). However, the behavior of photosynthesis on season long exposure to low temperatures in sorghum was not studied.

In response to studying the effects of temperature, Ludlow and Wilson (1971) reported the light response curves of tropical and subtropical C4 grasses and enumerated the cardinal temperatures for net photosynthesis which depend on illuminance, vapor pressure of the air, and leaf temperature. However, Baker and Rosenqvist (2004) showed that simultaneous measurement of chlorophyll fluorescence and photosynthesis can provide useful information on the performance of leaf photosynthesis. Furthermore, these measurements have been reported to evaluate the enzyme kinetics in response to $CO_2$ and PAR (Dwyer et al., 2007). However, photosynthesis and fluorescence responses to Ci and PAR of sorghum acclimated to wide range of temperatures have not been studied. The response functions of the intact leaves and the parameters derived from the light and $CO_2$ response curves can be used to quantify C4 photosynthesis to environmental change. In the present study, with the development and use of the sophisticated scientific instruments over time, it has been possible to study the fluorescence parameters associated with the induction of fluorescence along with the operation of photosynthesis.

Sorghum [(Sorghum bicolor L.) Moench] being a major cultivated species in the world due to its multipurpose nature and its potential use as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper) and fertilizer (utilization of organic by-products) is selected for the study (Tari, 2013). Since the crop is of majorly value, it is vital to gain the detailed insights of the basic physiology of the plant thus contributing to the increase in photosynthesis thus contributing to increased production and yield.

The objective of this research was to characterize effects of temperature on photosynthesis by measuring gas exchange and fluorescence parameters in response to $CO_2$ and PAR under controlled environment conditions. We hypothesize that leaf photosynthesis is affected due to varying temperature regimes and is caused by enzymatic and/or developmental changes including changes in activities of Rubisco and PEPC.

## 2. Materials and Methodology:

The experiments in the growth chambers are conducted at the Controlled Environmental Research Laboratory (CERL), Oklahoma State University in 2017. Six chambers in CERL were maintained at six different temperatures ranging from 12 °C to 40 °C. Seeds of grain sorghum (*Midland Genetics 4772*) were sown in four equally spaced rows in the growth chamber. Sand was used as the medium for plant growth in the pots (45 cm tall and 20 cm in diameter). Emergence was recorded 5 days after sowing. Plants were irrigated with standard Hoagland's nutrient solution for 3 min delivered at 08:00, 12:00 and 17:00 h to ensure favorable nutrient and water conditions for the plant growth and development. Irrigation is provided through an automated computer controlled drip system, and the amount of irrigation provided during the growing season was adjusted based on the evapotranspiration measured in each chamber. In addition, the water intervals were increased to 5 minutes later on during the reproductive phases in the warmer temperature chambers. Photoperiod was adjusted to 12 hours' light and 12 hours' dark period.

### 2.1. Treatments:

The temperatures were maintained within ±0.5 ℃ of treatment set points of 28/20 °C (day/night) in all units until the seedlings had emerged and were uniformly established. At 15- 20 d after the seedling (DAS), each of the six chambers were assigned one of six treatments. The treatments consisting of six day/night temperatures of 20/12, 24/16, 28/20, 32/24, 36/28, and 40/32 °C. A dedicated computer with in-house coded software monitored and controlled the environmental

variables. Three sets of one to two topmost fully expanded leaves in each of the six treatments were selected for photosynthesis measurements. Each set of leaves selected for measuring photosynthesis was considered as one replicate.

2.2. Gas exchange measurements:

These measurements were made on attached leaves using an open gas exchange LI-6400 photosynthesis systems (LICOR, Lincoln, Nebraska, USA) fitted with a 6400-40 leaf chamber flurometer (LCF) that provides LED-based fluorescence and irradiation. Measurements like Pn, gs, Ci, ETR were made on attached leaves between 9 AM to 1 PM with the instrument. Care is taken to cover the 2 $cm^2$ area of the leaf cuvette of the leaf chamber with youngest fully opened leaves on the plant. Temperature in the leaf cuvette was set in accordance with the daytime temperature of the chambers. The leaf chamber reference $CO_2$ was set to 400 μmol $m^{-2}$ $s^{-1}$. The leaves were artificially irradiated with a blue-red LED radiation source attached to the sensor head set at 1200 μmol $m^{-2}s^{-1}$ for uniform light. Photosynthesis is driven by the "actinic light" source that uses 3 blue LEDs (470 nm) and all red LEDs. To measure steady-state fluorescence ($F_s$), LCF uses two red LEDs (center wave-length about 630 nm) and a detector (detects radiation at 715 nm in the photosystem 2 fluorescence band). A flash light achieved by 27 red LEDs is used to measure the maximal fluorescence ($F_m$'). The software in the instrument provides data on the fluorescence parameters and also calculates derived parameters such as electron transport rate (ETR), photochemical yield of photosystem 2 (PSII) electron transport ($\boldsymbol{\Phi}_{PSII}$), and the quantum yield of $CO_2$ assimilation ($\boldsymbol{\Phi}_{CO2}$). The equation used to derive these values are below:

$$\boldsymbol{\Phi}_{PSII} = (F_m' - F_s)/F_m' \qquad \text{[unitless]}$$

$$\boldsymbol{\Phi}_{CO2} = (P_N - P_{dark})/I\alpha_{leaf} \qquad [\mu mol(CO_2)\ \mu mol(photon)^{-1}]$$

$\boldsymbol{\Phi}_{CO2} = P_N/absorbed\ PPFD$, assuming a leaf absorptivity of 85% (Oberhuber and Edwards, 1993)

Where $P_N$ is net photosynthetic rate, $P_{dark}$ is dark assimilation rate, both [μmol ($CO_2$) $m^{-2}s^{-1}$], I is the incident PAR [μmol $m^{-2}s^{-1}$], and $\alpha_{leaf}$ is leaf absorptance rate. $P_{dark}$ is the same magnitude, but opposite in sign, of dark respiration rate.

2.2.1. Fluorescence and net photosynthesis/internal carbon dioxide (F−$P_N$/Ci) curves:

The automatic program in *LI-6400* photosynthesis systems for F-$P_N$/Ci curves was used to generate the response of $P_N$ to $C_i$. Net photosynthesis and chlorophyll fluorescence characteristics were determined simultaneously. The top most expanded young leaves were selected for the measurements after 50 to 65 days old plants. Measurements were taken between 09:00 and 11:00 h by changing [$CO_2$] in leaf chamber fluorometer in 9 steps (400, 300, 200, 100, 50, 400, 400, 600 and 800 μmol $mol^{-1}$) under a constant PAR of 1000 μmol $m^{-2}s^{-1}$. The block temperature was set to corresponding growth chamber day time temperature. The instrument was given 240 s to reach the steady state at each PAR level, after which it logged values when the coefficient of variation was ≤ 5 %. Curve fitting software (Sigma Plot for Windows 12.5) was used to analyze the F-$P_N$/Ci responses using a three component exponential to maximum function of the term

$$P_N = a\,(1 - e^{-bx}) + y_0$$

Where $P_N$ = steady-state assimilation rate and x = $C_i$.

Using this equation, $P_{sat}$ was calculated as $a + y_0$ and phosphoenolpyruvate carboxylase (PEPC) efficiency as the slope of $P_N$ =0, calculated *as* $b\,[a + y_0]$. Likewise, saturated values of ETR ($ETR_{sat}$) were calculated by fitting exponential to maximum function to ETR and $C_i$.

2.2.2. Fluorescence and net photosynthesis/PAR (F−$P_N$/ PAR) curves:

Starting from total darkness, in which there can be no photosynthesis, the first few photons, absorbed by the leaf will be used with greatest efficiency. As light increases, the efficiency drops, and eventually subsequent increases in light yield little or no increase in photosynthesis. The

parameters derived from the light response curves measures dark respiration rate, light compensation point, the quantum efficiency (initial slope), and the maximum photosynthetic rate.

Sunfleck/Shade method is one of the approaches offered to separate each new light level with the starting light value, with time to equilibrate. Data collected in this manner might be most appropriate for addressing light dynamics in canopies.

These measurements were made between 09:00 and 11:00 h on top most fully expanded leaves by reducing PAR in 9 steps from 2000 to 0 µmol $m^{-2}s^{-1}$. The [$CO_2$] was kept constant at 400 µmol $mol^{-1}$ and the block temperature inside the leaf cuvette was set to the treatment day time temperature in the corresponding growth chamber. The instrument was given 120 to 240 s to reach the steady state at each PAR level, after which it logged values when the coefficient of variation was ≤ 5 %. The photosynthetic irradiance response curves were fit using non-rectangular hyperbola least square fitting procedure and the model is described as:

$$P_N = \frac{\Phi Q + P\,max - \sqrt{(\Phi Q + Pmax)2 - 4\,\Phi Q k Pmax}}{2\,k} - R_D$$

Where $\boldsymbol{\Phi}$ is the apparent quantum efficiency, Q is the PAR, $P_{max}$ is the PAR saturated rate of gross $CO_2$ assimilation, k is the curvature factor, and $R_D$ is the dark respiration rate. Maximum values of ETR ($ETR_{max}$) were calculated by fitting exponential to maximum function to ETR and PAR. R code is used to derive the parameters from irradiance response curves.

2.3. Estimation of cardinal temperatures for the response parameters:

The response parameters like $P_{max}$, $P_{sat}$, $ETR_{max}$, $ETR_{sat}$ and *PEPC* are derived from the light and $CO_2$ response curves. The $P_{max}$, and $ETR_{max}$ are computed from the light response curve by fitting the quadratic curve using SigmaPlot v. 12.5. While *the* $P_{sat}$, $ETR_{sat}$ and *PEPC* are derived from the *A-Ci* response curve. The cardinal temperatures are computed from the values, derived using the SigmaPlot.

3. Results and discussion:

There were strong interactions of temperature on photosynthesis and photochemical properties of grain sorghum. The maximum photosynthesis was observed at daytime and night time temperature of 32/ 24 °C. There were significant differences observed between the LCF block and leaf temperature for F-$P_N$/$C_i$ curves. The mean $T_L$ ''s recorded were 22.57, 26.60, 31.04, 33.78, 37.00 and 39.63 °C, indicating the increase in $T_{leaf}$ with increase in chamber temperature. Similar is the $T_{leaf}$ measured with F-$P_N$/PAR curves with the mean $T_{leaf}$ 21.72, 25.66, 29.50, 32.9, 36.48 and 37.94 °C.

3.1. Leaf $P_N$ responses to $C_i$:

$P_N$ responses to $C_i$ of grain sorghum acclimated to different temperatures followed an exponential rise to maximum function (Fig. 1). There was a significant increase in photosynthesis at above 15 $C_i$ pa. The $P_N$ values were significantly different when Ci values were above 15 Pa. The temperature responses of grain sorghum were similar at temperatures 32/24 and 36/28 °C (mean temperatures being 28 and 32 °C respectively). The maximum photosynthesis is observed at a temperature of 32/24 °C. Our results are in agreement with earlier studies conducted with sorghum to high temperature stress (Loreto et al. 1995).

In general, increase in temperature increases photosynthesis in $C_4$ species between 0 and 25% lower than that for $C_3$ species (Patterson and Flint 1990). However, the increase is limited to a certain extent where in the photosynthesis begins to drop when temperature exceeds 33 °C. Although $C_4$ plants have a higher temperature optimum than $C_3$ plants, $P_N$ is usually inhibited when leaf temperatures exceed about 38 °C (Berry and Bjorkman, 1980; Edwards and Walker, 1983).

The initial slope which is an indicator of PEPC efficiency (Caemmerer 2000) and $P_{sat}$ –$CO_2$ saturated rate (indicator of either RUBPCO activity or rate of PEP regeneration or electron transport or PEPC efficiency if it is very low) showed a quadratic response to temperature stress.

The response of $P_{sat}$ to temperature was also quadratic (Fig. 6). Highest $P_{sat}$ was observed at temperature 32/24 °C followed by 36/28 °C. This is further supported by the residual electron transport observed at higher temperatures.

3.2. Leaf $P_N$ responses to PAR:

The $C_4$ photosynthesis is characterized by light response curves that saturate at very high PAR. $P_N$ significantly increased with increase in PAR. The highest $P_N$ was achieved at high PAR in all the temperature regimes. The response curves saturated at PAR of approximately 1400 μ mol $m^{-2}s^{-1}$. Further, increase in temperature resulted in greater $P_N$ and the maximum $P_N$ was recorded at a leaf temperature of 32/24 °C. The response curves with $P_N$ at 24/16 and 36/28 °C showed similar responses (Fig. 3). The reason that photosynthetic rates were not significantly affected by season-long growth temperatures of 30/20 °C and 36/26 °C (Prasad et al. 2006) explains the maximum $P_N$ not being at 36/28 °C or 40/32 °C in our results. This is further supported by other studies that $C_4$ plants undergo thermal acclimation by reallocating nitrogen sources between photosynthetic components and, as predicted will not simply increase their photosynthetic rates (Dwyer et al. 2007). The response of irradiance-saturated maximum photosynthesis ($P_{max}$) was similar to that of $P_{sat}$ where the maximum is observed at 32/24 °C (Fig. 5).

The dark respiration ($R_D$) significantly increased with increasing temperatures (Fig. 10). Similar observations were made by Nagy et al. (2000) and V.G. Kakani et al. (2008). Influence of elevated temperature on $R_D$ was directly related to temperature effects on metabolism.

Quantum efficiency is yet another light parameter derived from the light response curves. It measures the efficiency of Photosystem II photochemistry (Genty et al., 1989) and calculated as:

$$\Phi_{PSII} = (F_m' - F_s)/F_m'$$

This parameter measures the proportion of the light absorbed by chlorophyll associated with PSII that is used in the photochemistry. It can also give a measure of the rate of linear electron transport and so an indication of overall photosynthesis. There is a strong linear relationship between quantum yield and the efficiency of carbon fixation under laboratory conditions, however, a discrepancy between the two parameters may occur under certain stress conditions, due to changes in the rate of photorespiration or pseudocyclic electron transport (Fryer et al., 1998). The initial slope of irradiance-response curves in current study showed that quantum yield of $C_4$ photosynthesis was independent of temperature because of lack of photorespiration (Fig. 9). Likewise, either [$CO_2$] or temperature did not have any significant effect on quantum efficiency in leaves of big bluestem, a $C_4$ species (V.G. Kakani, 2008). Similarly, sorghum, grown at AC (ambient [$CO_2$]) and EC (elevated [$CO_2$]) did not show any difference in $\Phi$ (Watling et al. 2000).

3.3. Photochemical responses:

The fluorescence measurements provide evidence for tolerance of grain sorghum photosynthesis to temperature stress. Measured leaf fluorescence parameters, minimal fluorescence ($F_0'$), maximal fluorescence ($F_m'$) and steady-state fluorescence ($F_s$) responded to changes in $C_i$ and PAR. Chlorophyll (Chl) fluorescence measurements indicated that PSII efficiency ($\Phi$ PSII) varied with $C_i$ in a similar way to photosynthesis in ambient $CO_2$ grown plants subjected to temperature stress. However, when $C_i$ was below 50 ppm, the ratio of $CO_2$ fixation ($\Phi CO_2$) to $\Phi$PSII, which is a measure of the energy efficiency of $CO_2$ fixation was lower. Thus, it can be attributed that at low values of $C_i$, less $CO_2$ was fixed per electron transported.

June (2004) in earlier studies reported that strong feedback links are observed between $CO_2$ fixation and ETR. The ETR responses to temperature stress was similar to that seen in $CO_2$ and irradiance curves. The highest ETR was achieved under high PAR and at 32/24 °C. However,

highest ETR was not observed at maximum temperatures since the $C_4$ plants undergo thermal acclimation when subjected to season-long temperature stress.

The response to temperature of $ETR_{sat}$ and $ETR_{max}$ derived from $F/C_i$ and $F/P_N$ is considered to be quadratic. These values were higher at 32/24 °C which are in accordance with earlier results. Loreto (1994) earlier reported that no further increase in electron transport rate was observed at temperatures greater than 40℃ and assumed the process to be temperature dependent. The change in the ratio of $CO_2$ fixation may indicate the extra requirement of electrons by carbon metabolism. The activation of a pseudo cyclic electron flow may be required to satisfy the high demand for ATP under high photosynthesis (Edwards and Baker 1993). Alternatively, it is possible that $CO_2$ leakage from bundle sheath cells (Henderson 1992) is also temperature dependent. However, it is evident that the electron requirement of $CO_2$ fixation undergoes variations when the environmental conditions are changed.

## 4. Conclusions

Our results show that $C_4$ photosynthesis under constant set of environmental conditions would acclimate o temperature. The parameters derived from the light and intercellular $CO_2$ response curves would allow development of crop simulation models to better assess the agrometeorological adaptation strategies and crop production methodologies under different management practices. Temperature had much greater effect on photosynthesis parameters and fluorescence responses to PAR. Both photosynthetic-light and A-Ci response curves suggested that the grain sorghum acclimatize its photosynthetic rate to heat stress by allowing a higher rate of electron transport. However, further research is required to understand the different mechanisms causing $P_N$ limitations at 40/32 °C in sorghum. The potential of grain sorghum surviving the future climatic regimes would have been made possible with the help of RNA-Seq studies, thereby identifying the enzymatic activity.

REFERENCES:

Ashraf, M. and P. J. Harris (2013). "Photosynthesis under stressful environments: an overview." *Photosynthetica 51(2)*: 163-190.

Baker, N. R. and E. Rosenqvist (2004). "Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities." *Journal of experimental botany 55(403)*: 1607-1621.

Berry, J. and O. Bjorkman (1980). "Photosynthetic response and adaptation to temperature in higher plants." *Annual Review of plant physiology 31(1)*: 491-543.

Centritto, M., et al. (2011). "Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (Populus nigra) saplings." *Tree physiology 31(3)*: 275-286.

Change, I. C. (2007). "The physical science basis." *Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change 996*.

Dwyer, S. A., et al. (2007). "High temperature acclimation of C4 photosynthesis is linked to changes in photosynthetic biochemistry." *Plant, cell & environment 30(1)*: 53-66.

Edwards, G. and D. Walker (1983). C3, C4: mechanisms, and cellular and environmental regulation, of photosynthesis, *Univ of California Press*.

Fryer, M. J., et al. (1998). "Relationship between CO2 assimilation, photosynthetic electron transport, and active O2 metabolism in leaves of maize in the field during periods of low temperature." *Plant Physiology 116(2)*: 571-580.

Genty, B., et al. (1989). "The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence." *Biochimica et Biophysica Acta (BBA)-General Subjects 990(1)*: 87-92.

Henderson, S. A., et al. (1992). "Short-term measurements of carbon isotope discrimination in several C4 species." *Functional plant biology 19(3)*: 263-285.

June, T., et al. (2004). "A simple new equation for the reversible temperature dependence of photosynthetic electron transport: a study on soybean leaf." *Functional plant biology 31(3)*: 275-283.

Kakani, V., et al. (2008). "Photosynthesis and fluorescence responses of C 4 plant Andropogon gerardii acclimated to temperature and carbon dioxide." *Photosynthetica 46(3)*: 420-430.

Kalra, N. and M. Kumar (2019). Simulating the impact of climate change and its variability on agriculture. Climate Change and Agriculture in India: Impact and Adaptation, *Springer*: 21-28.

Loreto, F., et al. (1994). "Measurements of mesophyll conductance, photosynthetic electron transport and alternative electron sinks of field grown wheat leaves." *Photosynthesis Research 41(3)*: 397-403.

Loreto, F., et al. (1995). "On the relationship between electron transport rate and photosynthesis in leaves of the C4 plant Sorghum bicolor exposed to water stress, temperature changes and carbon metabolism inhibition." *Functional Plant Biology 22(6)*: 885-892.

Ludlow, M. and G. Wilson (1971). "Photosynthesis of tropical pasture plants I. Illuminance, carbon dioxide concentration, leaf temperature, and leaf-air vapour pressure difference." *Australian Journal of Biological Sciences 24(3)*: 449-470.

Maiti, R. (1996). "Germination and seedling establishment." Sorghum science. *Science Publishers, Inc., Lebanon, NH*: 41-98.

Mathur, S., et al. (2014). "Photosynthesis: response to high temperature stress." *Journal of Photochemistry and Photobiology B: Biology 137*: 116-126.

Nagy, M., et al. (2000). "Interactive effect of CO 2 enrichment and temperature on the photosynthesis of field-grown hinoki cypress (Chamaecyparis obtusa) branches." *Trees 14(5)*: 282-288.

Oberhuber, W., et al. (1993). "Light dependence of quantum yields of Photosystem II and CO 2 fixation in C 3 and C 4 plants." *Photosynthesis Research 35(3)*: 265-274.

Patterson, D. T. and E. P. Flint (1990). "Implications of increasing carbon dioxide and climate change for plant communities and competition in natural and managed ecosystems." *Impact of carbon dioxide, trace gases, and climate change on global agriculture (impactofcarbond): 83-110*.

Prasad, P. V., et al. (2006). "Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [Sorghum bicolor (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures." *Agricultural and Forest Meteorology 139(3-4)*: 237-251.

Taylor, A. and J. Rowley (1971). "Plants under climatic stress: I. Low temperature, high light effects on photosynthesis." *Plant Physiology 47(5)*: 713-718.

Von Caemmerer, S. (2000). Biochemical models of leaf photosynthesis, *Csiro publishing*.

Wang, L.-J., et al. (2010). "Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves." *BMC plant biology 10(1)*: 34.

Watling, J. R., et al. (2000). "Elevated CO2 induces biochemical and ultrastructural changes in leaves of the C4 cereal sorghum." *Plant Physiology 123(3)*: 1143-1152.

Table 1. Cardinal temperatures for light and $CO_2$ response parameters in grain sorghum.

| Parameter | T min (°C) | T opt (°C) | T max (°C) |
|---|---|---|---|
| $P_{max}$ | 2.2 | 24.9 | 47.6 |
| $P_{sat}$ | 8.3 | 26.8 | 45.3 |
| $ETR_{max}$ | 7.7 | 26.2 | 44.6 |
| $ETR_{sat}$ | 6.6 | 26.8 | 47.0 |
| PEPC | -48.2 | 5.9 | 59.9 |

Fig. 1: Effect of six different temperatures on net $CO_2$ assimilation rate (A) of top most fully expanded leaves of grain sorghum in response to internal $CO_2$ concentration (Ci). Vertical bars indicate ± standard error of means (n = 3).

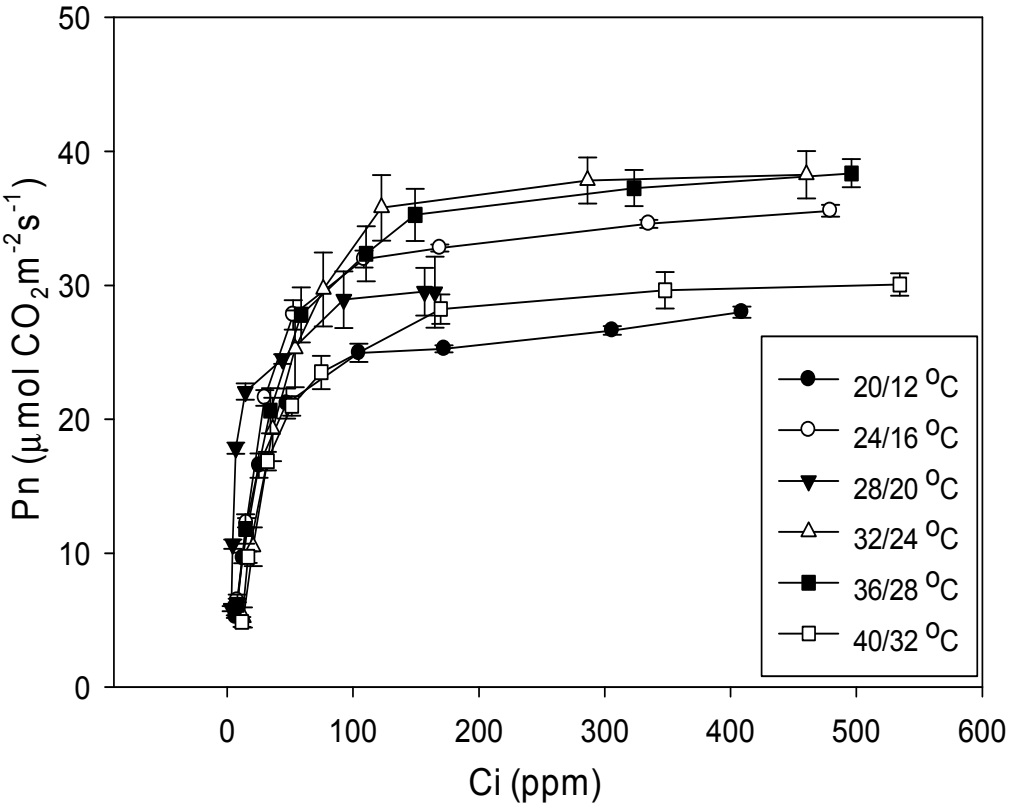


Fig. 2: Effect of six different temperatures on electron transport rate (ETR) of top most fully expanded leaves of grain sorghum in response to internal $CO_2$ concentration (Ci). Vertical bars indicate ± standard error of means (n = 3).

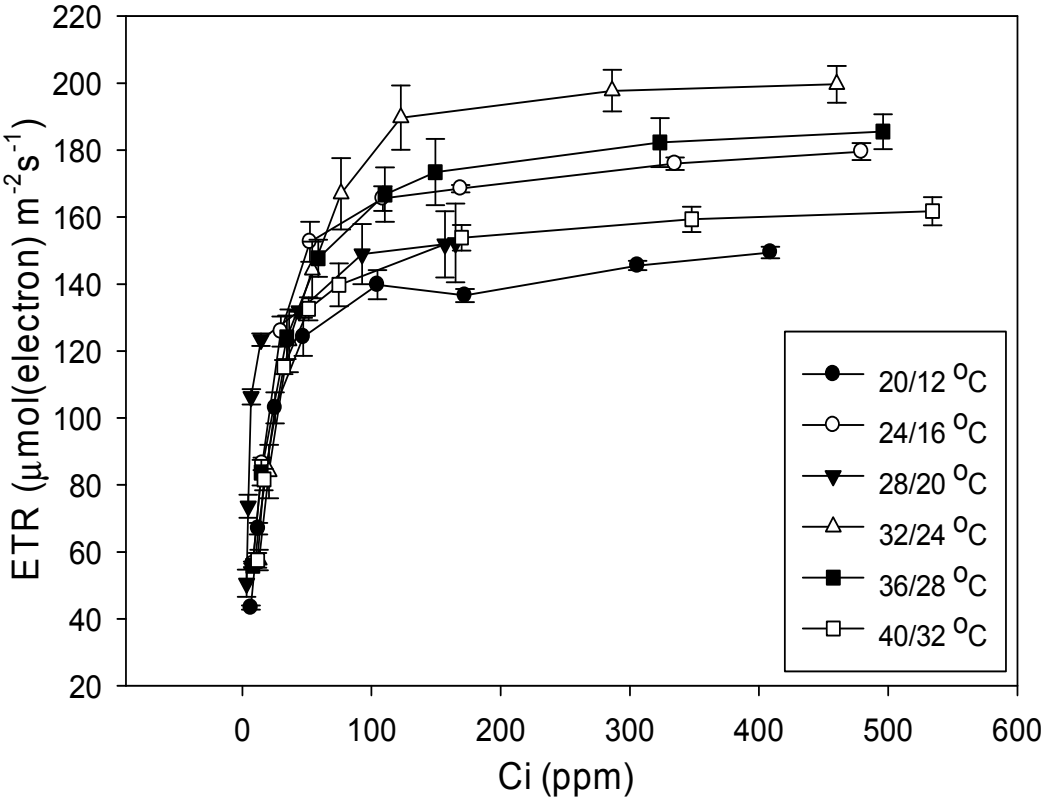


Fig. 3: Effect of six different temperatures on net $CO_2$ assimilation rate (A) of top most fully expanded leaves of grain sorghum in response to photosynthetic photon flux density (PPFD). Vertical bars indicate ± standard error of means (n = 3).

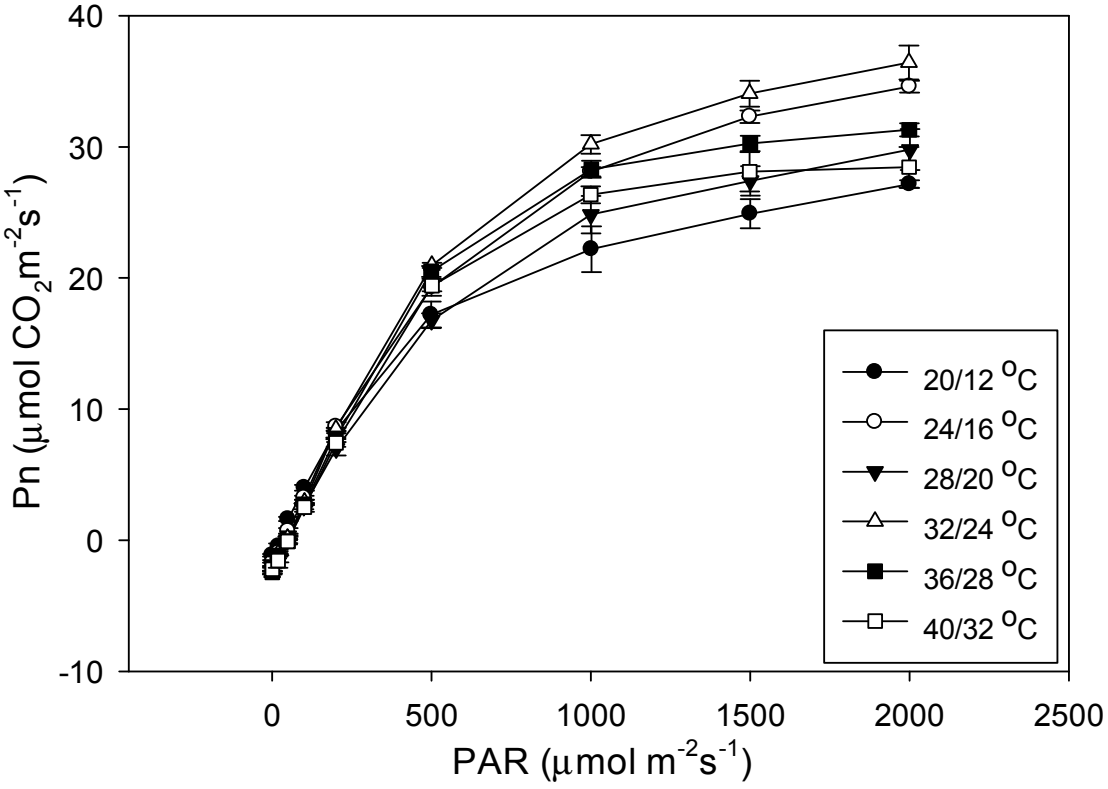


Fig. 4: Effect of six different temperatures on electron transport rate (ETR) of top most fully expanded leaves of grain sorghum in response to photosynthetic photon flux density (PPFD). Vertical bars indicate ± standard error of means (n = 3).

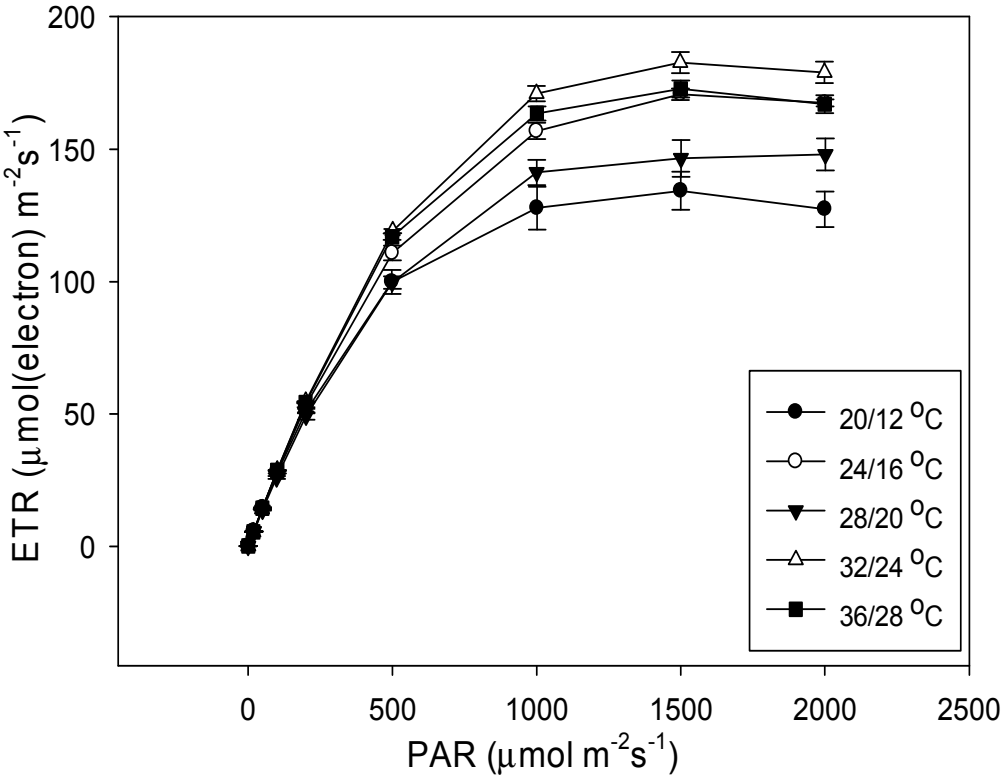


Fig. 5: Effect of maximum photosynthesis of top most fully expanded leaves of grain sorghum across six different temperatures.

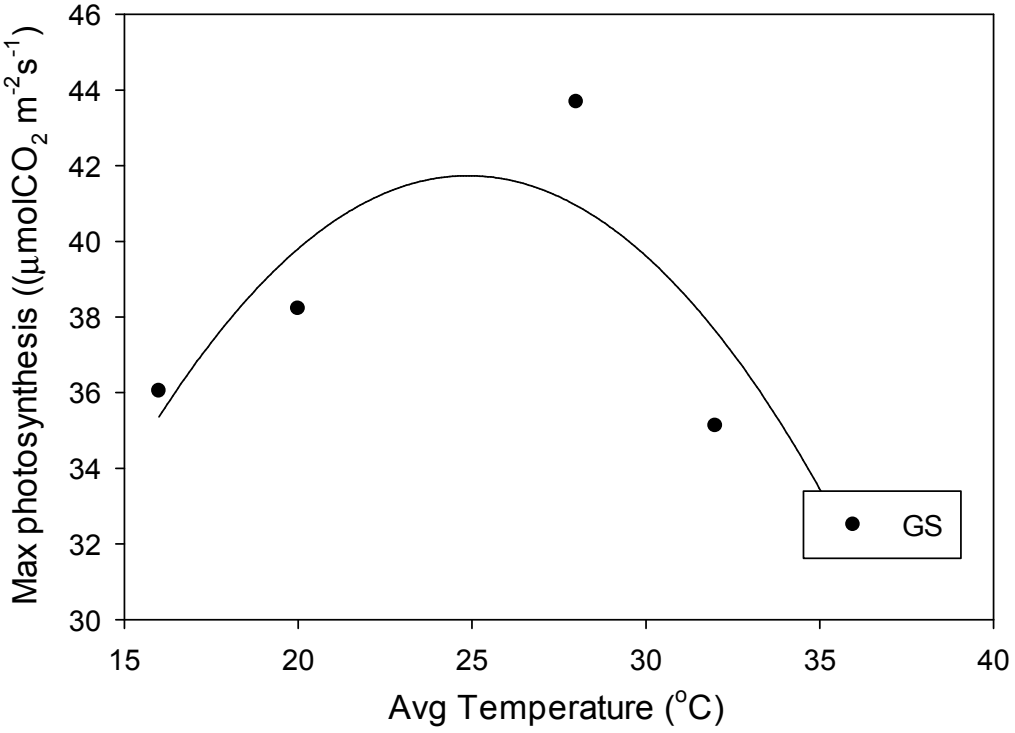


Fig. 6: Effect of saturated photosynthesis of top most fully expanded leaves of grain sorghum across six different temperatures.

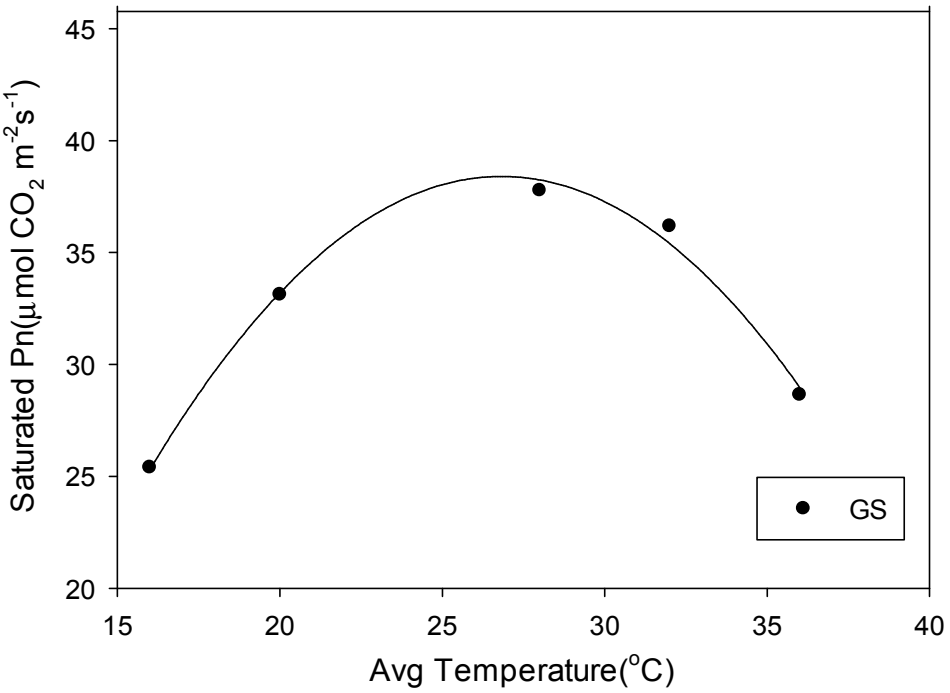


Fig. 7: Effect of saturated electron transport rate (ETR) of top most fully expanded leaves of grain sorghum across six different temperatures.

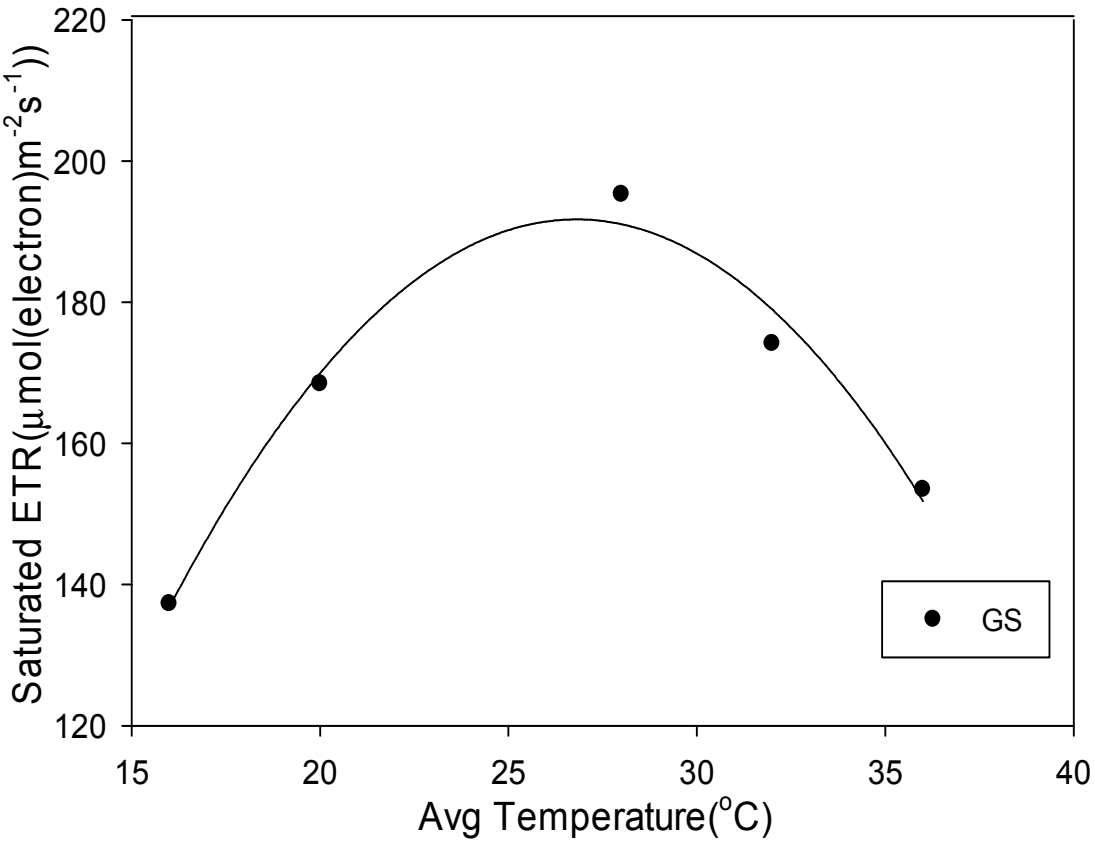


Fig. 8: Effect of maximum electron transport rate (ETR) of top most fully expanded leaves of grain sorghum across six different temperatures.

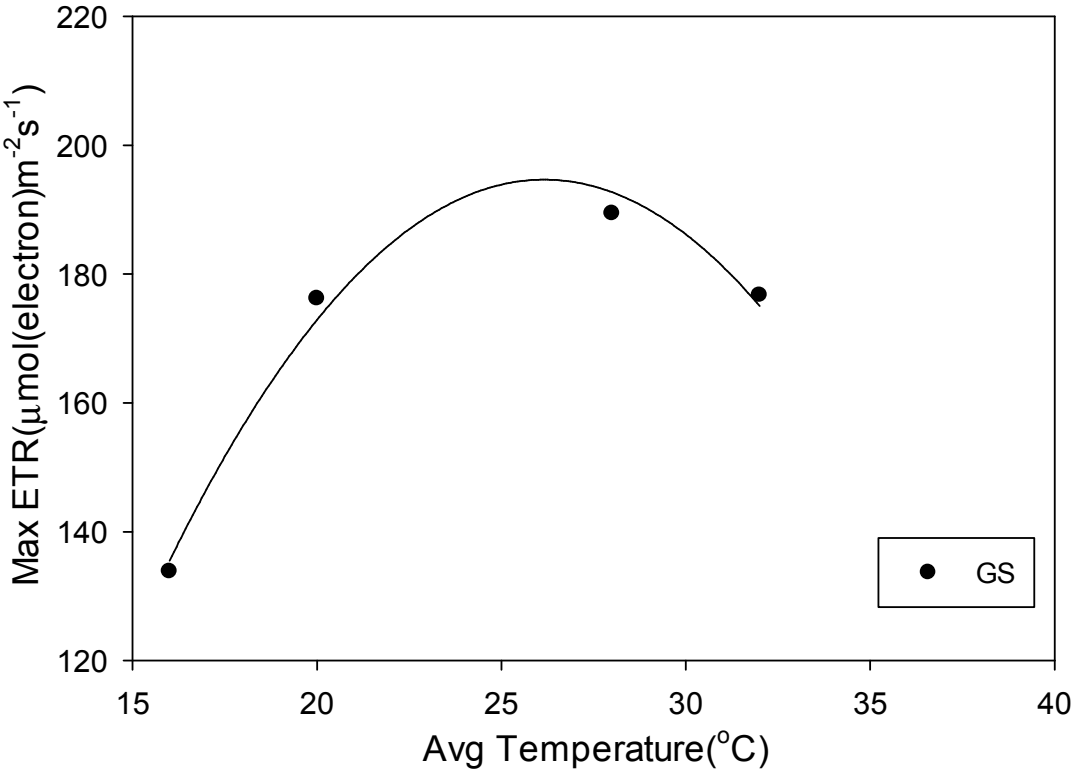


Fig. 9: Effect of maximum quantum efficiency of photosystem 2 (***Φ***) of top most fully expanded leaves of grain sorghum a cross six different temperatures.

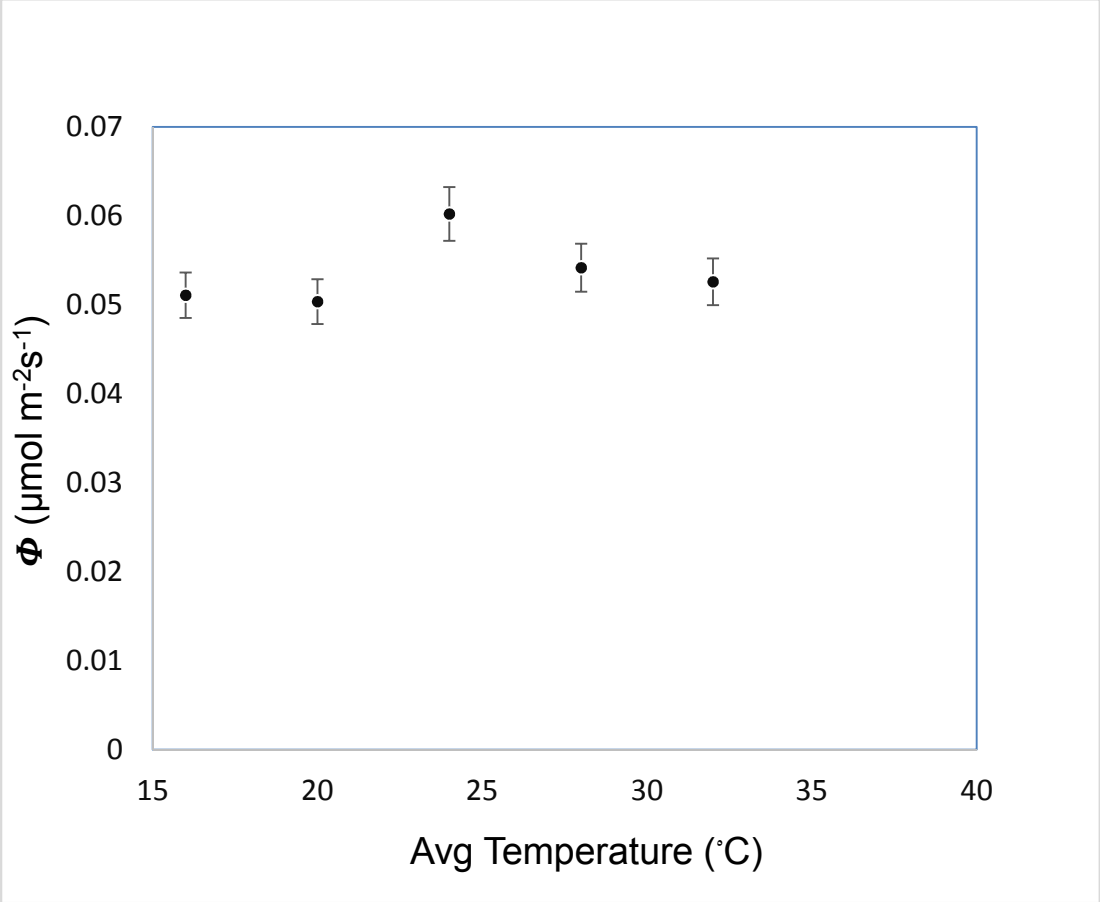
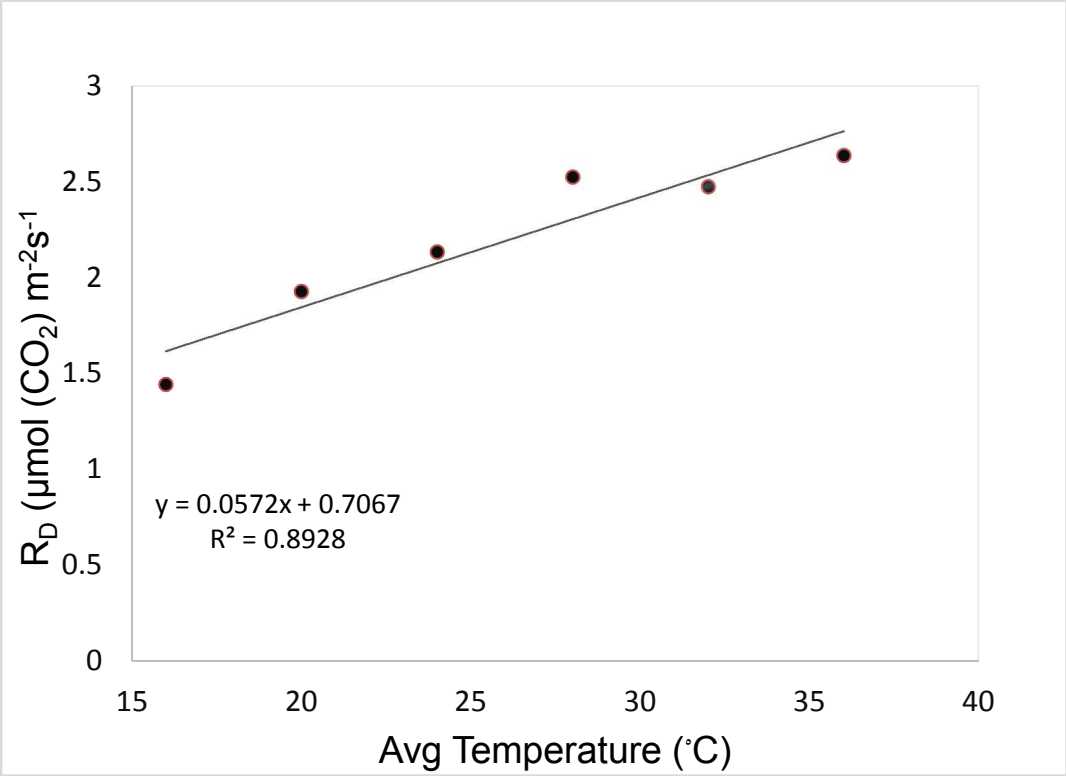


Fig. 10: Effect of dark respiration ($R_D$) of top most fully expanded leaves of grain sorghum across six different temperatures.



# CHAPTER III

## SOYBEAN RESPONSES TO TEMPERATURE: EFFECTS ON PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE


### Abstract

Temperature stress is a major environmental factor and there are limited studies elucidating its long-term impact on soybean (*Glycine max*. L. MG 3926 NRS2). The objective of present study was to quantify the effect of high temperature on gas exchange and chlorophyll fluorescence parameters in soybean. An experiment was conducted using walk-in growth chambers in Controlled Environment Research Facility at Oklahoma State University to study the effects of six different temperatures (20/12, 24/16, 28/20, 32/24, 36/28, and 40/32 °C) to evaluate the photosynthetic responses to temperature gradient. The rates of photosynthesis declined as the growing temperatures increased; whereas intercellular $CO_2$ and transpiration rates were increased. Soybean carbon assimilation would perform well under rising atmospheric temperature, provided they are well irrigated through an increased rate of electron transport. The photosynthesis parameters can be used to develop mechanistic simulation models and adaptation strategies to be able to thrive in future climates. However, further behavior of photosynthetic apparatus at extreme temperature stress can be quantified by analysis of molecular samples to identify the desirable genes responsible for the severe heat stress tolerance.

1. Introduction

Temperatures have been on the rise globally. The increase in temperatures are attributed to the effects of global warming. The effects of global warming on agricultural productivity is not new to this era. Studies have enumerated the causes and effects on global farming in various crops. Soybean is a major agricultural crop of the United States where the total production accounts to about 4.55 billion bushels in the year 2018 alone.

Soybean crop is often subjected to temperature stress particularly in tropical and semiarid tropical regions. The reduction in photosynthesis, abscission and abortion of flowers, development of seeds and young pods at high temperatures is considered to be the main cause of yield reduction in soybean (Prasad et al. 2006, 2008). The optimum temperature is at vegetative phase is reported to be 30 °C (Hesketh et al. 1973). The reproductive growth is more sensitive to temperature. The optimum temperature for post-anthesis period is about 23 °C and above this temperature seed growth rate, seed size and intensity of partitioning to seed decreases (Sionit et al. 1987; Pan, 1996; Thomas et al., 2003).

The optimum range of temperatures for the growth and development of soybean has been reported as 20-30 °C (Egli and Wardlaw, 1980; Hofstra, 1972; Hesketh and Wiley, 1973). The growth, yield and quality of soybean are greatly influenced by temperature (Liu et al. 2008). Low day or night temperatures during the growing season reduce vegetative growth, prolong the time period between R1 (i.e., the appearance of first open flower (Fehr and Caviness, 1977) R2 stages (i.e., the appearance of flowers at the node immediately below the uppermost node (Fehr and Caviness, 1977), decrease the seed yield of soybean plants (Seddigh and Gary, 1984).

Both high and low temperatures are responsible for causing stress in crop plants, significantly decreasing the yields. Heat stress is defined as the rise in temperature beyond a threshold level to cause irreversible damage to plant growth and development (Wahid and Close, 2007).

Photosynthesis is regarded as important indicator of growth in plants because of its direct association with net productivity (Ashraf 2004). The primary process affected due to temperature stress is photosynthesis. High growth temperatures appeared to be more deleterious to the soybean plants grown at ambient CO2 conditions (JCV Vu et al., 2001). The chlorophyll fluorescence parameters such as the ratio of variable fluorescence to maximum fluorescence (Fv/Fm), and the base fluorescence (Fo) were reported to show a negative correlation with heat tolerance (Yamada et al. 1996). Therefore, chlorophyll fluorescence techniques are useful for eco-physiological studies in assessing plant responses to environmental conditions such as water and temperature stress (Krause and Weis 1991, Srinivasan et al. 1996).

Soybean when subjected to low but non-freezing temperatures adversely affects a wide range of physiological processes, including photosynthesis (Purcell et al. 1987), carbohydrate metabolism (Judith, 1981), leaf water potential (David Wolfe. 1991), cellular lipid composition (De Kok. 1977) and the integrity of cell membrane (Chen and Chin-Ho Lin. 1993). Caulfield and Bunce (1988) showed that the leaf photosynthesis was decreased in soybean plants exposed to short-term cold temperatures of 5-8 °C in greenhouse and growth chamber studies. This can be attributed to the feedback inhibition by carbohydrates during the cold period (Azcón-Bieto, J. 1983).

Soybean (cv. Maple Arrow) grown at a temperature of 20 °C and exposed to 5 °C showed significantly less photosynthesis at the low temperature than plants grown at a temperature of 12.5 °C (Marowitch and Hoddinott, 1986). Opposite results were obtained in two $C_4$ Bouteloua species by Bowman and Turner (1993) who reported that when plants were exposed to a cold day/night temperature of 10/-2 °C for 2 days the reduction in photosynthesis was greater for plants grown in the cool (20/6 °C) than the warmer growth temperatures (30/16 °C). The sensitivity of cold injury depends on the plant developmental stages (Purcell et al., 1987; Chen

and Chin, 1993). However, season long exposure to cold temperatures and its effects on photosynthesis were not studied.

Despite the well documented effects of short-term cold growing season temperature on plant growth and development, there are only limited data available on the long-term growing season and evaluating the study by comparing the cold and warmer temperatures across the growing season. Additionally, the majority of studies have focused on long-term temperature effects on physiology and observing the ultrastructure of leaves. The objectives of this study were to understand the effects of high and low temperatures on chlorophyll fluorescence and photosynthesis when the crop is grown for whole life cycle under different temperature regimes.

## 2. Materials and methods:

### 2.1. Experimental conditions and plant culture:

The experiment was conducted during the fall of 2017 at the Controlled Environment Research Laboratory (CERL), Stillwater, Oklahoma State University (36.125161, -97.076602), OK, USA, using 6 large Conviron controlled environment growth chambers (32 sq. ft. growth space). The large chambers are capable of providing low temperatures to -10 °C lights off, -5 °C lights on. Large chambers generally provide 960 μmol $m^{-2}$ $s^{-1}$ lighting but the high intensity models can provide 1320 μmol $m^{-2}$ $s^{-1}$. A full color spectrum of light is supplied by a combination of high intensity fluorescent bulbs and incandescent bulbs. Lights are programmable in 4 or 8 lighting levels to simulate natural dawn to dusk. Several of the chambers have humidity control.

Seeds of soybean (*Glycine max*. L. MG 3926 NRS2) were sown in pots measuring 45 cm tall and 20 cm in diameter. The rooting medium used to fill the pots was pure, fine sand. Emergence was observed 5 days later. Plants were fertilized by irrigating three times a day with Hoagland's nutrient solution delivered at 0800, 1200 and 1600 hours to ensure favorable nutrient and water

conditions for plant growth. Irrigation with Hoagland's nutrient solution was provided through an automated computer-controlled subsurface drip system.

2.2. Treatments

Soybean was grown in all chambers at 28/20 °C until the treatments were initiated. The temperatures were maintained within 4 °C of treatment set points of 28/20 °C (day/night) in all units until the seedlings had emerged and were uniformly established. Each of the six chambers were assigned one of six treatments at 15-20 d after the seedling (DAS). The treatments corresponding to six chambers consisted of 20/12, 24/16, 28/20, 32/24, 36/28, and 40/36 °C. A dedicated computer with in-house coded software monitored and controlled the environmental variables. The chambers have in-built humidity controls. Three sets of one to two topmost fully expanded leaves in each of the six treatments were selected for photosynthesis measurements.

2.3. Gas exchange measurements:

Gas exchange measurements were made on attached leaves using an open gas exchange LI-6400 system (Li-Cor Inc.) fitted with a 6400-40 leaf chamber fluorometer (LCF) that provides LED-based fluorescence and light. Three sets of two topmost (positioned at 3$^{rd}$ and 4$^{th}$), fully expanded leaves of similar age in each growth chamber were selected for photosynthesis measurements.

Parameters were derived from the measured photosynthesis and fluorescence data using equations described in the LI-6400 manual. These parameters include fraction of photons absorbed by lead adapted to dark ($F_v/F_m$), efficiency of energy harvesting by oxidized PSII reaction centers in the light ($F_v'/F_m'$), the fraction of absorbed photons that are used in the photochemistry for a light adapted leaf ($\boldsymbol{\Phi}_{PSII}$) and the apparent quantum yield of $CO_2$ assimilation at any given irradiance ($\boldsymbol{\Phi}_{CO2}$), photochemical quenching (qP) and electron transport rate (ETR). The equations used to derive these values are shown below:

$$\Phi_{PSII} = (F_m' - F_s)/F_m' \qquad \text{[unitless]}$$

$$\Phi_{CO2} = (P_N - P_{dark})/I\alpha_{leaf} \qquad [\mu mol(CO_2)\ \mu mol(photon)^{-1}]$$

$$q_N = (F_m - F_m')/(F_m - F_o')$$

$$q_P = (F_m' - F_s)/(F_m' - F_o')$$

$$NPQ = F_m - F_m'/F_m'$$

Where $F_s$ is steady-state fluorescence, and $F_m'$ is the maximal fluorescence during a saturating light flash.

2.3.1. Fluorescence and net photosynthesis/internal carbon dioxide ($F-P_N/Ci$) curves:

The unique software program in *LI-6400* photosynthesis systems for $F-P_N/Ci$ curves was used to generate the response of $P_N$ to $C_i$. Net photosynthesis and chlorophyll fluorescence characteristics were determined simultaneously. The top most expanded young leaves were selected for the measurements after 50 to 65 days old plants. Measurements were taken between 09:00 and 11:00 h by changing [$CO_2$] in leaf chamber fluorometer in 9 steps (400, 300, 200, 100, 50, 400, 400, 600 and 800 µmol $mol^{-1}$) under a constant PAR of 1000 µmol $m^{-2}s^{-1}$. The block temperature was set to corresponding growth chamber day time temperature. The time allowed for the instrument to reach the steady state at each [$CO_2$] was 240 seconds. The instrument logged values when the steady state indicated by total coefficient of variation was ≤ 5 %. Curve fitting software (Sigma Plot for Windows 12.5) was used to analyze the $F-P_N/Ci$ responses using a three component exponential to maximum function of the term

$$P_N = a\ (1 - e^{-bx}) + y_0$$

Where $P_N$ = steady-state assimilation rate and $x = C_i$.

Using this equation, $P_{sat}$ was calculated as $a + y_0$ and phosphoenolpyruvate carboxylase (PEPC) efficiency as the slope of $P_N$ =0, calculated *as* $b[a + y_0]$. Likewise, saturated values of ETR ($ETR_{sat}$) were calculated by fitting exponential to maximum function to ETR and $C_i$.

2.3.2. Fluorescence and net photosynthesis/PAR ($F-P_N$/ PAR) curves:

These measurements were made between 09:00 and 11:00 h on top most fully expanded leaves by reducing PAR in 9 steps from 2000 to 0 µmol $m^{-2}s^{-1}$. The [$CO_2$] was kept constant at 400 µmol $mol^{-1}$ and the block temperature inside the leaf cuvette was set to the treatment day time temperature in the corresponding growth chamber. The nine values of PAR are 2000, 1500, 1000, 500, 200, 100, 50, 20, and 0 µmol $m^{-2}s^{-1}$. The time allowed for the instrument to reach steady state at each PAR level is 240 s. The instrument logged values when the steady state indicated by total coefficient of variation was ≤ 5 %. The photosynthetic irradiance response curves were fit using non-rectangular hyperbola least square fitting procedure and the model is described as:

$$P_N = \frac{\Phi Q + P\,max - \sqrt{(\Phi Q + Pmax)2 - 4\,\Phi Q k Pmax}}{2\,k} - R_D$$

Where $\boldsymbol{\Phi}$ is the apparent quantum efficiency, Q is the PAR, $P_{max}$ is the PAR saturated rate of gross $CO_2$ assimilation, k is the curvature factor, and $R_D$ is the dark respiration rate. Maximum values of ETR ($ETR_{max}$) were calculated by fitting exponential to maximum function to ETR and PAR. R code is used to derive the parameters from irradiance response curves.

2.4. Estimation of cardinal temperatures for the response parameters:

The response parameters like $P_{max}$, $P_{sat}$, $ETR_{max}$, and $ETR_{sat}$ are derived from the light and $CO_2$ response curves. The $P_{max}$, and $ETR_{max}$ are computed from the light response curve by fitting the quadratic curve using SigmaPlot v. 12.5. While *the* $P_{sat}$, and $ETR_{sat}$ are derived from the *A-Ci* response curve. The cardinal temperatures are computed from the values, derived using the SigmaPlot.

3. Results:

Strong interactions of temperature on photosynthesis and photochemical properties of soybean were observed. As mentioned, the temperature inside the leaf cuvette was set to the treatment day time temperature in the corresponding growth chamber. There were significant differences observed between the LCF block and leaf temperature for F-$P_N$/Ci curves. The mean $T_L$'s recorded were 23.50, 26.27, 30.32, 32.94, 36.15, and 37.69 °C, indicating that increase in $T_{leaf}$ with increase in chamber temperature. Likewise, the mean $T_{leaf}$ measured with F-$P_N$/PAR curves are 21.58, 23.93, 25.98, 30.06, 34.26, and 38.65 °C.

3.1. Leaf photosynthetic-light response:

The measurements of the photosynthesis light response curves indicated that the $P_N$ and ETR values is same across all temperature regimes and did not differ significantly when the PAR is less than 500 μmol $m^{-2}s^{-1}$. As the PAR kept increasing, the photosynthesis and ETR accelerates at slower rate in respective chambers. The $P_N$ and ETR shows the least recorded values in the chamber maintained at 20/12 °C at all values of PAR (Fig. 4). Measurements at 32/24, 28/20 and 24/16 °C had same higher ETR values at all levels of PAR. While, $P_N$ values at 32/24, 28/20 and 24/16 are significantly different at all levels of PAR where the optimum temperature (28/20 °C) has the maximum photosynthesis (Fig. 3). In response to the parameters developed from the light response curves, the maximum photosynthesis ($P_{max}$) exhibited a quadratic trend (Fig. 5). The light saturated point (Fig. 7) also followed a quadratic response with respect to temperature increase along with light compensation point (LCP) (Fig. 8).

3.2. Leaf photosynthetic-$CO_2$ ($P_N$-$C_i$) response

The responses of $P_N$ to Ci showed an exponential rise to maximum developed from A-Ci curves (Fig. 1). The $P_N$ values are significantly different when Ci is above 200 ppm. Conversely, there is significant difference between the ETR values across different temperatures irrespective of Ci

(Fig. 2). However, both $P_N$ and ETR values kept increasing with increasing Ci. 20/12 °C has the lowest ETR and $P_N$ across Ci followed by 40/32 °C. While, ETR and $P_N$ almost attained stability at about 450 ppm, there is no significant increase observed beyond 500 ppm. 24/16 and 36/28 and 32/24 °C has similar photosynthesis while the ETR values were found significantly different. 32/ 24 and 36/28 °C has similar ETR. The response parameters derived from the $P_N$-Ci curves showed that saturated photosynthesis exhibited a quadratic trend (Fig. 6).

### 3.3. Quantum yield of PS II Photochemistry

The maximum quantum yield of the PS II photochemistry ($\boldsymbol{\Phi}_{PSII}$) was estimated by measuring the modulated chlorophyll a fluorescence in dark-adapted leaves (Genty et al., 1989). Irrespective of the temperature to which leaves had been exposed, the $\boldsymbol{\Phi}_{PSII}$ range is 0.1. This is in agreement with the results in wheat exposed to temperature stress where $\boldsymbol{\Phi}_{PSII}$ was close to 0.8 between 5 °C and 35 °C (Yamasaki et al., 2002). Therefore, the difference in the maximum photosynthetic rate among the different treatments may not be attributed to the difference in the magnitude of photo inhibition.

## 4. Discussion

Crop responses to temperature depend on the specific optimum temperature for photosynthesis, growth and yield (Conroy et al. 1994). If the temperature is below optimum, a slight increase in temperature may lead to increased plant growth and development, but if the temperature is close to maximum, a small increase in temperature can negatively affect crop growth and in turn, decrease yield (Baker and Allen., 1993). Crop yield is greatly affected by elevated temperature stress and has been directly correlatd with decreased photosynthetic efficiency (Georgieva et al., 2000). This experiment was conducted to study the physiology of soybean plants subjected to a wide range of temperature stress. Chlorophyll fluorescence analysis is one of the widely used

techniques to study the effects of temperature stress on photosynthetic processes. The measurements helped to evaluate the response of PSII to variable temperature stresses.

The A-Ci curves exhibited a linear response of increase in photosynthesis with internal $CO_2$ concentration upto 300 ppm irrespective of temperature. After the Ci reached 350 ppm, there was an effect of temperature stress where in the curve with temperature 36/28 °C showed maximum photosynthesis followed by 24/16 °C.

Rate of photosynthesis in soybean is significantly affected by temperature, as seen from the photosynthetic light and $CO_2$ response curves. The maximum rate of photosynthesis in soybean was observed in plants grown under ambient temperature and increase in temperature upto 40/32 °C resulted in marginal decline. Similar results were observed by Jumrani. K et al (2017) where the interaction of temperature * genotype was significant indicating that the impact of temperature on rate of photosynthesis differed among the genotypes. Previous studies provided the basis for decrease in assimilation efficiency to the inactivation of Rubisco. In cotton (*Gossypium hirsutum*), wheat (*Triticum aestivum*), tobacco (*Nicotiana tabacum*), and maize (*Zea mays*), a decrease in Rubisco activation under moderate stress correlated with reduced rates of net photosynthesis and was accompanied by increased levels of RUBP and decreased levels of 3-phosphoglycerate (Kobza and Edwards, 1987). A similar relationship was observed between the responses of $P_N$ and the extent of inhibition of $P_N$ and Rubisco activation to elevated temperature has been reported in spinach (Weis., 1981b) and maize (Crafts-Brandner and Salvucci., 2002). The reason because photosynthesis declines in the current study above the thermal optimum is the capacity of Rubisco activase to maintain Rubisco in an activated state declines to limiting levels. As a result, Rubisco deactivates to a point where its ability to consume RuBP limits $CO_2$ assimilation (Salvucci & Crafts 2004a).

The phenomenon of photoinhibition has been studied well by using biophysical and genetic approaches. As according to Murchie and Lawson (2013), chlorophyll fluorescence is one of the most utilized ecophysiological techniques to study the photosynthetic process in plants. Photoinhibition defines as the reduction in photosynthetic efficiency of a plant as a result of damage to photosystem II (PSII), which occurs when the absorbed photons exceed the requirement of photosynthesis processes under high light conditions (Mathur et al., 2014). Recent studies have recommended that high temperature did not make grave damage to PSII; alternatively, it suppressed its repair mechanism (Tyystjarvi, 2012; Lu et al., 2017). The primary damage due to the temperature stress is loss of stability and the disorganization of membranes (Vitolo et al. 2012). However, the $\boldsymbol{\Phi}_{PSII}$ values showed that there is no damage to the photosystem II (PSII) due to photo inhibition. Increasing $R_d$ is found which might have added to the energy supply for cellular repair process (Fig. 9). This higher cost of maintenance respiration could have been supported by greater $CO_2$ assimilation rate; $P_{max}$ in the treatment 28/20 °C. Photorespiration and mitochondrial respiration increase with rising temperature (Brooks & Farquhar, 1985; Sage et al., 1990a).

In addition, the moderately high temperature treatments impair the activation of Rubisco by its catalytic chaperone, Rubisco activase (Rca). This becomes the primary cause of the decrease in carbon-dioxide assimilation in response to elevated temperatures (Kim and Portis, 2005; Galmes et al., 2013).

## 5. Conclusions

The soybeans of the future will face fundamentally different patterns of control over photosynthetic carbon gain with respect to rising temperatures. The photosynthetic light and carbon dioxide response curves show that the assimilation efficiency acclimates to rising temperatures. However, there is an increased rate of electron transport rate and unaffected $\boldsymbol{\Phi}_{PSI}$ due to photo inhibition. The light and $CO_2$ response parameters were studied across temperatures.

The decline in photosynthetic rate at severe heat stress can be studied further to understand the possible role of the different mechanisms in soybean leaves.

REFERENCES:

Ashraf, M. (2004). "Some important physiological selection criteria for salt tolerance in plants." *Flora-Morphology, Distribution, Functional Ecology of Plants 199(5)*: 361-376.

Azcón-Bieto, J. (1983). "Inhibition of photosynthesis by carbohydrates in wheat leaves." Plant Physiology 73(3): 681-686.

Azcón-Bieto, J. (1983). "Inhibition of photosynthesis by carbohydrates in wheat leaves." *Plant Physiology 73(3)*: 681-686.

Baker, J. and L. Allen (1993). "Contrasting crop species responses to CO 2 and temperature: rice, soybean and citrus." *Vegetatio 104(1)*: 239-260.

Bowman, W. D. and L. Turner (1993). "Photosynthetic sensitivity to temperature in populations of two C4 Bouteloua (Poaceae) species native to different altitudes." *American journal of botany 80(4)*: 369-374.

Brooks, A. and G. Farquhar (1985). "Effect of temperature on the CO 2/O 2 specificity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light." *Planta 165(3)*: 397-406.

Campbell, D. A. and E. Tyystjärvi (2012). "Parameterization of photosystem II photoinactivation and repair." *Biochimica et Biophysica Acta (BBA)-Bioenergetics 1817(1)*: 258-265.

Caulfield, F. and J. Bunce (1988). "Comparative responses of photosynthesis to growth temperature in soybean (Glycine max [L.] Merrill) cultivars." *Canadian Journal of Plant Science 68(2)*: 419-425.

Chen, Y. Y. and C.-H. Lin (1993). "Effect of LAB 173 711, an ABA analogue, on low-temperature resistance of mung bean seedlings." *Journal of plant growth regulation 12(1)*: 51-55.

Conroy, J., et al. (1994). "Influence of rising atmospheric CO2 concentrations and temperature on growth, yield and grain quality of cereal crops." *Functional Plant Biology 21(6)*: 741-758.

Crafts-Brandner, S. J. and M. E. Salvucci (2002). "Sensitivity of photosynthesis in a C4 plant, maize, to heat stress." *Plant Physiology 129(4)*: 1773-1780.

De Kok, L. J. and P. J. Kuiper (1977). "Glycolipid degradation in leaves of the thermophilic Cucumis sativus as affected by light and low□temperature treatment." *Physiologia Plantarum 39(2)*: 123-128.

Egli, D. and I. Wardlaw (1980). "Temperature Response of Seed Growth Characteristics of Soybeans 1." *Agronomy Journal 72(3)*: 560-564.

Fehr, W. R. and C. E. Caviness (1977). "Stages of soybean development."

Galmes, J., et al. (2013). "Variation in Rubisco content and activity under variable climatic factors." *Photosynthesis Research 117(1-3)*: 73-90.

Galmés, J., et al. (2013). "Variation in Rubisco content and activity under variable climatic factors." *Photosynthesis Research 117(1-3)*: 73-90.

Genty, B., et al. (1989). "The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence." *Biochimica et Biophysica Acta (BBA)-General Subjects 990(1)*: 87-92.

Georgieva, K., et al. (2000). "Photosynthetic activity during high temperature treatment of pea plants." *Journal of plant physiology 157(2)*: 169-176.

Hesketh, J., et al. (1973). "Temperature control of time intervals between vegetative and reproductive events in soybeans 1." *Crop Science 13(2)*: 250-254.

Hofstra, G. (1972). "Response of soybeans to temperature under high light intensities." *Canadian Journal of Plant Science 52(4)*: 535-543.

Jumrani, K., et al. (2017). "Impact of elevated temperatures on specific leaf weight, stomatal density, photosynthesis and chlorophyll fluorescence in soybean." Photosynthesis Research 131(3): 333-350.

Kim, K. and A. R. Portis Jr (2005). "Temperature dependence of photosynthesis in Arabidopsis plants with modifications in Rubisco activase and membrane fluidity." *Plant and Cell Physiology 46(3)*: 522-530.

Kobza, J. and G. E. Edwards (1987). "Influences of leaf temperature on photosynthetic carbon metabolism in wheat." *Plant Physiology 83(1)*: 69-74.

Krause, G. and E. Weis (1991). "Chlorophyll fluorescence and photosynthesis: the basics." *Annual review of plant biology 42(1)*: 313-349.

Liu, X., et al. (2008). "Soybean yield physiology and development of high-yielding practices in Northeast China." *Field Crops Research 105(3)*: 157-171.

Lu, T., et al. (2017). "Sub-high temperature and high light intensity induced irreversible inhibition on photosynthesis system of tomato plant (Solanum lycopersicum L.)." *Frontiers in plant science 8*: 365.

Marowitch, J., et al. (1986). "The influence of plant temperature on photosynthesis and translocation rates in bean and soybean." *Canadian journal of botany 64(10)*: 2337-2342.

Mathur, S., et al. (2014). "Photosynthesis: response to high temperature stress." *Journal of Photochemistry and Photobiology B: Biology 137*: 116-126.

Murchie, E. H. and T. Lawson (2013). "Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications." *Journal of experimental botany 64(13)*: 3983-3998.

Pan, D. (1996). Soybean responses to elevated temperature and doubled CO2, University of Florida.

Prasad, P. V., et al. (2006). "Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [Sorghum bicolor (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures." *Agricultural and forest meteorology 139(3-4)*: 237-251.

Prasad, P. V., et al. (2008). "Sensitivity of grain sorghum to high temperature stress during reproductive development." *Crop Science 48(5)*: 1911-1917.

Purcell, L., et al. (1987). "Effects of Chilling on Photosynthetic Capacity, and Leaf Carbohydrate and Nitrogen Status of Soybean 1." *Crop Science 27(1)*: 90-95.

Purcell, L., et al. (1987). "Effects of Chilling on Photosynthetic Capacity, and Leaf Carbohydrate and Nitrogen Status of Soybean 1." *Crop Science 27(1)*: 90-95.

Salvucci, M. E. and S. J. Crafts-Brandner (2004). "Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments." *Plant Physiology 134(4)*: 1460-1470.

Seddigh, M. and G. D. Jolliff (1984). "Night Temperature Effects on Morphology, Phenology, Yield and Yield Components of Indeterminate Field-Grown Soybean 1." *Agronomy Journal 76(5)*: 824-828.

Sionit, N., et al. (1987). "Interaction of temperature and CO2 enrichment on soybean: Growth and dry matter partitioning." *Canadian Journal of Plant Science 67(1)*: 59-67.

Srinivasan, A., et al. (1996). "Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques." *Euphytica 88(1)*: 35-45.

Thomas, J. F., et al. (1981). "Day and Night Temperature Effects on Nitrogen and Soluble Carbohydrate Allocation During Early Reproductive Growth in Soybeans 1." *Agronomy Journal 73(4)*: 577-582.

Thomas, J., et al. (2003). "Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance." *Crop Science 43(4)*: 1548-1557.

Tyystjärvi, E. (2013). Photoinhibition of photosystem II. *International review of cell and molecular biology, Elsevier. 300*: 243-303.

Vítolo, H. F., et al. (2012). "Cross-scale multivariate analysis of physiological responses to high temperature in two tropical crops with C3 and C4 metabolism." *Environmental and experimental botany 80*: 54-62.

Vu, J. C., et al. (2001). "Soybean photosynthesis, Rubisco, and carbohydrate enzymes function at supraoptimal temperatures in elevated CO2." *Journal of plant physiology 158(3)*: 295-307.

Wahid, A. and T. Close (2007). "Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves." *Biologia Plantarum 51(1)*: 104-109.

Weis, E. (1981). "The temperature□sensitivity of dark□inactivation and light□activation of the ribulose□1, 5□bisphosphate carboxylase in spinach chloroplasts." *FEBS letters 129(2)*: 197-200.

Wolfe, D. W. (1991). "Low temperature effects on early vegetative growth, leaf gas exchange and water potential of chilling-sensitive and chilling-tolerant crop species." *Annals of botany 67(3)*: 205-212.

Yamada, M., et al. (1996). "Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence." *Scientia Horticulturae 67(1-2)*: 39-48.

Yamasaki, T., et al. (2002). "Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter wheat." *Plant Physiology 128(3)*: 1087-1097.

Yamori, W., et al. (2005). "Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions." *Plant, cell & environment 28(4)*: 536-547.

Table 1. Cardinal temperatures for light and $CO_2$ response parameters in soybean.

| Parameter | T min (°C) | T opt (°C) | T max (°C) |
|---|---|---|---|
| $P_{max}$ | 9.08 | 25.83 | 42.57 |
| $P_{sat}$ | 9.28 | 31.72 | 54.15 |
| $ETR_{max}$ | 8.40 | 27.92 | 47.45 |
| $ETR_{sat}$ | -0.06 | 28.71 | 57.48 |

Fig. 1: Effect of six different temperatures on net $CO_2$ assimilation rate (A) of top most fully expanded leaves of soybean in response to internal $CO_2$ concentration (Ci). Vertical bars indicate ± standard error of means (n = 3).

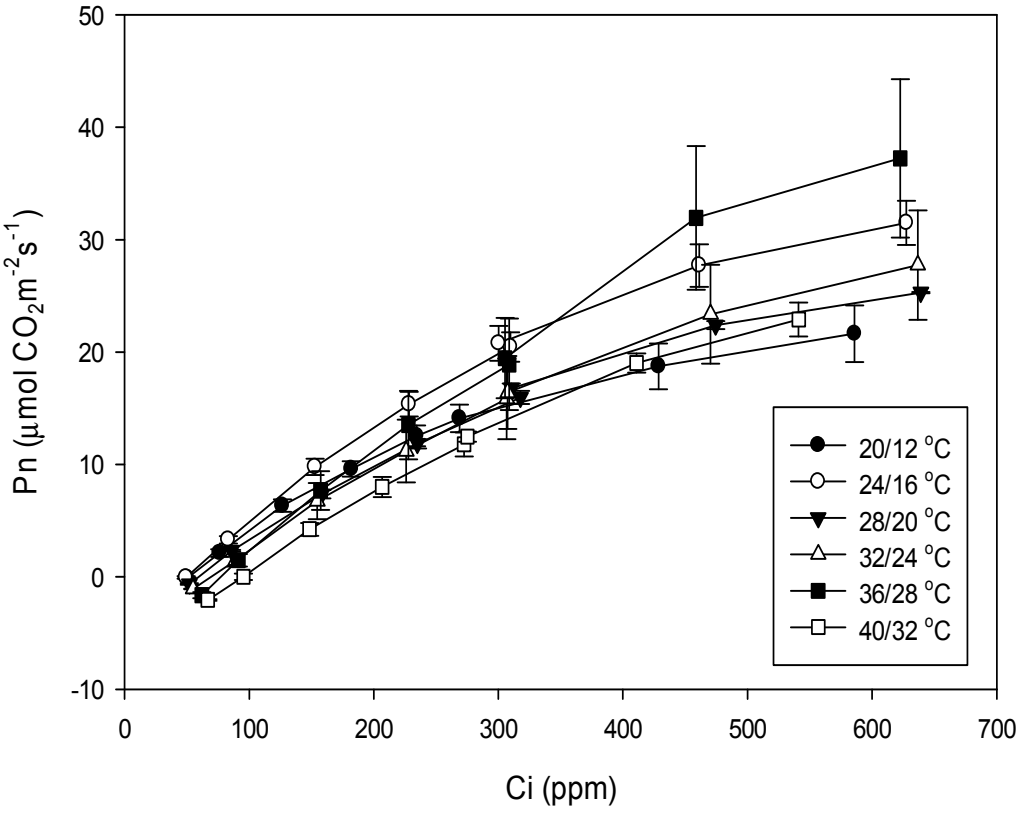


Fig. 2: Effect of six different temperatures on electron transport rate (ETR) of top most fully expanded leaves of soybean in response to internal $CO_2$ concentration (Ci). Vertical bars indicate ± standard error of means (n = 3).

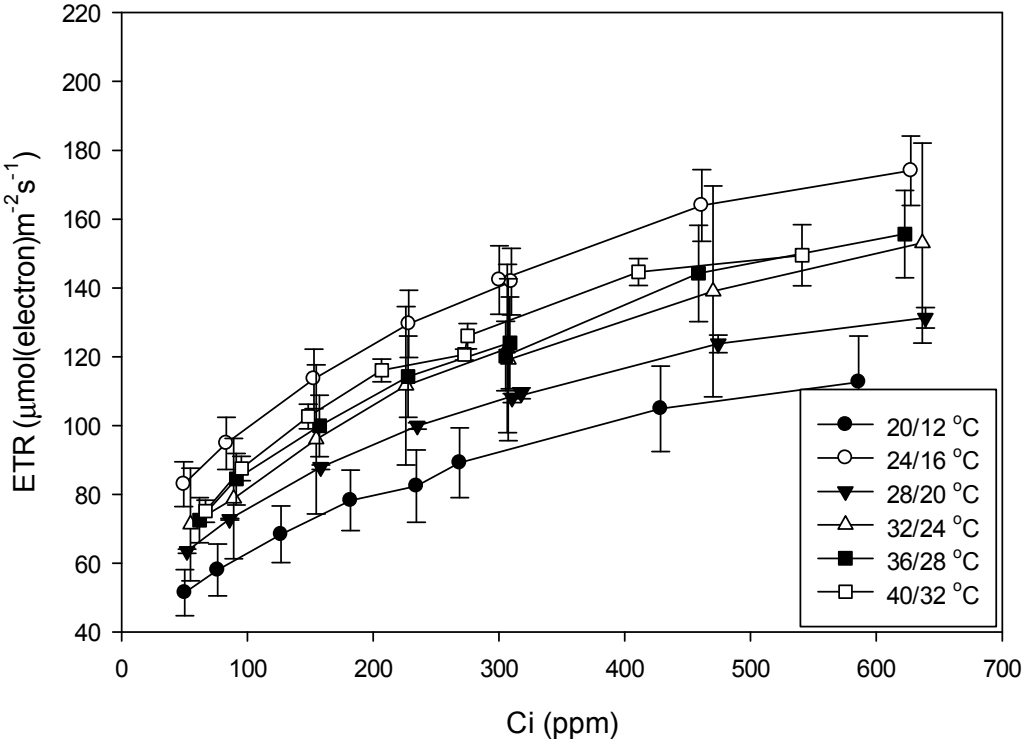


Fig. 3: Effect of six different temperatures on net $CO_2$ assimilation rate (A) of top most fully expanded leaves of soybean in response to photosynthetic photon flux density (PPFD). Vertical bars indicate ± standard error of means (n = 3).

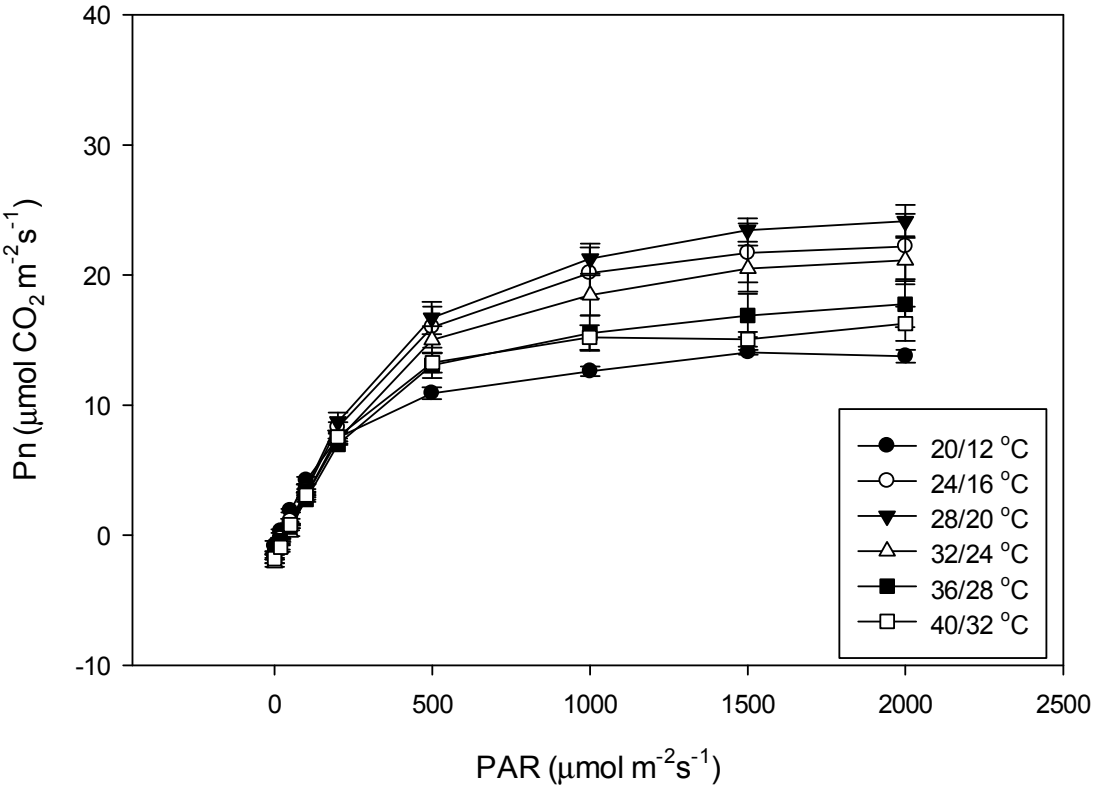


Fig. 4: Effect of six different temperatures on electron transport rate (ETR) of top most fully expanded leaves of soybean in response to photosynthetic photon flux density (PPFD). Vertical bars indicate ± standard error of means (n = 3).

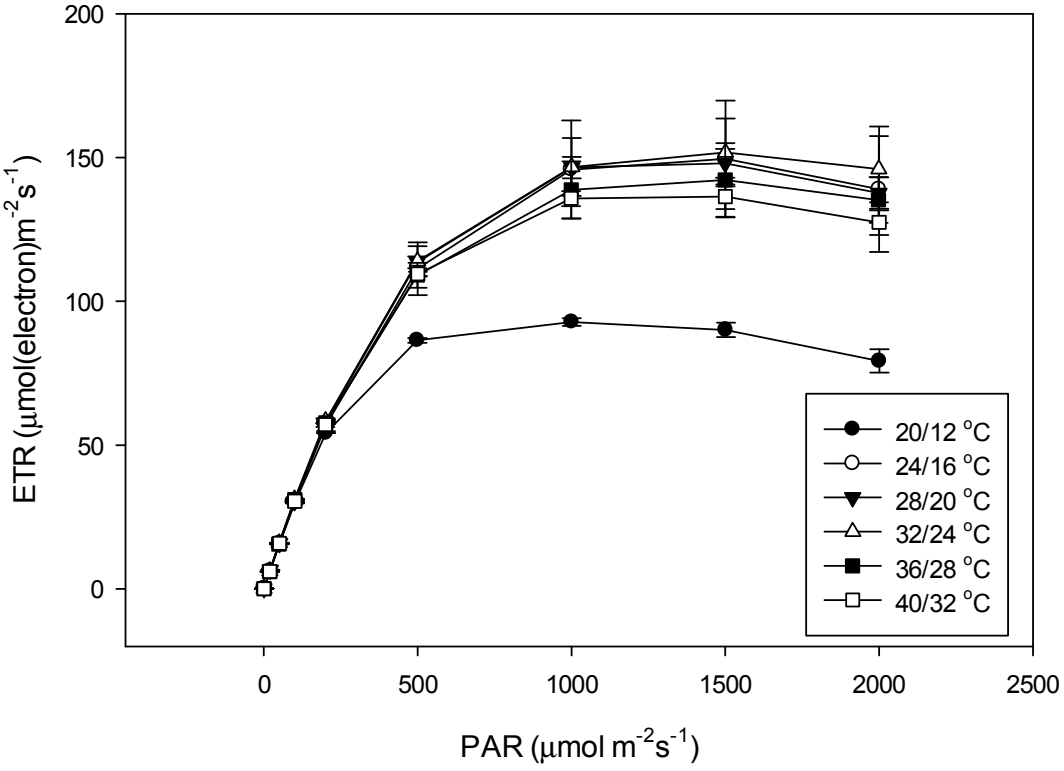


Fig. 5: Effect of maximum photosynthesis of top most fully expanded leaves of soybean across six different temperatures.

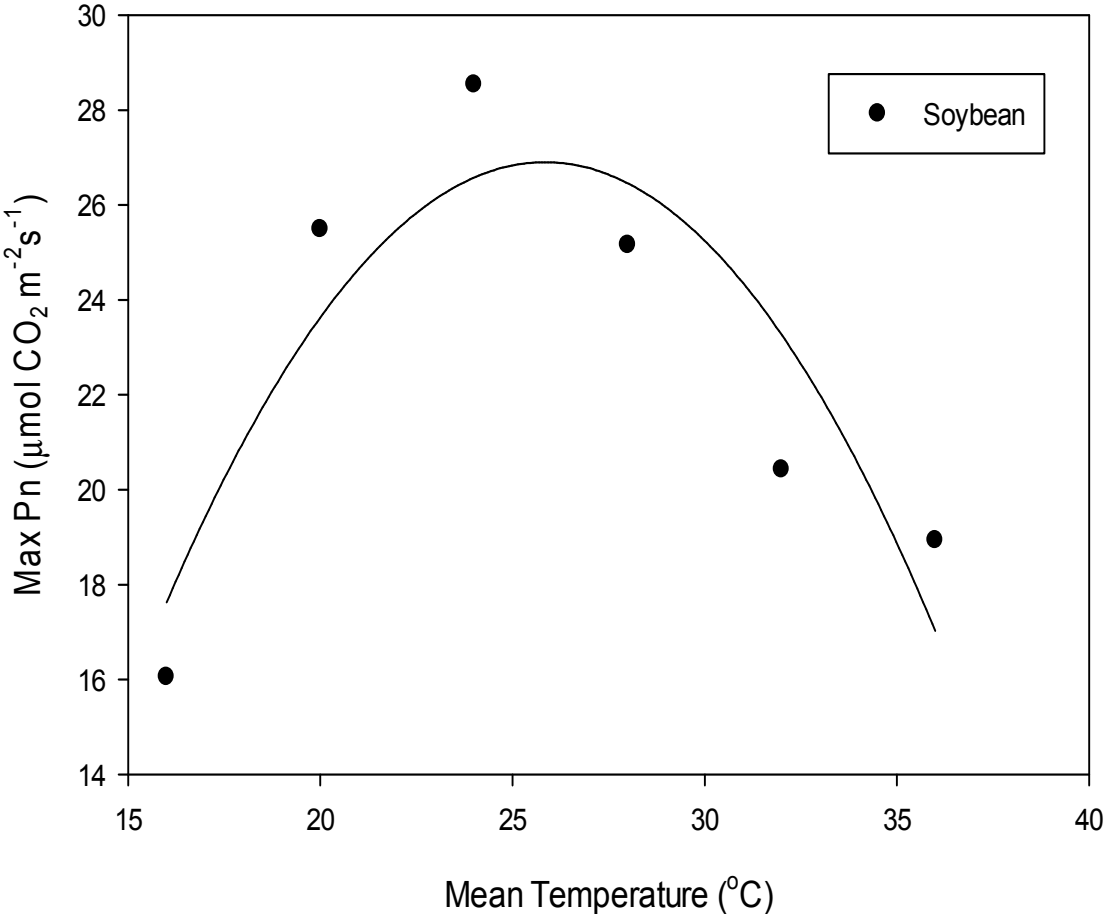


Fig. 6: Effect of saturated photosynthesis of top most fully expanded leaves of soybean across six different temperatures.

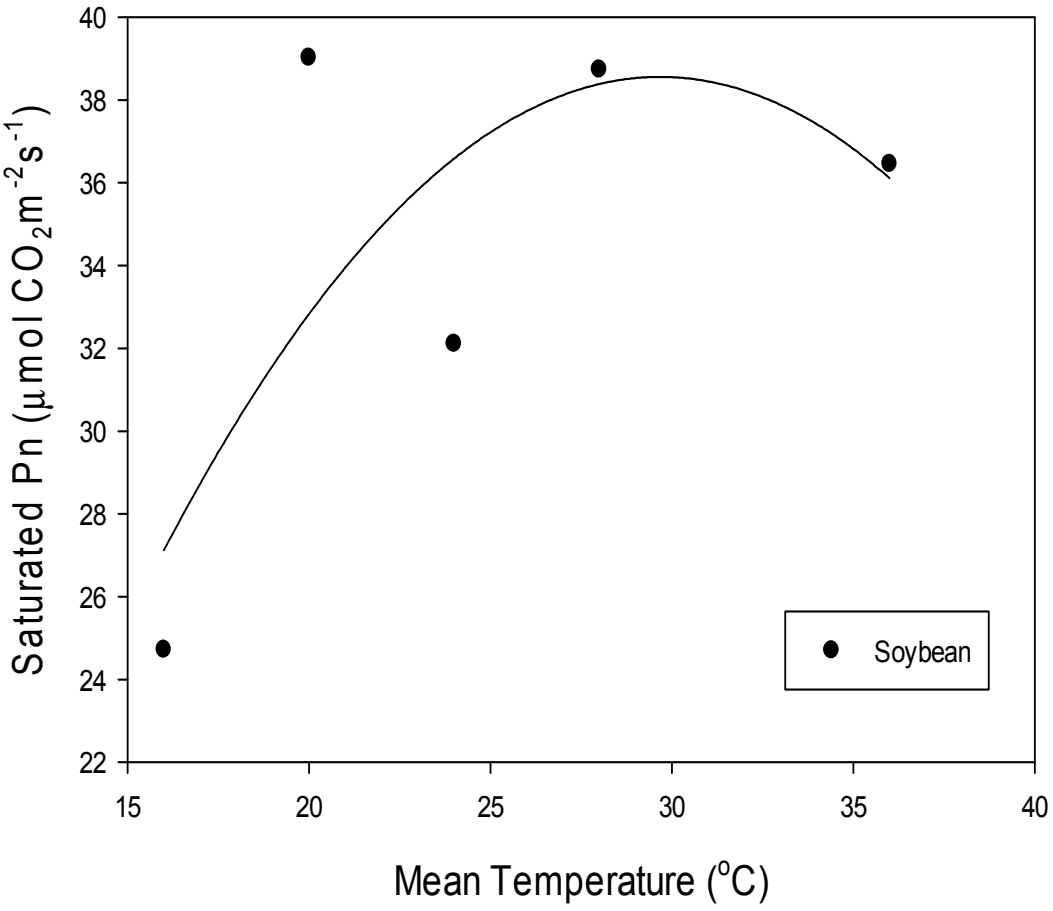


Fig. 7: Effect of light saturated point of top most fully expanded leaves of soybean across six different temperatures.

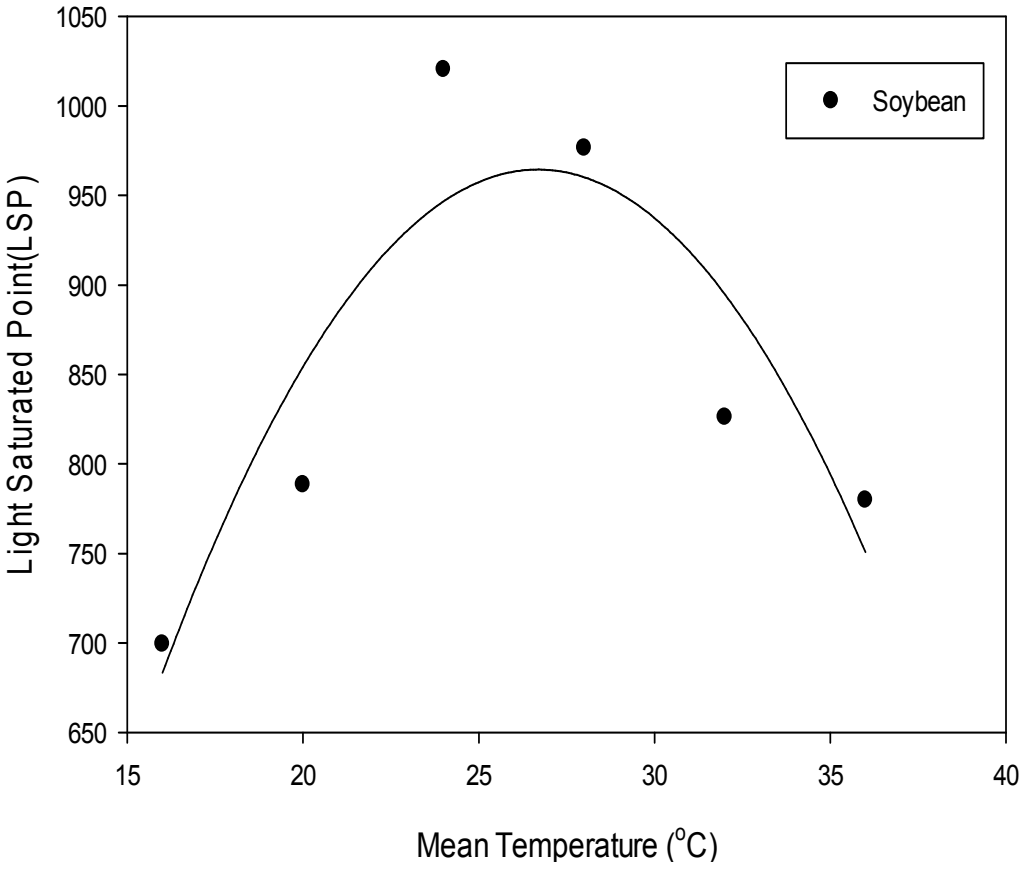


Fig. 8: Effect of light compensation point (LCP) of top most fully expanded leaves of soybean across six different temperatures.

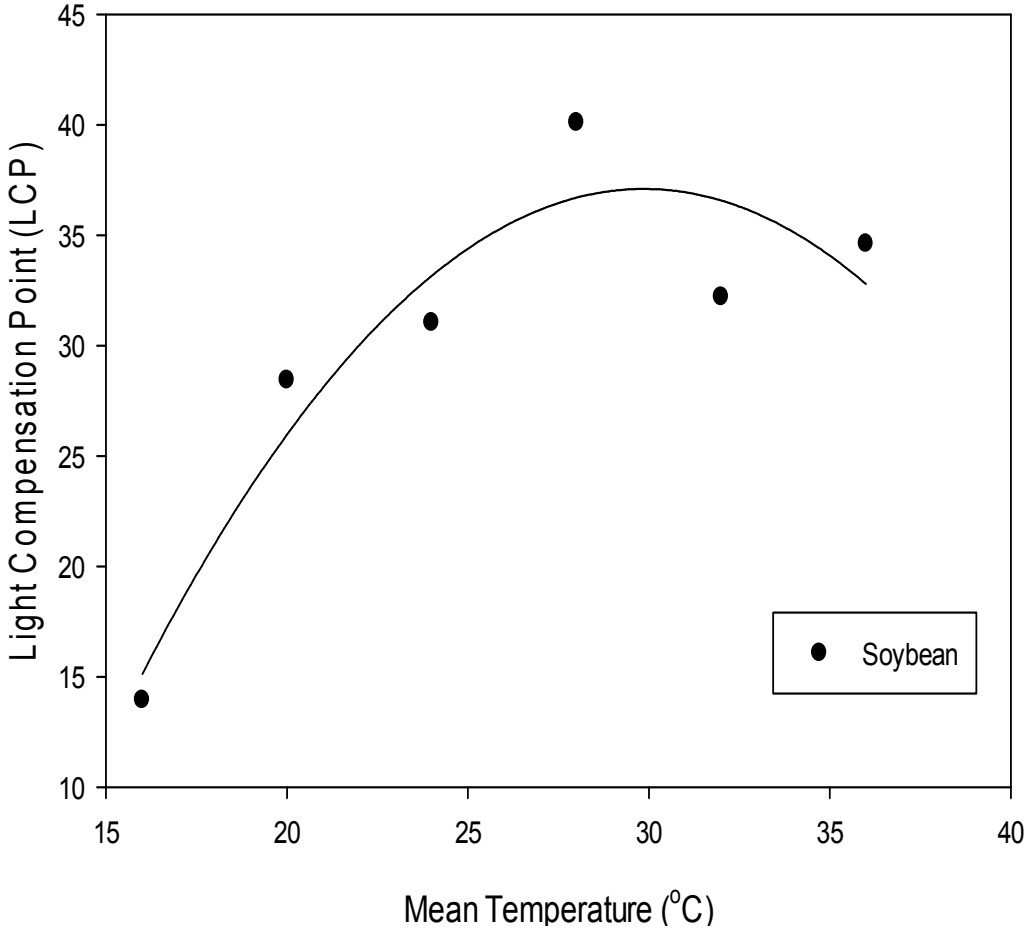
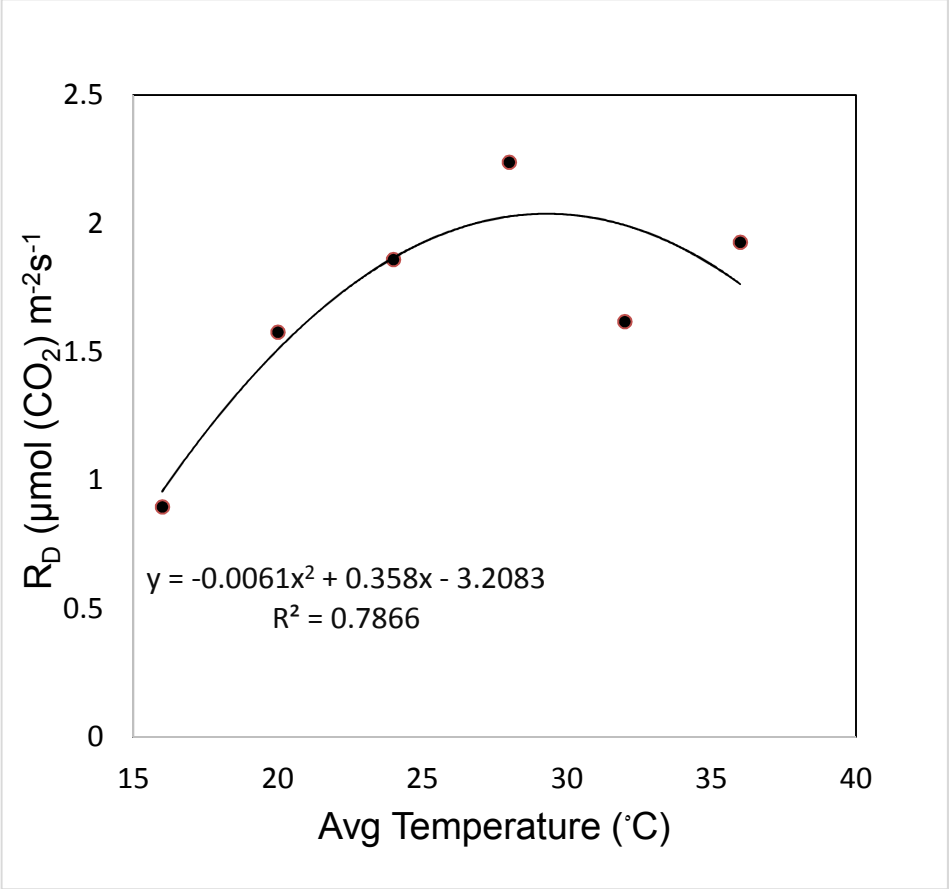


Fig. 9: Effect of dark respiration ($R_D$) of top most fully expanded leaves of soybean across six different temperatures.



# CHAPTER IV

## ANALYSIS OF LONG-TERM TEMPERATURE STRESS ON PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE OF FORAGE SORGHUM


### Abstract

Sorghum possesses wide range of ecological adaptability and is widely grown for feed and fodder in rainfed as well as irrigated regions. It is produced in the United States predominantly on the Southern Great Plains, although it is grown over 30 states, due to its high demand as a forage potential. However, information on its response to temperature along with chlorophyll fluorescence analysis is still lacking. An experiment was conducted using walk-in growth chambers in Controlled Environment Research Facility at Oklahoma State University to study the effects of six different temperatures (20/12, 24/16, 28/20, 32/24, 36/28, and 40/32 °C) to evaluate the photosynthetic responses to temperature gradient. The responses of both photosynthesises to light and internal $CO_2$ (A-Ci) suggested that assimilation rates of forage sorghum acclimates to high temperature through an increased rate of electron transport and unaffected PSII. The photosynthesis parameters can be used to develop mechanistic simulation models and adaptation strategies for forage sorghum. However, further behavior of photosynthetic apparatus and enzymatic actions can be quantified by analysis of molecular samples to determine the genes and hence develop breeding programs which would accelerate the development of temperature stress tolerance in forage sorghum.

1. Introduction

Sorghum is an important grain and forage crop in the semi-arid regions of the world. Sorghum producing regions often experience daytime/night-time temperatures of >32/22 °C (Prasad et al., 2006a). Furthermore, spring conditions can expose early stages to cold events that negatively affect germination and growth, reducing sorghum biomass production and yield (Franks et al., 2006; Maulana and Tesso, 2013). The mean optimum temperature range for sorghum is 21 to 35 °C for seed germination, 26 to 34 °C for vegetative growth and development, and 25 to 28 °C for reproductive growth (Maiti, 1996). Recent synthesis and analyses of past and future climate data suggest that we will experience greater climatic variability in terms of extreme temperature stresses. These changes could have significant influence on productivity of major crops, including sorghum. Therefore, it is important to understand the impacts of season-long and short episodes of temperature stress on physiology of forage sorghum.

Forage sorghum is a large, warm-season, annual grass that is adapted to particular climatic conditions in the United States and can be grown as a silage crop. It can be a profitable alternative crop, provided that is managed well under adverse climatic stresses. Its fodder is fed to almost every class of livestock and can be used as hay or silage (Azraf ul Haq et al., 2007).

The physiology of the crop can be better understood if specific behavioral traits of crop are evaluated and studied over a range of temperatures ranging from the least to highest treatments. In order to assess the photosynthetic damage caused due to the temperature stress, two approaches have been used.

a. Plants have been exposed to different stresses inclusive of cold and heat stress.
b. The stress factor has been applied till the end of the growing season so as to eliminate the possibility of recovery and hence enable a better assessment of the damage caused.

High temperatures coupled with water deficit predisposes plants to photo inhibition (Powles, 1984; Greer et al., 1986; Feierabend et al., 1992), besides affecting photosynthetic efficiency directly (Havaux, 1992). There are several target sites for elevated temperature-induced damage such as the $CO_2$ fixation system, photophosphorylation, the electron transport chain, and the oxygen-evolving complex (OEC) (Nash et al., 1985; Feller et al., 1998; Carpentier, 1999). Combined effect of injury to aforementioned sites results in decrease of photosynthetic efficiency.

Furthermore, decreases in growth can be explained by the sensitivity of the photosynthetic apparatus of sorghum to low temperatures (Taylor and Rowley, 1971; Long et al., 1983; Wang et al., 2008; Bekele et al., 2014). Temperatures below 20 °C causes chilling stress in sorghum, which greatly affect the agronomic performance of the crop (Peacock, 1982). Leaves that develop at a temperature of 15 °C or below are characterized by a very low photosynthetic capacity (Haldimann et al., 1996), altered leaf pigment composition (Haldimann et al., 1995; Haldimann, 1998) and impaired chloroplast development (Robertson et al., 1993) in C4 crops.

Therefore, our objective was to probe the effects of temperature stress on photosynthetic processes and PSII stability, in leaves of forage sorghum. We hypothesize that leaf assimilation rates are affected due to temperature stress.

Gas exchange measurements coupled with chlorophyll fluorescence has facilitated the characterization of the in vivo response of photosynthesis to a variety of stress conditions, including cold (Nie et al., 1992; Savitch et al., 2009; Strigens et al., 2013) and heat (Yan et al., 2012) in several C4 crops. Various parameters of fast Chl fluorescence transients, such as the ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$), the basal fluorescence ($F_0$), and fast and slow maxima of delayed Chl fluorescence, are physiological features that have been shown to correlate with heat tolerance.

## 2. Methodology

The experiment was conducted at Controlled Environment Research Laboratory (CERL), Oklahoma State University (36°7'N, 97°4'W), Oklahoma, USA. Six walk-in growth chambers were utilized for the study for the six treatment temperatures. The six treatments include the mean temperatures ranging from the lowest of 12 °C to highest of 40 °C. The seeds of forage sorghum were sown in cylindrical polyvinyl chloride (PVC) pots measuring 45 cm tall and 23 cm diameter. PVC pots were filled with gravels at the bottom to allow proper drainage and rest was packed with fine, pure sand. Seedling emergence was observed at ten days after planting (DAP). The six chambers were maintained at optimum temperature of 28/20 °C (day/night) until about 10-15 days of planting, after which respective temperatures were imposed to corresponding chambers. Plants in all the six chambers were grown at a day-time maximum/night-time minimum air temperature regime of 28/20 °C from sowing to appearance of fifth leaf to remove the effects of temperature on seedling emergence and establishment. Thereafter, plants in each chamber were exposed to an air temperature regime of 20/12, 24/16, 32/24, 36/28, and 40/32 °C. The treatments were kept constant until final harvest. A constant 12 hours' daylight (06:00 to 18:00 h) under a light intensity of 200 μmol $m^{-2}$ $s^{-1}$ PAR (Photosynthetically Active Radiation) was maintained from planting to final harvest. The watering to the chambers is provided with automated with drip irrigation system, controlled using a timing device to ensure optimum water and nutrient conditions for plant growth with Hoagland's solution, supplied three times a day (08:00, 12:00, and 16:00 h) for three minutes each.

The timing of watering was increased to five minutes each in the chambers maintained at temperatures 36/28 °C and 40/32 °C to avoid water stress. Temperature and humidity inside the chambers were logged every three minutes using data loggers (TP 425, The Dickson Company, Addison, IL).

### 2.1. Gas exchange measurements

The gas exchange measurements were conducted on plants using top most fully expanded leaf in all the chambers.

2.1.1. Fluorescent and net photosynthesis/PAR curves:

The response of net photosynthetic rate to photosynthetic photon flux density (PPFD) was generated by using the automatic program in LI-6400 photosynthetic system (*LICOR,* Lincoln, NE). This was fitted with a 6400-40 leaf chamber fluorometer (LCF) for generating irradiation and LED-based fluorescence.

Details on the operation and parameters generated from the curve were well described by Kakani et al. (2008). Fully expanded, uppermost leaves of three different plants (45-50 d old) were used for measurements taken between 09:00 to 11:00 h by altering PAR inside LCF in nine steps (2000, 1500, 1000, 500, 200, 100, 50, 20, and 0 μmol $m^{-2}$ $s^{-1}$). The leaf block temperature was adjusted according to the corresponding daytime (maximum) temperature for each chamber. The instrument was given 120 s at each PPFD level to attain a steady state, and it logged values at a coefficient of variation ≤5 %. Chlorophyll fluorescence characteristics were recorded simultaneously with A. The nonrectangular hyperbola was used to fit the photosynthetic-light response curve. The

$$P = \frac{\varphi I + P_{max} - \sqrt{(\varphi I + P_{max})^2 - 4\varphi I \theta P_{max}}}{2\theta} - R_d$$

where φ is the quantum yield at $I = 0$ μmol $m^{-2}$ $s^{-1}$ or termed as apparent quantum yield, $P_{max}$ is the asymptotic estimate of maximum net $CO_2$ assimilation, θ is the curvature factor, and $R_d$ is the rate of dark respiration. Light compensation point ($I_{comp}$) and light saturation point at a 75 percentile ($Q_{sat75}$) were calculated as described by Lobo et al. (2013):

$$I_{comp} = \frac{R_d(\varphi R_d - A_{max})}{\varphi(R_d - A_{max})}$$

$$Q_{sat75} = \frac{(P_{max}+R_d)(P_{max}-0.75\theta P_{max}-0.25\theta R_d)}{\varphi(P_{max}-R_d)}$$

2.1.2. Fluorescent and net photosynthesis/internal carbon dioxide curves:

The response of net photosynthetic rate (A) to internal carbon dioxide concentration (Ci) was generated by using automatic program in LI-6400 photosynthetic systems. The measurements was taken between 09:00 and 11:00 h by altering the $CO_2$ concentration in LCF in the following sequence: 400, 300, 200, 100, 50, 400, 400, 600, and 800 μmol $mol^{-1}$. All measurements were made at a constant PPFD of 1000 μmol $m^{-2}$ $s^{-1}$. The block temperature inside the leaf cuvette was set to the corresponding maximum air temperature for each chamber. The *A-Ci* response was fitted with a three-parameter exponential to maximum function (Kakani et al., 2008).

The function has the following form:

$$P_N = a\ (1 - e^{-bx}) + y_0$$

where $P_N$ is the net $CO_2$ assimilation rate, and x is internal $CO_2$ concentration ($C_i$). $P_{sat}$ was estimated as a + c using this equation.

Likewise, saturated values of ETR ($ETR_{sat}$) were calculated by fitting exponential to maximum function to ETR and Ci.

2.2. Estimation of cardinal temperatures for the response parameters:

The response parameters like $P_{max}$, $P_{sat}$, $ETR_{max}$, $ETR_{sat}$ and *PEPC* are derived from the light and $CO_2$ response curves. The $P_{max}$, and $ETR_{max}$ are computed from the light response curve by fitting the quadratic curve using SigmaPlot v. 12.5. While *the* $P_{sat}$, $ETR_{sat}$ and *PEPC* are derived from the *A-Ci* response curve. The cardinal temperatures are computed from the values, derived using the SigmaPlot.

3. Results

### 3.1. Leaf photosynthetic-light response

The measurements of the photosynthetic light response curve indicated that both *A* and *ETR* were similar among the six treatments when *PAR* level is 500 µmol $m^{-2}$ $s^{-1}$. Measurements at 28/20, 32/24, and 36/28 °C had similar A at three higher PAR levels (1000, 1500, and 2000µmol $m^{-2}$ $s^{-1}$). While measurements at 28/20, 32/24, 36/28 and 40/32 °C had almost similar ETR at 1000 and 1500 µmol $m^{-2}$ $s^{-1}$ light levels. There was a gradual decline in ETR at the highest PAR level for treatments 28/20 and 40/32 °C. The treatments 20/12 and 24/16 °C had the lowest ETR and A at all light levels.

The responses of photosynthetic capacity can be elaborated by explaining the parameters derived from the leaf photosynthetic-light response curve. The below mentioned parameters are computed using 'onls' package in R (Spiesss, 2015) through nonlinear least square procedure. The irradiance-saturated maximum photosynthesis ($P_{max}$) increased linearly with increasing temperatures upto 28/20 °C and thereafter started declining. Similar is the response of dark respiration ($R_D$) which is quadratic to increasing temperatures. However, the decrease in $R_D$ at higher temperatures is not quite significant. Other parameters derived from the photosynthetic-light response curve includes the light compensation point (LCP) and light saturation point at a 75 percentile ($Q_{sat75}$) both of which are linearly correlated with temperature increase. The LCP started to increase with increase in temperature while the $Q_{sat75}$ showed a declining trend. The initial slope of irradiance-response curves in current study showed that the quantum yield ($\phi$) of C4 photosynthesis was independent of temperature because of lack of photorespiration.

### 3.2. Leaf photosynthetic-$CO_2$ (A-$C_i$) response

The leaf photosynthetic $CO_2$ response curve followed exponential rise to maximum function. The response of photosynthesis was similar among all six treatments at a Ci<100 ppm. Increasing Ci gradually increased photosynthesis with increase in treatment temperatures. Measurements at

32/24, 36/28, and 40/32 °C had similar A at different levels of Ci. Similarly, the response of ETR was same for the six treatment temperatures at Ci<100 ppm. Measurements at 32/24 and 36/28 °C had the maximum ETR at all levels of Ci. The treatments at 20/12 and 24/16 °C had the lowest A and ETR for corresponding values of Ci.

For C4 plants, the saturation for $P_N$ is reached near the current [$CO_2$] level, with a small improvement in term of net photosynthetic rate (Taiz and Zeiger, 1991; von Caemmerer et al., 1997). In the case of C3 plants, the saturation for $P_N$ is reached by doubling the current [$CO_2$]. This can be explained by the constant trend in A/Ci curve for photosynthesis from 360 ppm irrespective of temperature in sorghum across changing internal carbon dioxide in the leaves.

The parameters derived from the A-Ci curve further explain the response of photosynthesis to extreme temperature changes as can be seen from the behavior of saturated photosynthesis. The response is linear when plotted against temperature which signifies the increase in the photosynthetic capacity with increase in temperature. The plants exhibited higher photosynthesis even at highest temperatures of 40 °C. The response could have been more reliable and consistent if an additional temperature of 45 °C is included.

Furthermore, the response of the activity of PEPC enzyme is quadratic (polynomial of order 2) with temperature increase. It can further be elucidated that the enzyme activity is minimum at the highest temperature.

4. Discussion

The productivity of forage sorghum (any crop, as a rule) is determined directly by the photosynthetic carbon assimilation. This in turn, is determined by the complex interplay between the photosynthetic apparatus and the growing environment. Exposure of plants above the normal physiological temperatures leads to subsequent decreases in photosynthesis. Likewise, plants developed at temperatures below the thermal optimum have reduced growth, subsequently

leading to reduced photosynthesis. However, plants exposed to season-long temperature stress may acclimate to temperatures, away from the thermal optimum. The main aim of the current research was to study the effect of season-long temperature stress on leaf-level photosynthesis in forage sorghum.

In general, the A/Ci response curve show a typical crossing over due to increases in the $CO_2$ compensation point and the RuBP-regeneration rate with increasing temperature. The response is similar to that found by Kirschbaum and Farquhar (1984) in *Eucalyptus pauciflora*.

Both the photosynthetic-light (Fig. 4) and A-Ci (Fig. 1) curves confirmed that the forage sorghum can tolerate high temperatures which can be explained by higher photosynthesis at 36/28 °C. The plants recorded the lowest photosynthesis at the temperatures 20/12 and 24/16 °C (Fig. 1).

Irradiance-saturated maximum photosynthesis ($P_{max}$) started declining at higher temperatures above the normal optimum (Fog. 5). These lower rates can be attributed to lower activities of PEPC and RuBPCO at higher temperatures in forage sorghum. Increase in $R_D$ was seen with increasing temperatures. Similar observations were made by Nagy et al. (2000) and Kakani et al. (2008) in hinoki cypress and big bluestem respectively, both of which are C4 species.

The quantum yield of photosynthesis ($\phi$) is a definitive measure of the energetic efficiency of photoautotrophy. The quantum yield for any defined light-dependent process is the rate at which that defined event occurs relative to the rate of photon absorption by the system (Skillman, 2008). The initial slope of irradiance-response curves in the present study showed that there were no significant interactive effects of temperature on $\phi$. This can be explained by the fact that plants possessing the $C_4$ pathway do not show oxygenase activity in vivo under atmospheric $O_2$ concentrations, and therefore, their quantum yields should not show and do not show a dependence on $CO_2$ concentration. Similarly, the absence of a temperature dependence of the quantum yield under low $O_2$ conditions in the $C_3$ plant and under normal atmospheric conditions

for the $C_4$ plant would suggest that under low light intensities, the carboxylase activity of RuBP carboxylase-oxygenase is temperature-independent between 13 and 39 C (Ehleringer and Bjorkman, 1977). Hence, the quantum yield of a $C_4$ plant, which is independent of the intercellular $CO_2$ concentration, is shown to be independent of leaf temperature over the ranges measured.

4.1. Photochemical responses:

The fluorescence measurements provide evidence for the tolerance of forage sorghum upto certain maximum temperatures owing to the data recorded to changes in $C_i$ and PAR for measured leaf fluorescence parameters such as minimal fluorescence ($F_0$'), maximal fluorescence ($F_m$'), and steady-state fluorescence ($F_s$') (data not shown).

The response to temperature of $ETR_{max}$ and $ETR_{sat}$ (Fig. 7&8) derived from photosynthetic-light and photosynthetic-$CO_2$ are both quadratic. The response was linear and increased with increase in temperature upto a temperature of 36/28 °C due to the absence of photorespiration in $C_4$ species. Similar is the response of $P_{max}$ and $P_{sat}$ to temperature (Fig. 5&6).

Photoinhibition is a phenomenon leading to a reduction of photosynthetic activity due to light-induced decreases in $CO_2$ assimilation (Baker, 1996). However, the extent of photoinhibition depends on the balance between photodamage and repair mechanisms of PSII core (Demmig-Adams et al., 2012). Recently, this hypothesis has changed and many researchers have demonstrated that the repair mechanism of PSII is more sensitive to environmental stresses than the process of photo damage itself (Nishiyama and Murata, 2014). In addition to these factors, Tikkanen et al. (2014) reported that PSII photoinhibition slows down the electron transport rate and prevents ROS generation and photodamage to PSI. However, there was not any evidence of decreased electron transport rate or inactivation of PSII caused by high temperature in this study.

The unaffected PSII and increased ETR exhibited the heat stability of its photosynthetic apparatus for photosynthetic-light and A-Ci response curves.

Although there was an increase in the electron transport rate upto a certain maximum temperature, the number of electrons needed to fix a $CO_2$ molecule stayed similar in both light and A-Ci response curves. This suggested an increase in activity of photosynthetic enzymes and their ability to fix $CO_2$ even at higher temperatures. The number of electrons needed to fix one molecule of $CO_2$ ranged from 4.9 to 5.2 across temperatures under saturated $CO_2$ conditions while it is in the range from 4.2 to 4.6 under light saturated conditions.

## 5. Conclusions

The present study evaluated the photosynthetic responses of forage sorghum to varying temperature regimes. Both photosynthetic-light and A-Ci response curves suggested that the forage sorghum acclimatize its photosynthetic rates to heat and cold stress by allowing a higher rate of electron transport. The parameters derived from the response curves are further evaluated. The quantum yield of forage sorghum is independent of intercellular $CO_2$ concentration and temperature. In addition, the unaffected PSII exhibited the heat stability of photosynthetic apparatus for response curves across temperatures. However, further research is required to understand the $P_N$ limitations to even high temperatures in leaves of forage sorghum.

REFERENCES

Azraf-ul-Haq, A., et al. (2007). "Performance of forage sorghum intercropped with forage legumes under different planting patterns." *Pakistan Journal of Botany 39(2)*: 431-439.

Baker, N. R. (1996). Photoinhibition of photosynthesis. Light as an Energy Source and Information Carrier in Plant Physiology, *Springer*: 89-97.

Bekele, W. A., et al. (2014). "Unravelling the genetic complexity of sorghum seedling development under low□temperature conditions." *Plant, cell & environment 37(3)*: 707-723.

Carpentier, R. (1999). "Effect of high-temperature stress on the photosynthetic apparatus." *Handbook of plant and crop stress 2*: 337-348.

Demmig-Adams, B. (1990). "Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin." *Biochimica et Biophysica Acta (BBA)-Bioenergetics 1020(1)*: 1-24.

Ehleringer, J. and O. Björkman (1977). "Quantum yields for CO2 uptake in C3 and C4 plants: dependence on temperature, CO2, and O2 concentration." *Plant Physiology 59(1)*: 86-90.

Feierabend, J., et al. (1992). "Photoinactivation of catalase occurs under both high-and low-temperature stress conditions and accompanies photoinhibition of photosystem II." *Plant Physiology 100(3)*: 1554-1561.

Feller, U., et al. (1998). "Moderately high temperatures inhibit ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco." *Plant Physiology 116(2)*: 539-546.

Franks, C. D., et al. (2006). "A comparison of US and Chinese sorghum germplasm for early season cold tolerance." *Crop Science 46(3)*: 1371-1376.

Greer, D., et al. (1986). "Photoinhibition of photosynthesis in intact bean leaves: role of light and temperature, and requirement for chloroplast-protein synthesis during recovery." *Planta 168(2)*: 253-260.

Haldimann, P. (1998). "Low growth temperature□induced changes to pigment composition and photosynthesis in Zea mays genotypes differing in chilling sensitivity." *Plant, cell & environment 21(2)*: 200-208.

Haldimann, P., et al. (1995). "Carotenoid composition in Zea mays developed at sub□optimal temperature and different light intensities." *Physiologia Plantarum 95(3)*: 409-414.

Haldimann, P., et al. (1996). "Photosynthetic performance and resistance to photoinhibition of Zea mays L. leaves grown at sub□optimal temperature." *Plant, cell & environment 19(1)*: 85-92.

Havaux, M. (1992). "Stress tolerance of photosystem II in vivo: antagonistic effects of water, heat, and photoinhibition stresses." *Plant Physiology 100(1)*: 424-432.

House, L. R., et al. (1982). "Sorghum in the Eighties Proceedings of the International Symposium on Sorghum Volume 1."

Kakani, V., et al. (2008). "Photosynthesis and fluorescence responses of C 4 plant Andropogon gerardii acclimated to temperature and carbon dioxide." *Photosynthetica 46(3)*: 420-430.

Kirschbaum, M. and G. Farquhar (1984). "Temperature dependence of whole-leaf photosynthesis in Eucalyptus pauciflora Sieb. ex Spreng." *Functional plant biology 11(6)*: 519-538.

Lobo, F. d. A., et al. (2013). "Fitting net photosynthetic light-response curves with Microsoft Excel—a critical look at the models." *Photosynthetica 51(3)*: 445-456.

Long, S. P., et al. (1983). "Chilling damage to photosynthesis in young Zea mays: I. Effects of light and temperature variation on photosynthetic CO2 assimilation." *Journal of experimental botany 34(2)*: 177-188.

Maiti, R. (1996). "Germination and seedling establishment." Sorghum science. *Science Publishers, Inc., Lebanon, NH*: 41-98.

Maulana, F. and T. T. Tesso (2013). "Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum." *Crop Science 53(2)*: 564-574.

Nagy, M., et al. (2000). "Interactive effect of CO 2 enrichment and temperature on the photosynthesis of field-grown hinoki cypress (Chamaecyparis obtusa) branches." *Trees 14(5)*: 282-288.

Nash, D., et al. (1985). "Heat inactivation of oxygen evolution in photosystem II particles and its acceleration by chloride depletion and exogenous manganese." *Biochimica et Biophysica Acta (BBA)-Bioenergetics 807(2)*: 127-133.

Nie, G. Y., et al. (1992). "The effects of development at sub□optimal growth temperatures on photosynthetic capacity and susceptibility to chilling□dependent photoinhibition in Zea mays." *Physiologia Plantarum 85(3)*: 554-560.

Nishiyama, Y. and N. Murata (2014). "Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery." *Applied microbiology and biotechnology 98(21)*: 8777-8796.

Peacock, J. (1982). "Response and tolerance of sorghum to temperature stress."

Powles, S. B. (1984). "Photoinhibition of photosynthesis induced by visible light." *Annual Review of plant physiology 35(1)*: 15-44.

Prasad, P. V., et al. (2006). "Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [Sorghum bicolor (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures." *Agricultural and forest meteorology 139(3-4)*: 237-251.

Robertson, E., et al. (1993). "Chloroplast thylakoid protein changes induced by low growth temperature in maize revealed by immunocytology." *Plant, cell & environment 16(7)*: 809-818.

Savitch, L. V., et al. (2009). "Effects of low temperature stress on excitation energy partitioning and photoprotection in Zea mays." *Functional Plant Biology 36(1)*: 37-49.

Skillman, J. B. (2008). "Quantum yield variation across the three pathways of photosynthesis: not yet out of the dark." *Journal of experimental botany 59(7)*: 1647-1661.

Strigens, A., et al. (2013). "Association mapping for chilling tolerance in elite flint and dent maize inbred lines evaluated in growth chamber and field experiments." *Plant, cell & environment 36(10)*: 1871-1887.

Taiz, L. and E. Zeiger (1991). "Plant physiology. Redwood City." California, USA: Benjamin/Cumming Company, USA. Trejo, C. and Davies, WJ (1991). Drought-induced closure of Phaseolus vulgaris stomata preceded leaf water deficit and any increase in xylem ABA concentration. *Journal of Experimental Botany 42*: 15071515.

Taylor, A. and J. Rowley (1971). "Plants under climatic stress: I. Low temperature, high light effects on photosynthesis." *Plant Physiology 47(5)*: 713-718.

Tikkanen, M., et al. (2014). "Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage." *Biochimica et Biophysica Acta (BBA)-Bioenergetics 1837(1)*: 210-215.

von Caemmerer, S., et al. (1997). "Reduction of ribulose-1, 5-bisphosphate carboxylase/oxygenase by antisense RNA in the C4 plant Flaveria bidentis leads to reduced assimilation rates and increased carbon isotope discrimination." *Plant Physiology 113(2)*: 469-477.

Wang, D., et al. (2008). "Cool C4 photosynthesis: pyruvate Pi dikinase expression and activity corresponds to the exceptional cold tolerance of carbon assimilation in Miscanthus× giganteus." *Plant Physiology 148(1)*: 557-567.

Yan, K., et al. (2012). "Responses of photosynthesis and photosystem II to higher temperature and salt stress in Sorghum." *Journal of Agronomy and Crop Science 198(3)*: 218-225.

Table 1. Cardinal temperatures for light and $CO_2$ response parameters in forage sorghum.

| Parameter | T min (°C) | T opt (°C) | T max (°C) |
|---|---|---|---|
| $P_{max}$ | 6.9 | 27.5 | 48.0 |
| $P_{sat}$ | 3.1 | 37.9 | 72.7 |
| $ETR_{max}$ | 4.3 | 29.6 | 55.0 |
| $ETR_{sat}$ | 6.0 | 31.5 | 57.1 |
| PEPC | -5.3 | 22.6 | 50.5 |

Fig. 1: Effect of six different temperatures on net $CO_2$ assimilation rate (A) of top most fully expanded leaves of forage sorghum in response to internal $CO_2$ concentration (Ci). Vertical bars indicate ± standard error of means (n = 3).

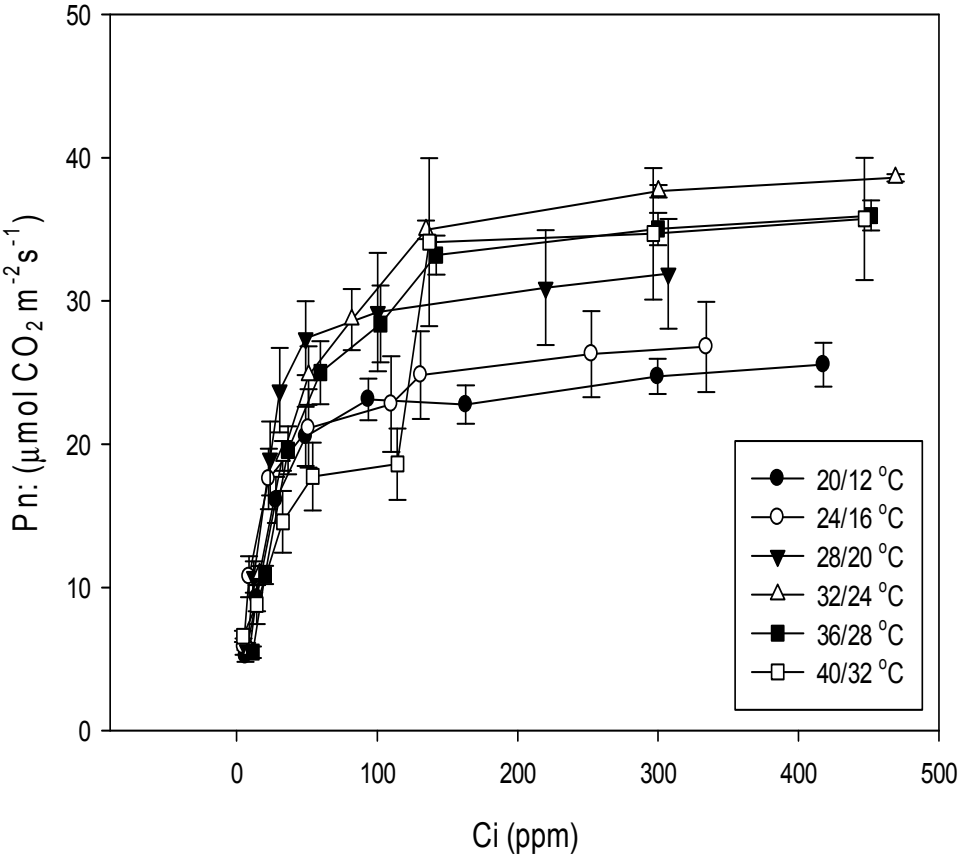


Fig. 2: Effect of six different temperatures on electron transport rate (ETR) of top most fully expanded leaves of forage sorghum in response to internal $CO_2$ concentration (Ci). Vertical bars indicate ± standard error of means (n = 3).

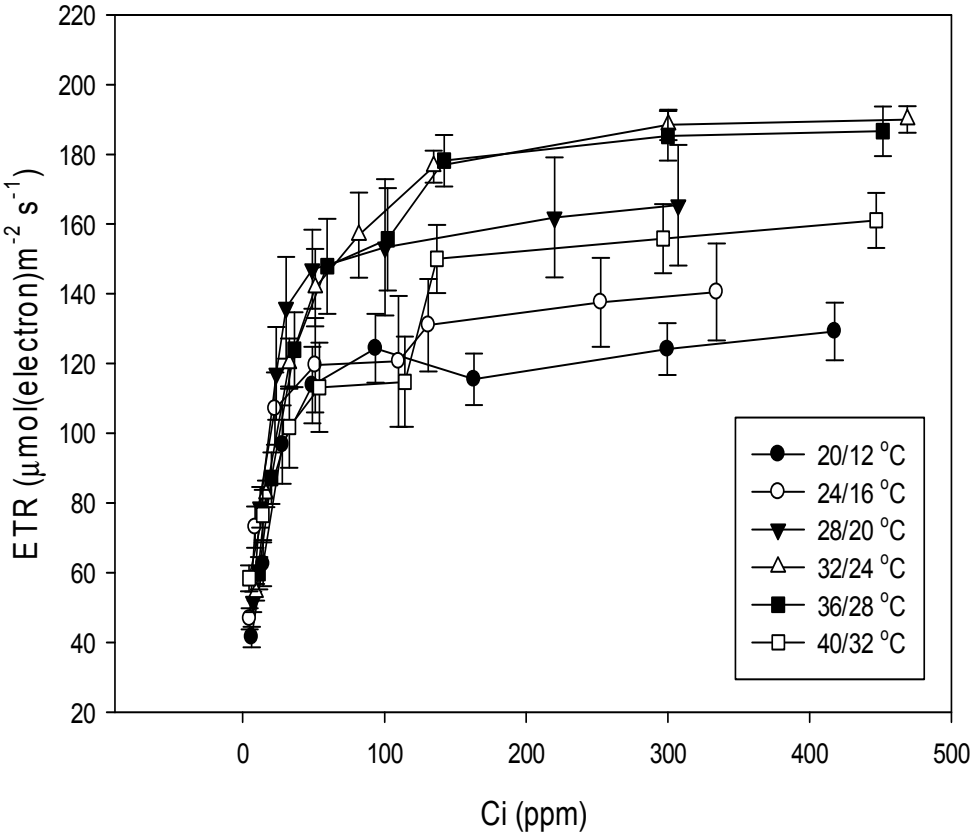


Fig. 3: Effect of six different temperatures on electron transport rate (ETR) of top most fully expanded leaves of forage sorghum in response to photosynthetic photon flux density (PPFD). Vertical bars indicate ± standard error of means (n = 3).

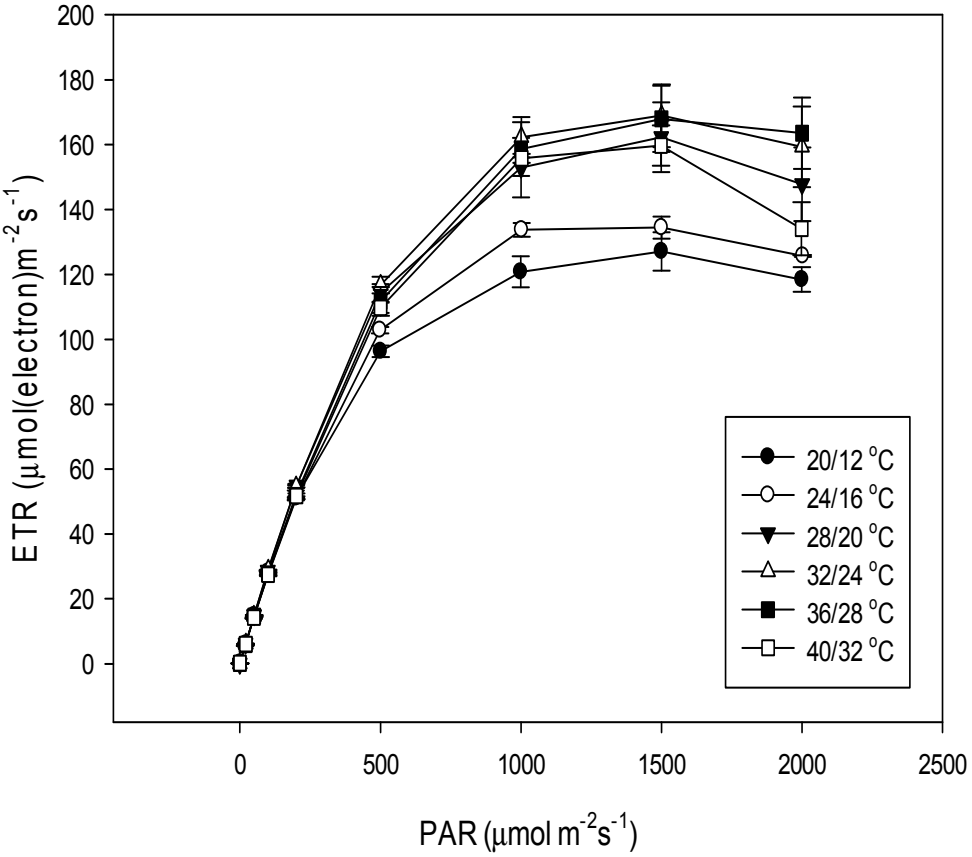


Fig. 4: Effect of six different temperatures on net $CO_2$ assimilation rate (A) of top most fully expanded leaves of forage sorghum in response to photosynthetic photon flux density (PPFD). Vertical bars indicate ± standard error of means (n = 3).

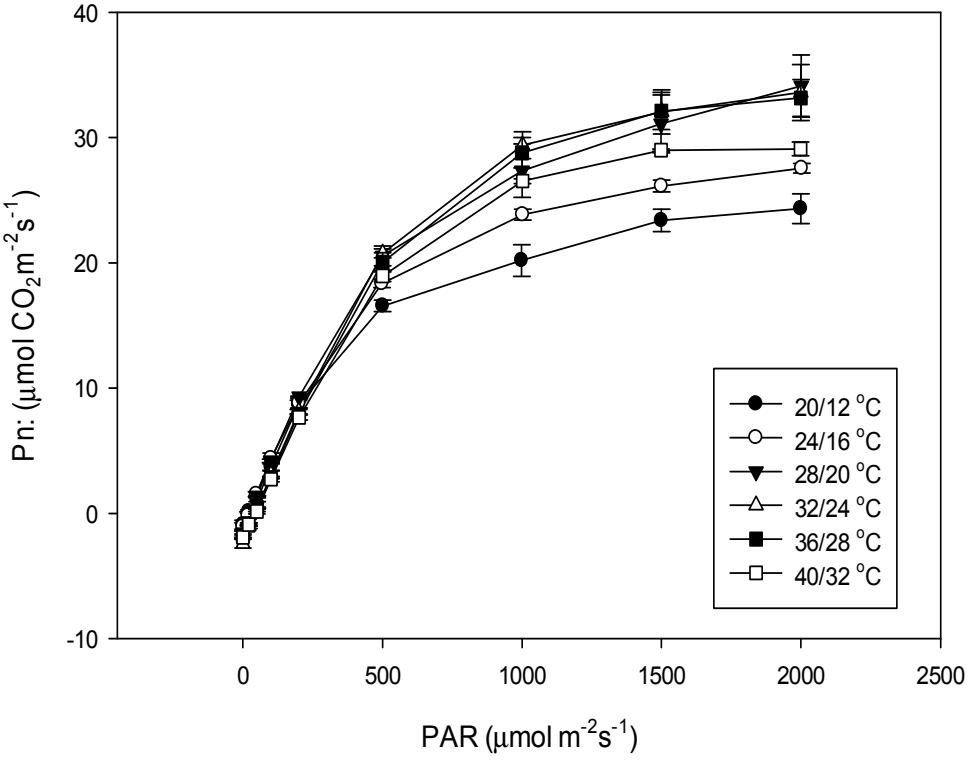


Fig. 5: Effect of maximum photosynthesis of top most fully expanded leaves of forage sorghum across six different temperatures.

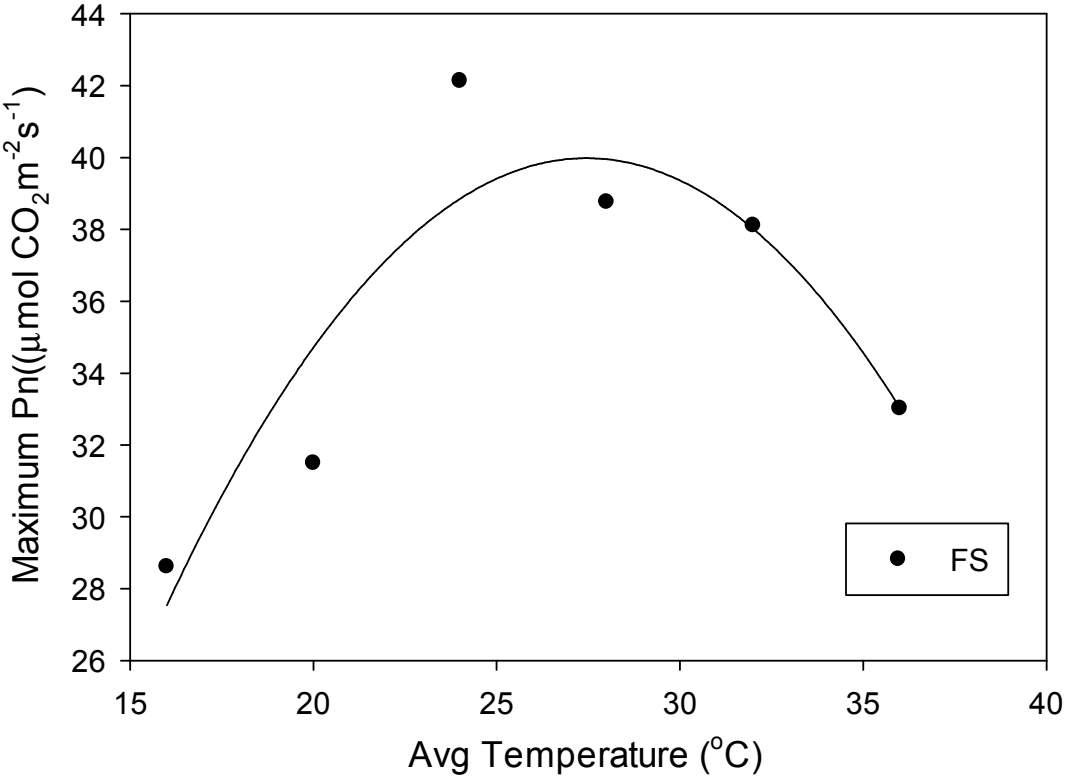


Fig. 6: Effect of saturated photosynthesis of top most fully expanded leaves of forage sorghum across six different temperatures.

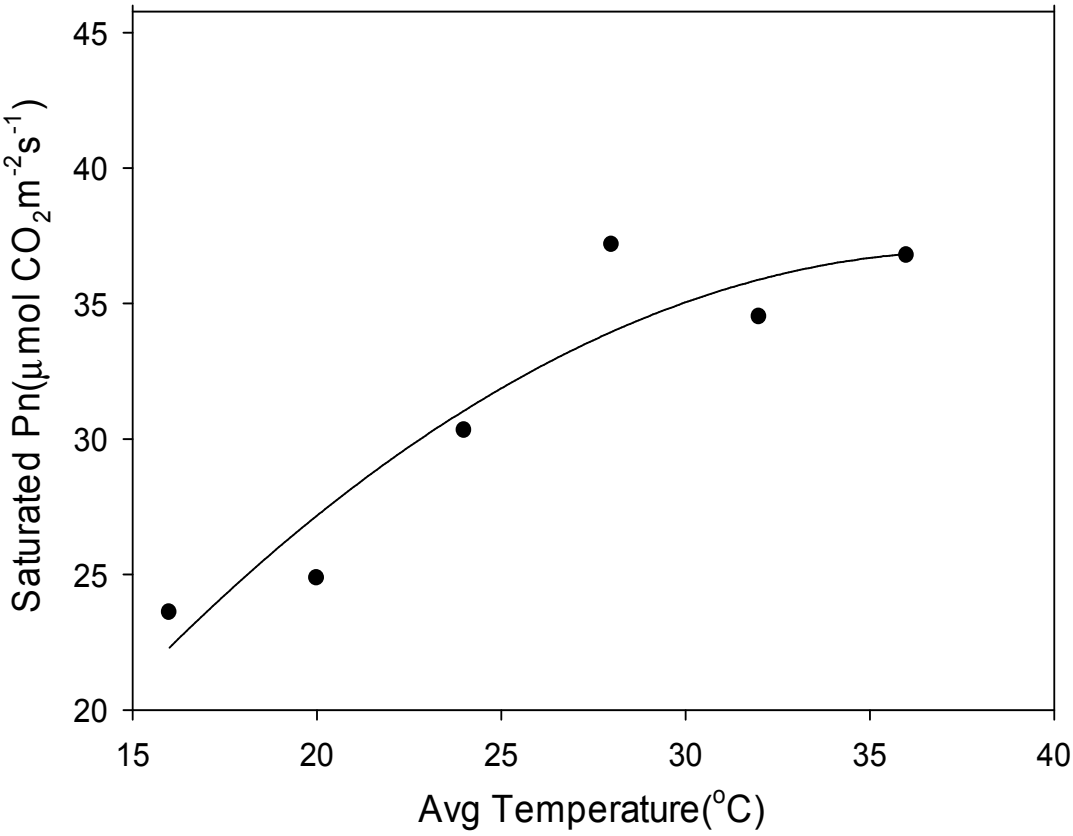


Fig. 7: Effect of saturated electron transport rate (ETR) of top most fully expanded leaves of forage sorghum across six different temperatures.

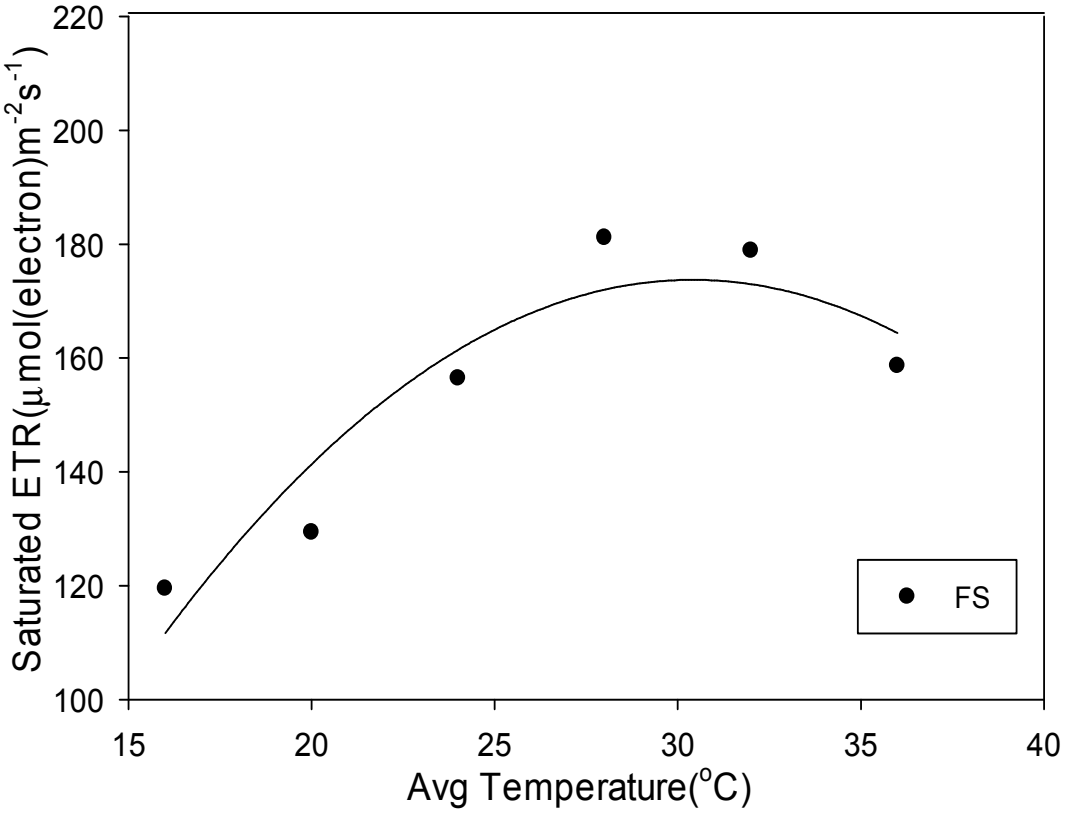


Fig. 8: Effect of maximum electron transport rate (ETR) of top most fully expanded leaves of forage sorghum across six different temperatures.

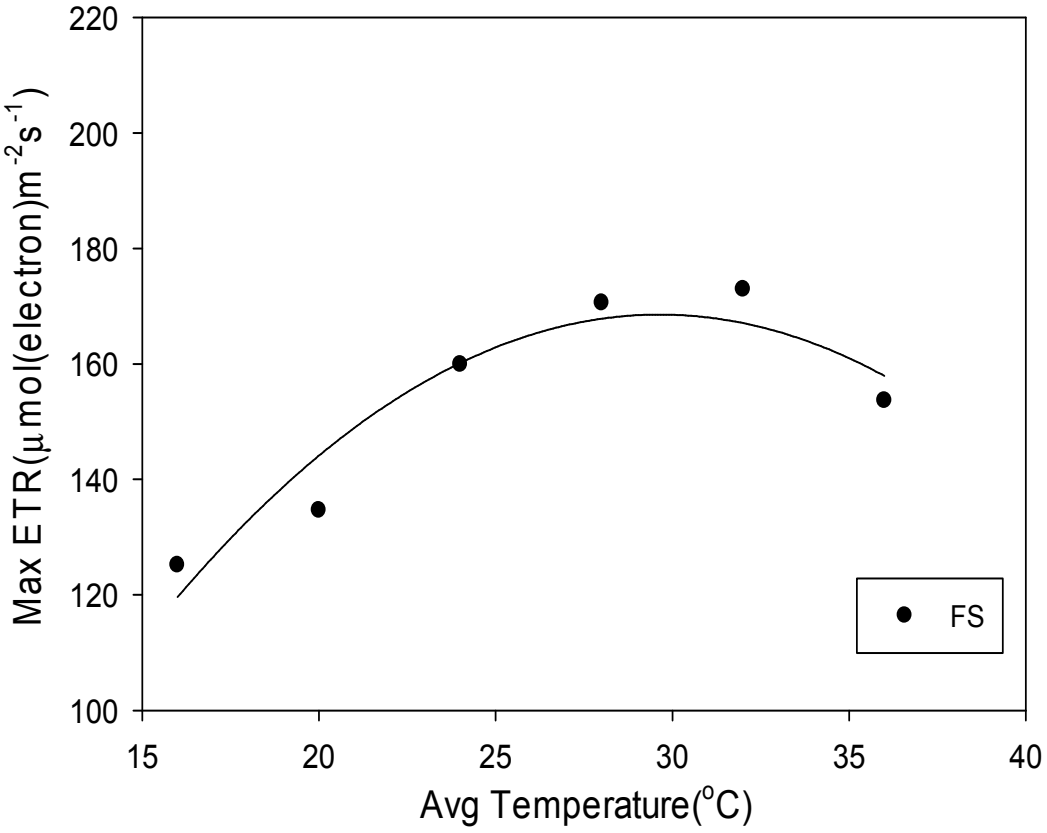


Fig. 9: Effect of light compensation point (LCP) of top most fully expanded leaves of forage sorghum across six different temperatures.

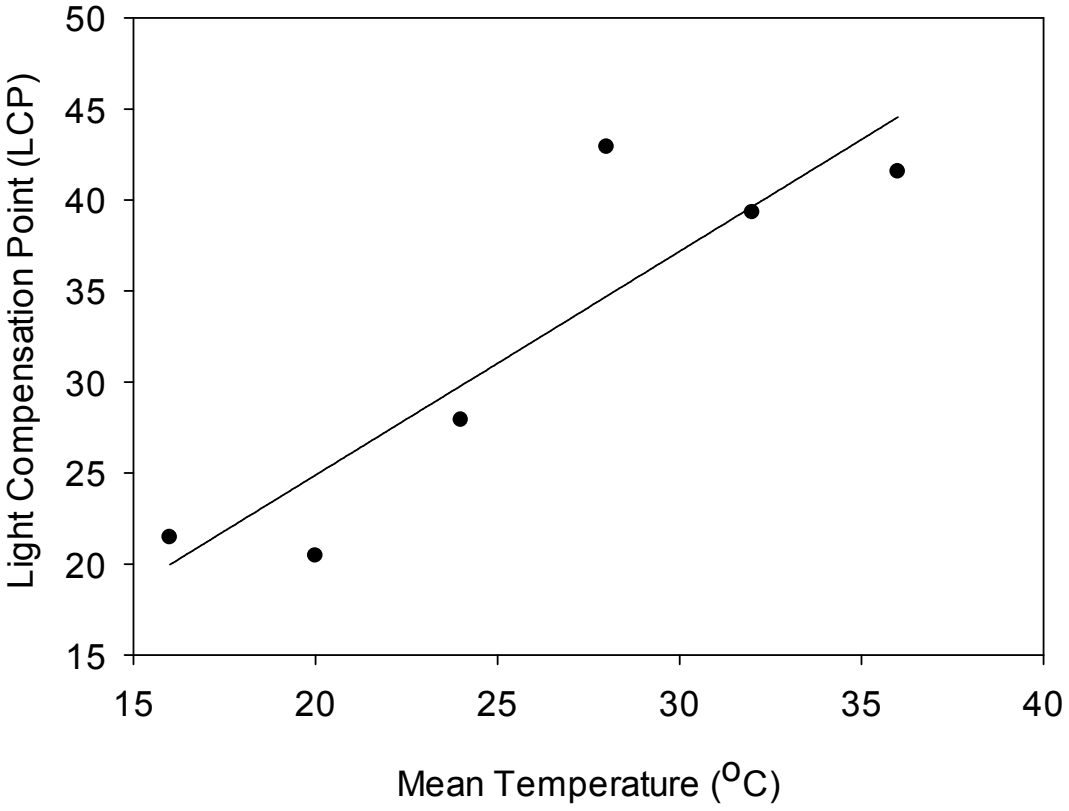
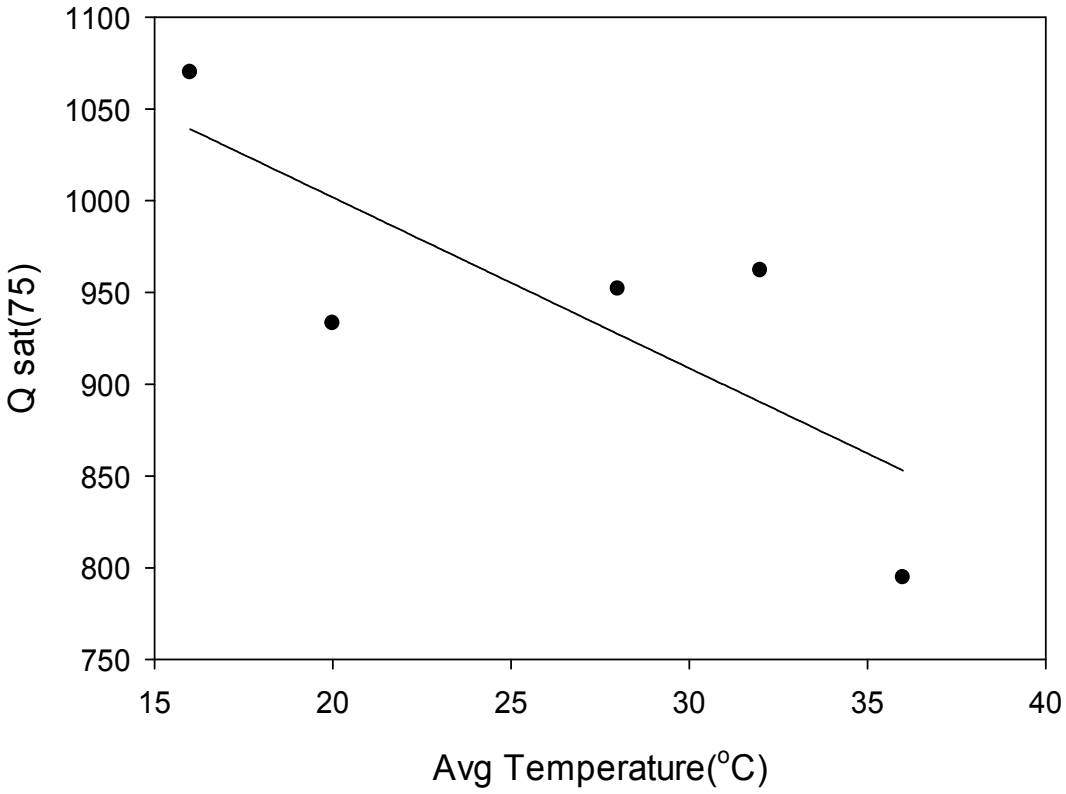


Fig. 10: Effect of light saturation point (LCP) of top most fully expanded leaves of forage sorghum across six different temperatures.



VITA

Jhansy Reddy Katta

Candidate for the Degree of

Master of Science

Thesis: EVALUATING PHOTOSYNTHETIC RESPONSES OF SORGHUM (GRAIN & FORAGE) AND SOYBEAN TO TEMPERATURE STRESS

Major Field: Plant and Soil Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in July, 2019.

Completed the requirements for the Bachelor of Science in Agriculture at Prof Jayashankar T.S. Agricultural University (formerly a part of Acharya N.G. Ranga Agricultural University (ANGRAU)), Hyderabad, India in 2016.

Experience:

Graduate Research Assistant at Oklahoma State University, Department of Plant and Soil Sciences from July 2017 to July 2019.

Professional Memberships:

American Society of Agronomy (ASA)
Crop Science Society of America (CSSA)
Soil Science Society of America (SSSA)
American Association for the Advancement of Science (AAAS)