NTNU
Norwegian University of
Science and Technology

Photosynthetic Responses of maize (Zea mays L.) seedlings to heat and drought stress

Michael Nii Adjah Torgbor

Acknowledgements

Trondheim, May 2017

MICHAEL NII ADJAH TORGBOR

Table of contents

Abbreviations

A .... Assimilation
ABA .... Abscisic acid
AIC .... Akaike information criterion
ATP .... Adenosine triphosphate
Chl .... Chlorophyll
Chl* .... Chlorophyll in the excited state
Ci .... Internal CO_2 concentrations
E .... Transpiration
F_o .... Minimal chlorophyll fluorescence from dark-adapted leaves
F_m .... Maximal chlorophyll fluorescence from dark-adapted leaves
F_v .... Variable chlorophyll fluorescence in dark adapted leaves
F_v/F_m .... Maximum quantum efficiency of PSII photochemistry in dark-adapted leave
gs .... Stomatal conductance.
H .... High temperature treatment
HD .... High temperature – drought (dry) treatments
HSPs .... Heat shock proteins
HW .... High temperature – moisture (wet) treatment
J_{max} .... Maximum electron transport rate
L .... Low temperature treatment
LD .... Low temperature – drought (dry) treatments
LW .... Low temperature – moisture (wet) treatment
M .... Moisture
ML .... Maximum likelihood
MT .... Measurement temperature.
NADPH .... Nicotinamide adenine dinucleotide phosphate
NPQ .... Non-photochemical quenching
P680 .... Central reaction center chlorophyll molecule of PSII
P700 .... Central reaction center chlorophyll molecule of PSI
PAM .... Pulse amplitude modulation
PC .... Plastocyanin
PEPC .... Phosphoenolpyruvate carboxylase
Pheo .... Pheophytin
PPFD .... Photosynthetic photon flux density
PQ .... Plastoquinone
PsbS .... PSII subunit and gene product of PsbS
PSI .... Photosystem I
PSII .... Photosystem II
Q_A .... Plastoquinone molecule at site A in PSII reaction centers
Q_B .... Plastoquinone molecule at site B in PSII reaction centers
qE .... Flexible Non-photochemical quenching
qI .... Sustained Non-photochemical quenching
REML .... Restricted maximum likelihood
ROS .... Reactive oxygen species
RuBP .... Ribulose 1,5 bisphosphate
Rubisco ....Ribulose 1,5 bisphosphate carboxylase/ oxygenase

T	…………...	Temperature
V	…………...	Variety
V_{cmax}	…………...	Maximum catalytic rate of ribulose 1,5 bisphosphate carboxylase/ oxygenase
V_{pmax}	…………...	Maximum catalytic rate of phosphoenolpyruvate carboxylase (PEPC)
WUE	…………...	Water use efficiency.

Abstract

Like all other plants, maize (*Zea mays* L.) depends on the environment for growth. It is also an important food source for the continuously increasing human population. However, due to the recent changes in climate, maize plants in current maize producing regions are faced with unfavorable conditions such as drought and high temperatures which adversely affect productivity. The response of plants to existing stress factors is, however, dependent on the developmental stage, duration of stress and interaction among stress factors.

The effect of temperature and moisture level on growth, photosynthetic parameters and chlorophyll fluorescence were investigated at the early seedling (young) stage of growth of two maize varieties (Obaatampa (OB) and SunSweet (SS)) from different geographic locations (tropical and temperate respectively). Seedlings were subjected to both high and low temperature under dry and moist water conditions in a 2x2x2 factorial experiment. The effect of measurement temperature on the photosynthetic responses was also determined since they were obtained at two different temperatures.

The biomass results showed that, though the varieties differed, high temperature and drought stress reduced the growth of both varieties with OB growing better than SS under low temperature. High temperature also adversely affected all the photosynthetic parameters with assimilation (A), transpiration (E) and stomatal conductance (g_s) following the same trend except water use efficiency (WUE). The observed trends showed that A and E were controlled by g_s.

The two varieties differed in their response to moisture with OB opening its stomata under moist conditions whiles SS did the opposite. This resulted in the difference in WUE which was better in SS as compared to OB indicating different mechanisms of survival under stress. Measurement temperature adversely affected only E and WUE at high measurement temperatures with variation in variety under different moisture treatment.

The various stress combinations didn't have any adverse effect on photosystem II, with both varieties maintaining a maximum photochemical efficiency (F_v/F_m) above 0.80 in all treatments though there were variation in varieties under the moisture treatment combinations.

In summary, results show that, although high temperature adversely affects stomatal conductance which in turn limit the Calvin cycle reaction, the varieties respond differently under contrasting

moisture conditions though the plant was healthy. However, though the tropical variety (OB) was expected to perform much better than the temperate variety (SS) at high temperature, OB was just slightly better than SS at high temperature under moist conditions but much better at low temperatures.

1. Introduction
1.1. Stress effect on maize

Maize (*Zea mays* L.) is an annual grass in the Poaceae family and currently one of the top three cereals, along with rice and wheat grown worldwide. Its current growth or production is due to its importance as a major source of food for both humans and animals, thus providing food directly and indirectly (via animals) for about 4.5 billion people only in developing countries. The seeds of maize are either eaten whole or dried and processed into flour. Corn oil is used in cooking while corn starch is used as a thickener in sauces and puddings or further processed to produce high-fructose corn syrups which are inexpensive sources of sugar in processed foods (De Groote *et al.*, 2013; Shiferaw *et al.*, 2011).

Plants, in general, depend on the environment for growth, where better conditions favor better growth and productivity thereby providing more food for the continuously increasing population of humans. Productivity is greatly reduced under poor or unfavorable environmental conditions such as cold, drought and heat irrespective of the nature of the plants grown. These environmental factors impose stress on the plants which adversely affects them (de Carvalho *et al.*, 2011; Shiferaw *et al.*, 2011; Wahid *et al.*, 2007).

Maize is no exception and suffers in the face of several environmental factors even though it is a C4 plant with better stress tolerance mechanisms as compared to C3 plants (de Carvalho *et al.*, 2011). Stress imposed on plants results in numerous physiological and biochemical changes leading to the adoption of various mechanisms to avoid or tolerate the stress to survive. While some changes include the synthesis and expression of compatible solutes (for example, proline and glycine betaine), carbohydrates and protective proteins (HSP), others affect the photosynthetic parameters upon exposure to stress (Bagley *et al.*, 2015; Close, 1996; S. Liu *et al.*, 2015; Shiferaw *et al.*, 2011).

Drought is one of the major causes of crop loss worldwide, bringing about a 20-40% reduction in average yields. This worsens through the synergistic interaction with other environmental stress factors such heat which also adversely affect plant as a single factor (Ashraf & Harris, 2013; Efeoğlu *et al.*, 2009; Hale & Orcutt, 1987; Shah & Paulsen, 2003). The synergistic interaction of both heat and drought stress results in the reduction of the turgor pressure leading to the cessation of cell expansion. This is also associated with decreased transpiration and photosynthesis due to

the partial or complete stomata closure (Bagley *et al.*, 2015; Benešová *et al.*, 2012; Sinsawat *et al.*, 2004).

Drought and temperature changes also affect plant distribution (Bita & Gerats, 2013; Correia *et al.*, 2006; Wahid *et al.*, 2007). Maize grown at low temperatures tends to have a reduced photosynthetic rate when exposed to a short period of heat stress. These plants tend to recover after the short exposure to high temperatures but completely fail to recover after extended periods. However, plants grown at relatively higher temperatures are not slightly affected by a sudden rise in temperatures and tend to adapt to the prevailing condition faster (Z. Liu *et al.*, 2013; Sinsawat *et al.*, 2004). The response exhibited by maize plants under stress depends on the stage of the growth of the plant (Karim *et al.*, 2000). Although the reproductive stages have a high window of sensitivity to stress, the juvenile stages are delicate and sensitive to harsh conditions which result in the death of the plant before reaching other stages of growth (Hale & Orcutt, 1987; Karim *et al.*, 2000).

In the light of the recent temperature changes leading to heat and drought conditions in the tropics and subtropics, it is vital to gain insight into the responses of maize (one of the world's important cereals) due to the repressive effect of stress on plant photosynthetic rate (Battisti & Naylor, 2009; Shiferaw *et al.*, 2011; Taiz *et al.*, 2015).

1.2. Photosynthesis

Photosynthesis is the primary process used by autotrophs for the synthesis of complex carbon compounds and is greatly affected in all growth phases by stress. Photosynthesis involves several components including photosynthetic pigments, photosystems and electron transport as well as the CO_2 reduction pathways. Damage caused by stress at any of these steps reduces the overall photosynthetic capacity of plants (Ashraf & Harris, 2013; Taiz *et al.*, 2015).

The mechanism of operation of photosynthesis is characterized by two major sets of reactions: the light reactions and the dark reactions (Figure 1.1). While the light reactions generate chemical energy (ATP and NADPH), the dark reactions entail the capture and assimilation of CO_2 in the midst of other inorganic components via the use of the chemical products generated via the light reaction (Ashraf & Harris, 2013; Taiz *et al.*, 2015).

Although plants use the two main stages of photosynthesis (light and dark reaction) for sugar production, there exists variation in the photosynthetic mechanism due to adaptation to specific climates. While C3 plants, which represent 95% of Earth plant species, grow well in a broad range of conditions apart from hot, dry and bright light conditions, C4 plants, including maize, in contrast, grow well in dry and hot climatic condition accompanied by high light intensities. Another group, Crassulacean acid metabolism plants (CAM) are mainly adapted to arid areas since they have better water conserving and limited photorespiration characteristics (Ashraf & Harris, 2013; Taiz *et al.*, 2015).

Irrespective of the plants, stress factors such as drought and unfavorable temperatures in one way or the other hinder the photosynthetic process (light and dark reaction) through the regulation of the stomata, alteration in the organelle structure as well as the concentrations of enzymes (Ashraf & Harris, 2013; Taiz *et al.*, 2015).
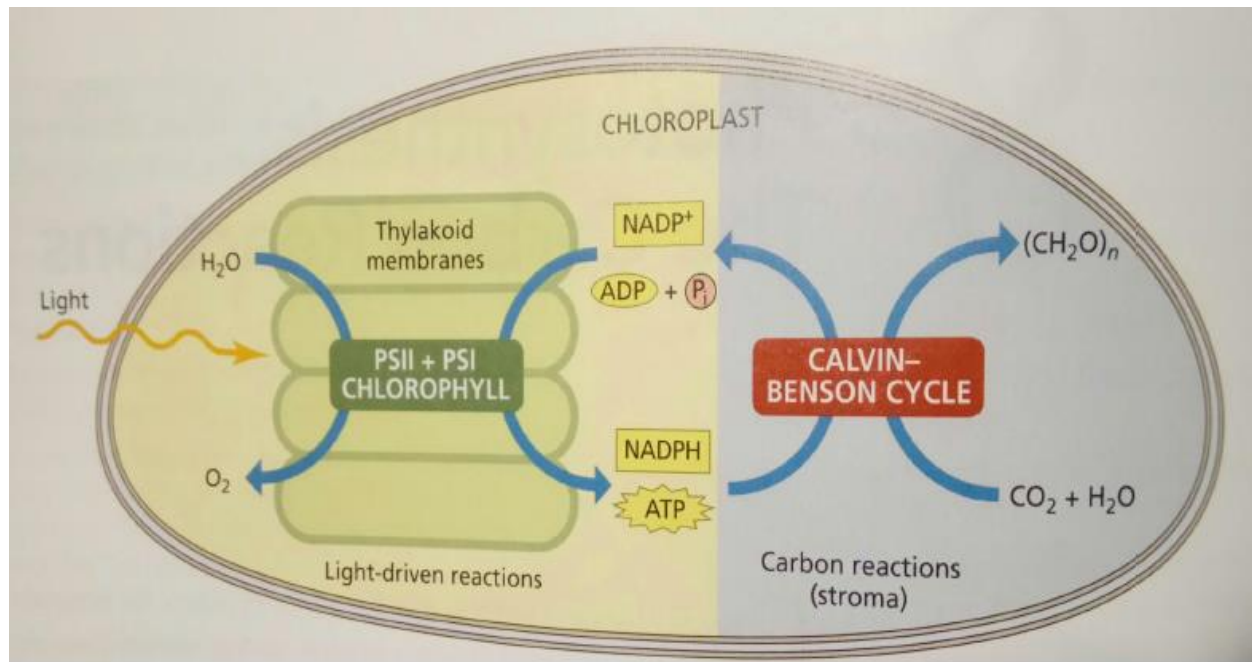
Figure 1.1. The diagrammatic representation of the light (left) and dark reactions (right). The light reactions produce NADPH and ATP used in the dark reactions for CO_2 assimilation (Taiz *et al.*, 2015).

1.2.1. Light reactions

Although plants are designed to take up large amounts of light energy for chemical energy through the chloroplast containing chlorophyll, the duration as well as the intensity of the light influences the capture and utilization of the energy. The light reaction is the initial stage of photosynthesis in the thylakoid and involves light harvesting complexes (antennae) which capture and transfer light energy to the photochemical reaction centers which receive the energy from the antennae for the necessary chemical oxidation - reduction reactions (Taiz *et al.*, 2015).

Chlorophyll containing antennae (integral and peripheral) absorb a photon of light leading to the transition of the chlorophyll (Chl) from the ground state or lower energy to an excited state (Chl*). Chlorophyll is highly unstable when it's in the higher excited state and rapidly gives up some of the energy to the surroundings as heat in order to enter the lowest excited state where it remains stable for a maximum of several nanoseconds (10^{-9} s) (Hale & Orcutt, 1987; Taiz *et al.*, 2015).

4

Due to the inherent instability of the chlorophyll in the excited state, the lowest excited state of the chlorophyll has four alternative pathways to dispose of excitation energy and return to the ground state. These include; fluorescence, which is the re-emission of the photon accompanied by heat; disposing of energy as heat only without fluorescence; chlorophyll energy transfer; and last but not the least, photochemical reactions (Figure 1.2) (Demmig-Adams *et al.*, 2015; Taiz *et al.*, 2015).

Although the light reaction complex is designed to take up large amounts of light energy for chemical energy, the mechanism of light harvesting and usage are negatively affected by existence of unfavorable conditions such as drought and unfavorable temperature, rendering the energy from a photon potentially damaging to the plant (Demmig-Adams *et al.*, 2015; Hale & Orcutt, 1987; Taiz *et al.*, 2015).

As a major environmental stress, drought causes not only stomatal closure and damage to the photosynthetic pigments, but also leads to the deterioration of the thylakoid membrane, resulting in the reduction of the Chl content (Efeoğlu *et al.*, 2009; Wahid *et al.*, 2007). This leads to a reduction in the growth of the leaves as well as the roots of maize and wheat under stress (Ashraf & Harris, 2013). The decrease in the chlorophyll is mainly attributed to the accelerated rate of breakdown rather the slow rate of Chl synthesis due to drought (Wahid *et al.*, 2007).

At high temperatures also, there is a reduction in the Chl biosynthesis leading to the reduction in the photosynthetic rate (Efeoğlu *et al.*, 2009; Wahid *et al.*, 2007). However, during periods of both stress factors, there is a synergistic effect which aggravates the negative impact of these factors on plant growth (Hale & Orcutt, 1987; Taiz *et al.*, 2015).
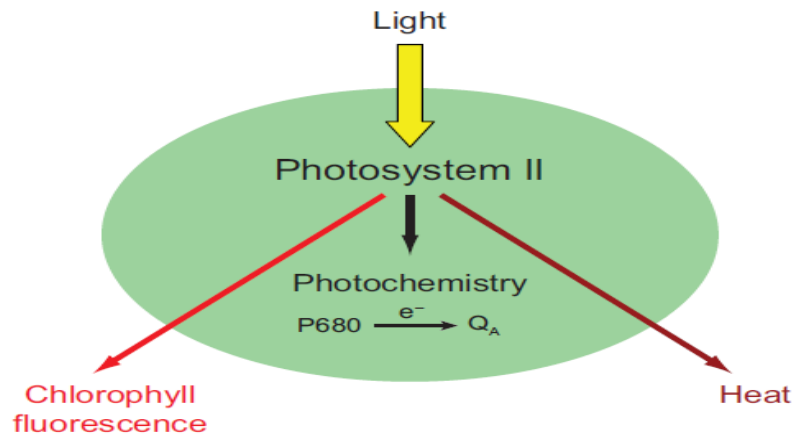
Figure 1.2. The diagrammatic representation of absorbed light energy (photon) by a chlorophyll. Light absorbed by plants could either be directed toward photosynthesis (photochemical), dissipated as fluorescence or heat, or both (Baker, 2008).

Photochemistry

The conversion of the energy from the excited state for chemical reactions (photochemical reaction) is amongst the fastest known chemical reactions. Its characteristic speed enables it to compete with the other three energy dissipating mechanisms (Figure 1.2). The photochemical reactions consist of two reaction centers, Photosystem I (PSI) and Photosystem II (PSII), which operate in series with independent antennae systems such as the light harvesting complexes LHCI and LHCII, respectively (Ashraf & Harris, 2013; Hale & Orcutt, 1987; Taiz *et al.*, 2015).

These reaction centers vary in the chlorophyll absorbance wavelength as well as function with PSII chlorophyll absorbing at 680nm (P 680) whiles that of PSI absorbs maximally at 700nm (P 700). PSII oxidizes water to produce two electrons which are transferred by pheophytin and two quinones (plastoquinone (Q_A) and plastohydroquinone (Q_B)) to cytochrome b_6f (Figure 1.3). The electrons are then transferred via another electron transporter (plastocyanin (PC)) to PSI. While the Cytochrome b_6f uses one electron to increases the number of protons pumped across the membrane to increase the generate ATP by the ATP synthase, the other is used by PSI in the reduction of $NAPD^+$ to NADPH (Taiz *et al.*, 2015).

In the presence of excess excitation energy which is not effectively dissipated (quenched), toxic species such as singlet oxygen, superoxide and peroxide are produced which negatively affect

photosynthesis by damaging the pigments, nucleic acids and proteins. Although reversible at the early stages, prolonged exposure to excess light results in the permanent damage of the D1 protein of the PSII reaction center complex leading to photoinhibition. The damaged DI has to be newly synthesized for the system to resume its normal photosynthetic function (Taiz *et al.*, 2015; Wahid *et al.*, 2007).

Stress results in the reduction of the light-absorbing efficiency of the photosystem mainly PSII due to its high susceptibility to stress (Ashraf & Harris, 2013; Taiz *et al.*, 2015). Prolonged stress leads to the modification of pigments which render the photochemical center less efficient. Moreover, drought stress doesn't just reduce the efficiency of PSII, but also of PSI leading to energy loss to remain healthy (Ashraf & Harris, 2013).
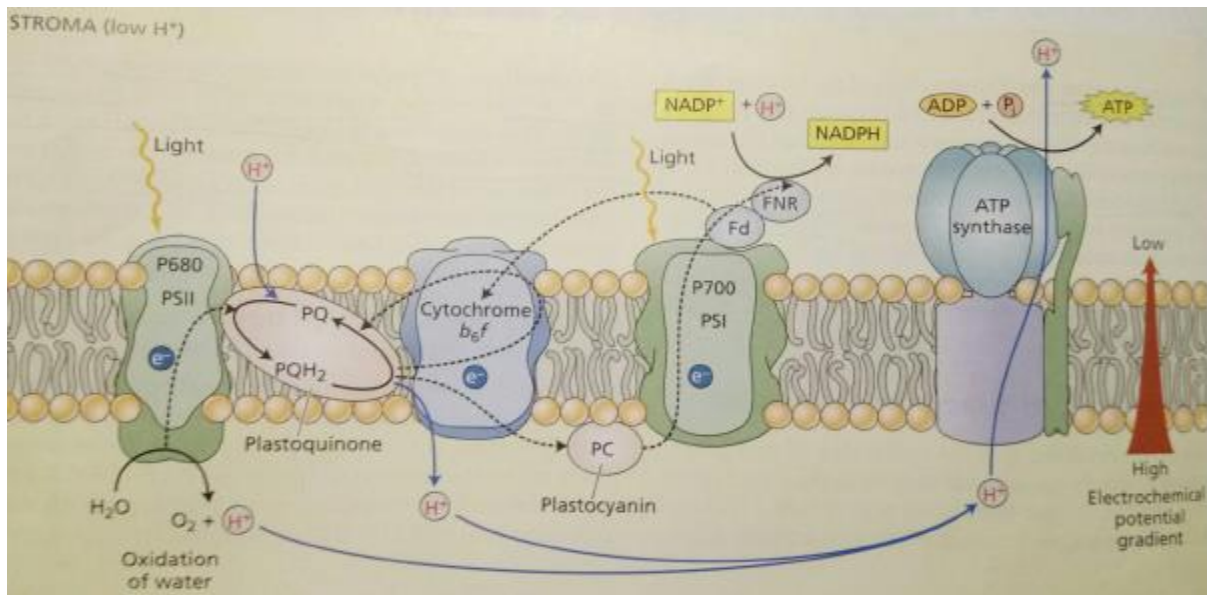

Figure 1.3. Diagrammatic representation of the light reactions leading to the production of ATP and NADPH for the dark reactions(Taiz *et al.*, 2015).

Non-photochemical quenching (NPQ)

When plants absorb more light than they can use in photochemical processes, the excess energy is dissipated to reduce the formation of excess reactive oxygen which is detrimental to plant growth (Taiz *et al.*, 2015). Non-photochemical quenching is the major process that serves as a "volume knob" that regulates the delivery of excitation energy to the reaction centers of plants. It is achieved through the dissipation of the energy as heat, thereby reducing the formation of reactive oxygen species (ROS) which negatively affect plant growth. However, NPQ is not a mutually exclusive process and occurs in the Light harvesting complexes (antennae) of the PSII system as well as PSII itself (Adams III *et al.*, 2008; Hale & Orcutt, 1987; Taiz *et al.*, 2015; Tardy & Havaux, 1996).

Non-photochemical quenching is divided into two main forms based on their flexibility (speed of recovery): flexible or rapidly reversible dissipation (qE type of NPQ) and the sustained dissipation (qI type of NPQ) (Adams III *et al.*, 2008). While rapidly reversible quenching deals with the recovery within a short duration upon exposure to light fluctuations, sustained quenching takes longer to relax in the face of prolonged environmental stress as well as light resulting in the decrease of the plants photosynthetic capacity. Although less is known about sustained dissipation as compared to rapid dissipation, there is a common mechanism underlying their dissipation of heat known as the xanthophyll cycle (Figure 1.4) (Adams III *et al.*, 2008; Pfundel, 2003).

The xanthophyll cycle is triggered by the reduction in the pH of the thylakoid lumen due to increased H^+ transported across its membrane. This results in the activation of the de-epoxidase enzyme which converts violaxanthin to antheraxanthin and finally to zeaxanthin as a heat dissipation mechanism during periods of light saturation which are also caused by stress factors. During dark adaptation periods, zeaxanthin is converted to back to violaxanthin by the epoxidase enzyme since the system is not primed for heat dissipation. This mechanism increases the concentration of violaxanthin during dark or favorable light absorption conditions as compared the intense light stress conditions (Adams III *et al.*, 2008; Baker, 2008; Demmig-Adams *et al.*, 2015; Taiz *et al.*, 2015).
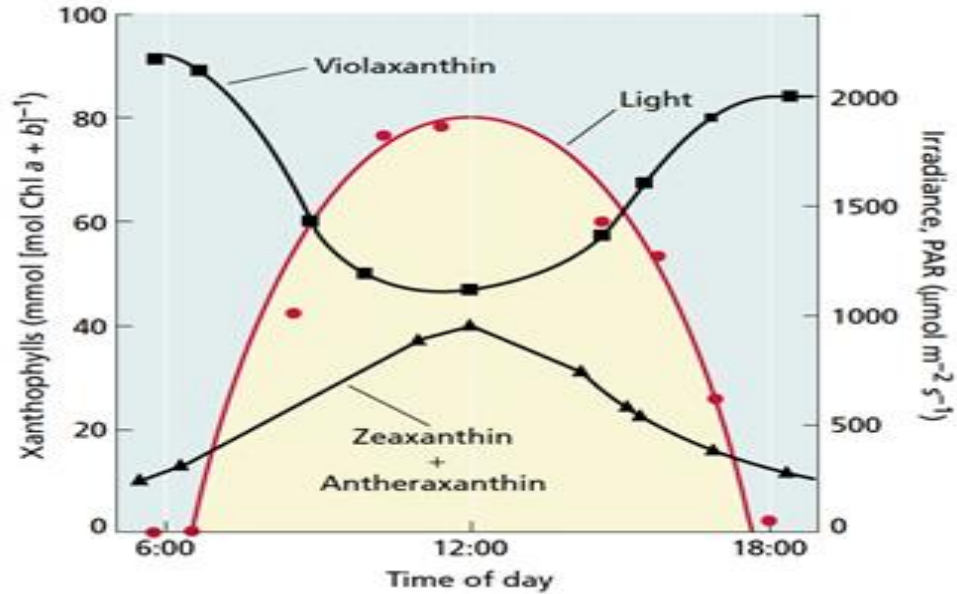
Figure 1.4. Diagrammatic representation of the changes in xanthophyll concentration's during high and low light intensities dissipating excess energy as heat (Taiz *et al.*, 2015).

Chlorophyll fluorescence

Chlorophyll fluorescence, the other alternative pathway for energy dissipation, entails the radiation of a longer wavelength (lower energy) as compared to the absorbed wavelength (higher energy) (Lazar, 2015; Taiz *et al.*, 2015). Although the processes following light absorption are competitive, changes in photochemical and non-photochemical quenching rates affect the yield of chlorophyll fluorescence. Due to this, measurement of chlorophyll fluorescence, which is a non-invasive procedure, provides insight into the energy division in the leaves of plants (Adams III & Demmig-Adams, 2004; Demmig-Adams *et al.*, 2015; Lazar, 2015).

The fluorescence of chlorophyll is an effective means used to determine the health of a plant even in the presence of stress factors. In the absence of light (dark conditions), healthy and non-stressed plants are not bothered by alternative means of dissipating energy since the chlorophylls are in their ground states (Adams III & Demmig-Adams, 2004). This makes the reaction center as well as the light harvesting complexes ready and open to photochemically process any incoming light energy or photon. Under (weak) non-actinic light which doesn't drive photosynthesis, plant leaves

9

emit a minimum fluorescence (F_o) (Adams III & Demmig-Adams, 2004; Demmig-Adams *et al.*, 2015).

After a flash of an intense or saturating pulse of red light (actinic light) for a short period, the photochemical reactions PSII are driven leading to the full reduction of the plastoquinones. At this state, PSII is said to be closed (Baker, 2008). Due the closure of the photochemical pathway (PSII) as well as no NPQ, the absorbed energy (photon) is released as a fluorescence known as the maximum fluorescence (F_m) which represents the unused portion of the absorbed photons (Demmig-Adams *et al.*, 2015; Pfundel, 2003; Taiz *et al.*, 2015).

The difference between the minimal (F_o) and maximum (F_m) fluorescence yields the variable fluorescence (F_v) which represents the total amount of energy absorbed by the plant that is available for photosynthesis by the photosystem II (PSII). This is used in the computation of the maximum photochemical efficiency of the PSII using the ratio F_v/F_m. The maximum photochemical efficiency of the plant gives a clear indication of the state of the photosynthetic performance of a plant. Plants having a maximum photochemical efficiency or maximum quantum yield of 0.80 and above are regarded as healthy while those below are known to be unhealthy (stressed) since they don't capture enough light energy for photosynthesis (Demmig-Adams *et al.*, 2015; Pfundel, 2003; Taiz *et al.*, 2015).

Light response curves.

The response of plants to both varied light and CO_2 concentration provides vital insight of about the photosynthetic performance of the plant.

Although light is a critical resource that limits plant growth, the exposure to too much or too little vary in the contribution to plant growth. Measuring the net CO_2 fixation in intact leaves across varying photosynthetic photon flux density (PPFD) level generates light-response curves. However, in the dark, there is no photosynthetic CO_2 assimilation, but due to the mitochondrial respiration, CO_2 is given off by the plant thereby shifting the light response curve to the negative part of the graph (Baker, 2008; Taiz *et al.*, 2015).

As the light increases, photosynthetic CO_2 assimilation eventually reaches a point at which the CO_2 uptake exactly balances CO_2 produced by respiration. This is known as the light compensation point since the net assimilation of CO_2 is zero due to the balance between the photosynthesis and respiration. There is some variation in light compensation point within species as well as developmental stages with the sun plants having light compensation points range of 10-20 µmol $m^{-2} s^{-1}$ whiles that of shade plants range from 1-5 µmol $m^{-2} s^{-1}$. This is due to the fact that, little respiration is required in the shade plants to bring the light compensation to zero (Taiz *et al.*, 2015).

There exists a linear relationship between PPFD and the photosynthetic rate as the light level increases above the light compensation point. Photosynthesis is light limited throughout the linear portion of the light curve, where more light stimulates more photosynthesis which provides a slope known as the maximum quantum yield. This represents the ratio of a given light dependent product to the number of absorbed photons (Taiz *et al.*, 2015).

At a higher PPFD along the light- response curve, a point is reached where the where the curve starts to level off and eventually approaches saturation. At the light saturation point, net photosynthesis no longer increases, indicating that other factors other than light, such as rubisco activity, RuBP regeneration or the metabolism of triose phosphate has become the limiting factor. The light response curves of most plants saturate in the range of 500-1000 µmol $m^{-2} s^{-1}$ which is well below the full sunlight 2000 µmol $m^{-2} s^{-1}$. However, the plants grown in well-fertilized conditions have saturation point above 1000 µmol $m^{-2} s^{-1}$ (Taiz *et al.*, 2015). This indicates that, plants with reduced health as a result of stress are have reduced assimilation of CO_2 since they have lower light compensation point (Taiz *et al.*, 2015).

1.2.2. Dark reactions

These are stroma based reactions that follow the light reactions. Although they were initially thought to be independent, they are dependent on ATP and NADPH produced by the light reactions. These dark reactions are now more properly referred to as carbon reactions of photosynthesis since the products of the photochemical process not only provides a substrate for enzymes but also controls the catalytic rate. The dark reactions entail a series of processes known

as the Calvin/Benson cycle which incorporates atmospheric CO_2 into organic compounds (Taiz *et al.*, 2015).

The Calvin-Benson cycle.

The Calvin-Benson cycle is the predominant pathway used by autotrophs to manufacture their own food source through the fixation of the atmospheric CO_2 into the skeletons of organic compounds. This pathway leads to the decrease in the oxidative state of carbon from the highest values CO_2 (+ 4), to (+2) and (0) in sugar and secondary alcohols respectively. In the light of the ability to lower the oxidative state of carbon, the Calvin cycle is also known as the reductive pentose phosphate cycle and the photosynthetic carbon reduction cycle (Taiz *et al.*, 2015).
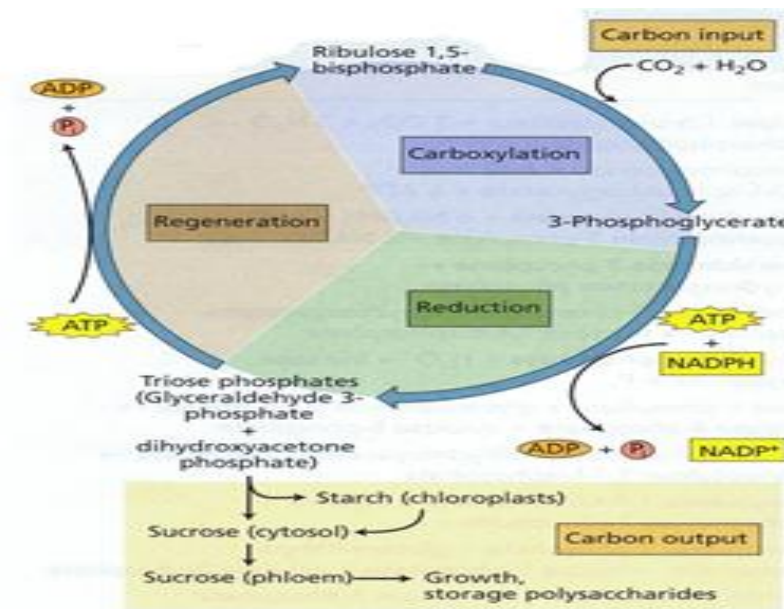
Figure 1.5. Calvin-Benson cycle showing the three main stages of carbon dioxide (CO_2) capture and assimilation (Taiz *et al.*, 2015).

The Calvin cycle occurs in three highly regulated stages in the stroma, namely carboxylation, reduction and regeneration (Figure 1.5). The first enzymatic step (carboxylation stage) entails the uptake of CO_2 by a 5-carbon molecule (ribulose 1,5-bisphosphate) catalyzed by the ribulose 1,5-bisphosphate carboxylase oxygenase enzyme (Rubisco). This results in an unstable 6-carbon molecule which separates into two molecules of 3-Phosphoglycerate upon the reaction with water H_2O. The reduction phase entails the reduction of the 3-Phosphoglycerate molecules to triose

phosphates. This step is driven by two enzymes (3-phosphoglycerate kinase and NADP-glyceradehyde-3-phosphate dehydrogenase) as well as photochemically generated ATP and NADPH. This is followed by the regeneration of CO_2 acceptor molecule (ribulose 1,5-bisphosphate) through enzyme catalyzed reactions as well as ATP consumption for the next carbon fixation cycle (Baker, 2008; Taiz *et al.*, 2015).

Since plants vary in their makeup, there exists variation in the rate at which they handle unfavorable conditions to survive. C4 plant species, including maize, possess higher temperature optimum for photosynthesis as compared to C3 plants. C4 and CAM plant also have adaptations that favour growth under reduced atmospheric CO_2 concentrations and low water availability respectively. The adaptation of C4 and CAM plants involves the initial uptake of the CO_2 by a three carbon molecule (phosphoenol-pyruvate) by the aid of the phosphoenol-pyruvate carboxylase (PEPCase) to four carbon molecules such as malate and aspartate. These products are either stored in the vacuole in CAM plants or channeled to the internal regions of the cell where NADP-malic enzyme breaks down the 4-carbon intermediates to their initial 3-carbon molecule to release the CO_2 which enters the normal Calvin-Benson cycle (Bagley *et al.*, 2015; Crafts-Brandner & Salvucci, 2002; Taiz *et al.*, 2015). This adaptation of C4 and CAM plants prevents them from undergoing photorespiration (Taiz *et al.*, 2015).

Though photosynthesis is affected by several environmental factors, the effects of these factors are determined via certain measurement parameters of such as gas exchange, stomatal conductance, leaf internal CO_2, net and gross photosynthesis, dark respiration as well as light and CO2 response curves since photosynthesis is affected by the properties of the leaves (Taiz *et al.*, 2015).

Stomatal conductance and Gas exchange.

Stomata are microscopic pores on plant leaves and serve as a channel between their interior and the exterior environment (Lehmann & Or, 2015). The regulation of the entry as well as the exit of substances from the leaf via the guard cells surrounding the stomata is due to plant response to environmental signals. Plant adopt various mechanism to survive in environments through the regulation of the opening and closing as well as distribution of the stomata on the leaves which

directly affect the gas exchange of the plant (Lehmann & Or, 2015; Taiz *et al.*, 2015). The prolonged closure of the stomata results in the reduction of the internal CO_2 since diffusion is prevented. This negatively affects photosynthesis since CO_2 serves as an activator for Rubisco as well as the substrate for photosynthesis (Taiz *et al.*, 2015).

Stomata of plants are not only controlled by the reduced concentration of CO_2 in the environment, but also other factors such as humidity, light intensity and soil moisture via abscisic acid (ABA) from roots. These factors also affect conductance, internal CO_2 and photosynthesis since the rate of entry as well as the exit of gases are dependent on the stomata within a particular environment (Efeoğlu *et al.*, 2009; Taiz *et al.*, 2015).

During drought and high temperature, atmospheric moisture decreases resulting in the closure of the stomata to prevent excessive water loss through transpiration. This decreases gas exchange as well as internal CO_2 which negatively affects photosynthesis (Benešová *et al.*, 2012; Efeoğlu *et al.*, 2009). The closure of the stomata in water-stressed environments (low soil water potential), is a mechanism to prevent the excessive loss of water (transpiration) leading to the reduction CO_2 uptake and an increase in internal O_2 within the leaves which can result in photorespiration (Efeoğlu *et al.*, 2009).

Light, on the other hand, plays a dominant role in stomatal regulation through its rapid and reversible influence on stomatal opening and closure. The characteristic movement of the stomata is attributed to mainly blue light which occurs throughout the life of the plant (Taiz *et al.*, 2015). Stomata opens as the level of light reaching the surface of the leaf increases and closes as the level of light reaching the decreases. Blue light activates a proton pump that affects guard cell osmotic balance through the lowering of the pH of the apoplastic spaces surrounding the guard cells and generates the driving force for ion uptake as well as stomatal opening (Hale & Orcutt, 1987; Taiz *et al.*, 2015).

Effect of stress on dark respiration, net and gross photosynthesis, internal CO_2.

As the substrate for photosynthesis, CO_2 is vital for assimilation (A) inside the leaf (Bagley *et al.*, 2015). The internal leaf CO_2 concentration will always be less than the ambient when net photosynthesis is positive since CO_2 is consumed through photosynthesis and the diffusion into the leaf is restricted by the regulation of the stomata (Lehmann & Or, 2015; Taiz *et al.*, 2015). Upon gaseous entry of CO_2 in the cell, it dissolves in the cell water and diffuses as bicarbonate ion to the stroma of the chloroplast to produce sugar. (Taiz *et al.*, 2015). Plants growing in adequate water, light, nutrients as well as CO_2 enriched environments above the natural atmospheric conditions results in increased photosynthesis and enhanced productivity. The expression of photosynthesis as a function of the partial pressures of CO_2 in the air spaces or internal CO_2 (Ci) in the leaf makes possible the evaluation of limitations of photosynthesis imposed by the CO_2 supply, indicating that, photosynthesis is greatly reduced at low Ci concentrations (Taiz *et al.*, 2015).

Following the process of production of the needed sugar for plant growth through photosynthesis, is the breakdown of the sugar to generated energy for other cellular processes. This occurs through a process called dark respiration, providing the plants with the ability to perform other functions to sustain growth. Net photosynthesis which gives the rate of carbon fixation is determined from the difference between the gross photosynthesis and the respiration of the plant. (Taiz *et al.*, 2015).

CO_2 response curves

CO_2 response curves are analogous to light response curves, but deal with the determination of the effect of varied CO_2 concentration on the photosynthetic ability of the plant (Taiz *et al.*, 2015).

Under favorable conditions, CO_2 enrichment above that of the natural atmosphere results in increased photosynthesis and enhanced productivity. The expression of photosynthesis as a function of partial pressure of CO_2 in the intercellular spaces (Ci) within the leaf makes it possible to evaluate effects of the imposed CO_2 supply. At low Ci concentrations, photosynthesis is strongly limited, but in the absence of atmospheric CO_2, the leaves of plants give out CO_2 due to the mitochondrial respiration (Taiz *et al.*, 2015).

As the Ci concentration increases, a point is reached where the rate of photosynthesis balances the respiration rate known as the CO_2 compensation point. At this point, the net assimilation of CO_2 by the leaf is zero (Taiz *et al.*, 2015).

At low to intermediate concentration of CO_2, photosynthesis is limited by the carboxylation ratio of rubisco. However, at higher Ci concentrations, photosynthesis start to reach a saturation point where net photosynthetic rate becomes limited by other factors aside CO_2, such as light. By analysis of the CO_2 response curve, the maximum catalytic rate of ribulose 1,5 bisphosphate carboxylase/ oxygenase (Rubisco) (V_{cmax}), the maximum catalytic rate of phosphoenolpyruvate carboxylase (PEPC) (V_{pmax}) as well as the maximum electron transport rate (J_{max}) can be determined (MASSAD *et al.*, 2007; Taiz *et al.*, 2015).

Plants in general, regulate their Ci values by regulating their stomata opening to prevent reaching their saturation point. The plot of CO_2 assimilation (A) to Ci shows how photosynthesis is regulated by CO_2, independent of the functioning of the stomata (Taiz *et al.*, 2015).

For C3 plants, the initial slope of the response of the rate of A to the Ci concentration represents the V_{cmax}, while the J_{max} is calculated from the plateau of the response of the A to PPFD. For C4 plants, the calculation of the V_{cmax}, V_{pmax} and J_{max} cannot be done graphically due to their complex biochemical mechanisms (MASSAD *et al.*, 2007; Sharkey *et al.*, 2007).

In C4 plant which includes maize, the photosynthetic rate of the plant is saturated at Ci values of about 100-200 ppm. Due to this, C4 plants do not benefit much from increased in atmospheric concentrations of CO_2 (Taiz *et al.*, 2015). C4 plants, although known to possess better survival mechanism than other plants (C3 plants), the exposure to stress (heat and drought) negatively affects their stomatal conductance which affects the intake of CO_2 (Z. Liu *et al.*, 2013).

1.3. Aim of study

As seen above, the photosynthetic capability of a plant is greatly dependent on its state of health. The climatic conditions existing within an area remains a strong contributing factor which determines the health a plant.

Owing to the current change in climatic conditions, maize plants like all other plants, although having the capability to adapt to changes, are negatively affected during the exposure to harsh conditions for extended periods. These negatively affect the photosynthetic parameters which ultimately reduce the rate of photosynthesis and overall growth of the plant.

Although there exists some research on maize, most of these researches are focused on the latter stages of development and yield. Also, there is reduced knowledge on the effect of harsh or stress conditions over extended periods mainly in the early stages of developments. Furthermore, although there are only a few works on maize at the early growth stage, most research only considered a single stress factor and short exposure of the maize to the stress conditions. Lastly, even though most research has been focused on the latter stages of growth, it is known that, alteration due to harsh conditions at the early stage of plant growth negatively affect the final yield of the plant.

In view of this, this project seeks not just to determine the effect of single factors (drought and heat stress) faced by the maize plant, but also the interactive effect of these stress factors on the photosynthetic rate in the young leaves of two maize varieties exposed to stress conditions.

1.3.1. Hypotheses

- Drought and heat forces stomatal closure thereby limiting gas exchange.

- Heat limits carboxylation rate.

- Prolong heat and drought saturates heat dissipation capacity, resulting in damage to photosynthesis system and photo-inhibition.

- The tropical variety performs better than temperate variety at high temperature

2. Materials and Methods

2.1. Materials and sample

Two maize varieties (Obaatampa (OB) and Sun Sweet F1 (SS)) were obtained from geographically different locations, namely tropical and temperate region respectively. OB was obtained from a seed shop in Ghana while Sun Sweet was from LOG AS, Oslo, Norway. OB is one of the top maize varieties in Ghana and is mainly known for its high yielding quality as well as relatively better survival in harsh conditions as compared to other local varieties. Sun Sweet is a preferred maize variety in the temperate countries and is consumed fresh as corn on the cob. The high yielding quality of OB as well as the sweetness of SS renders them important since they satisfy human wants.

2.1.1. Growth conditions

The Seeds of both varieties were sown in perlite at $22°C$ and 16 hours light in the greenhouse at the Norwegian University of Science and Technology (NTNU) for a period of 10 days. After emergence, healthy seedlings were transplanted into 3:1 potting soil: vermiculite filled pots to provide better soil conditions with 60 seedlings per variety with 15 seedlings per seed tray.

Seedlings were then transferred into growth chambers set at $25/20°C$ (day/ night) with 12 hours photoperiod with irradiance (400 µmol $m^{-2}s^{-1}$) supplied by fluorescent tubes for acclimatization for a week. Seedlings were kept healthy by regular watering to make the soil moist before the start of the experimental treatments.

After the acclimatization period, seedlings were randomized with a minimum of 7 pots per variety per tray; totaling 15 plants per variety for each treatment in both chambers. The temperatures were maintained at $35/20°C$ (day/ night; High temperatures) for one chamber and $25/20°C$ (day/night; low temperatures) for the other with a 12-hour photoperiod and light irradiance (400 µmol $m^{-2}s^{-1}$).

Well-watered (W) labelled plants were watered every other day in the ratio (25ml-15ml-25ml) while drought labelled (D) plants were watered on the first and last days of the well-watered plants with ratios (20ml-15ml). For all treatments, equal volumes of Hoagland solution were administered without alteration to the watering pattern.

Growth chambers were closely monitored and controlled before and during the experiment with no change in the set program. Each chamber had an upper and a lower part with different light banks making all the plants in each treatment group not independent, but however, they treated as such for the statistical analysis. The limitations in growth chamber prevented the repetition of the whole experiment creating a confounding of temperature with the possible chamber effects.

2.1.2. Experimental design

The experiment was conducted using the 2x2x2 factorial design in growth chambers. The fixed factors, levels and treatment combinations investigated are shown in Table 2.1 and 2.2.

Table2.1 Fixed factors and the various levels in the growth chamber.

FACTORS	LEVELS	SET LEVELS
VARIETIES	Obaatampa (OB)	
	Sweetcorn (SS)	
TEMPERATURE	High temperature (H)	35°C (Heat stress)
	Low temperature (L)	25°C
WATER / MOISTURE	High water / Wet (W)	75ml/ week
	Low water / Dry (D)	35ml/week (Drought stress)

Table2.2. The 4 main treatment combinations used for the experiment.

TEMPERATURE	WATER (MOISTURE)	
	High water/ Wet (W)	**Low water/ Dry (D)**
LOW TEMP. (L)	Wet and Low temp.	Dry and Low temp.
HIGH TEMP. (H)	Wet and High temp.	Dry and High temp.

2.2. Data collection

The parameters measured include chlorophyll fluorescence, photosynthetic response (A, E, g_s, WUE) as well as shoot dry weight (biomass). Whiles chlorophyll fluorescence and photosynthetic response measurements were taken on the 7th and 14th day after the start of the experiment, biomass samples were obtained only on the 14th day (end of the experiment).

For data analysis, the biomass data taken on the 14th day and only the 7th day fluorescence and photosynthetic response measurements were used since plants in some treatment groups were highly stressed by the 14th day of the experiment.

For each variety, 7 plants were sampled per treatment for fluorescence, 4 plants for photosynthetic responses while a minimum of 4 plants were used for biomass measurements.
Though CO_2 response curves data was taken on the 10th day, it was not analyzed due to the lack of C4 response software and poor data quality.

2.2.1. Chlorophyll fluorescence measurements

A pulse amplitude modulated fluorometer (PAM 210, Heinz Walts GmbH) was used to obtain the fluorescence data with leaves face down on the aperture. The maximum (F_m) and minimum (F_o) fluorescence were obtained from two dark adapted maize leaves per plant with 3 spot measurements per leaf. While F_o indicates that the fluorescence level at which plastoquinone (Q_A) is fully oxidized by far-red light, the F_m indicates the fluorescence level at which Q_A is fully reduced after a saturation pulse of red light (Baker, 2008; Taiz *et al.*, 2015). Under each treatment, 7 sample plants per variety were obtained per data taking day.

The maximum quantum yield ($F_v/F_m = (F_m - F_o)/F_m$) for each plant was obtained by averaging the measurements for each plant.

2.2.2. Photosynthetic measurements.

A PP Systems CIRAS 3 portable gas exchange system was used in measuring the photosynthetic rate at different temperatures (25°C and 35°C) with the aid of a script as well as CO_2 response curve data at random. A single maize leaf was measured with reference air from the CO_2 cartridge delivering a maintained concentration of 400 ppm of CO_2 for photosynthetic rate measurements at 400 µmol m^{-2}s^{-1} PAR with 5 minutes acclimatization time and three instantaneous measurements 10 seconds apart. The three measurements were averaged to get a single estimate of each photosynthetic parameter per plant.

For the CO_2 response, a pre-programmed C4 responses script with periodic variation in reference CO_2 was used (Systems, 2014). This provided the varied response exhibited by the plants under conditions of different CO_2 concentrations.

2.2.3. Shoot dry weight

The above ground parts of each plant were harvested into envelopes and dried at 70 $^\circ$C in a drying oven for 14 days. Samples were then weighed to the nearest 0.001g to obtain the dry biomass of samples.

2.3. Statistical analysis

All statistical analysis was performed using (R) software version 3.3.1 (2016) and plots generated from R.

A linear model in a 2x2x2 factorial analysis of variance (ANOVA) was used in the determination of the relationship between the fixed factors (variety (V), moisture (M) and temperature (T)) for the various responses (dry weight, chlorophyll fluorescence and photosynthetic responses). Multiple measurements per plant for chlorophyll parameters were averaged as well as photosynthetic rates for at both measuring temperatures to obtain a single measurement per plant. The variety, temperature, moisture as well as their interactions were included in the model to identify the various responses under the different treatments after checking the data based the assumptions of a linear model. The Tukey's Honestly Significant Difference (HSD) test was used as a multiple comparison for the parameters.

In the determination of the effect of measuring temperature on the photosynthetic response, as a fourth factors, the linear mixed effect model (LME) was fitted using the restricted maximum likelihood (REML) to account for the repeated measures with the (Lmer) function. The factors temperature (T), moisture (M), variety (V) and measurement temperature (MT) were included as fixed factors while plant was treated as a random effect. The Akaike Information Criterion (AIC) was then used in deciding between competing models. The maximum likelihood (ML), not REML, was used in the model selection to fit the models to estimate the correct AIC values. The model with the lowest AIC has the most support. The estimates of the best model was obtained using the REML. The difference between AICbest model and AICcompeting model is the ΔAIC. Competing models having $|\Delta$AIC$| \leq 2$ have substantial support and should be considered. Those in which $4 \leq |\Delta$AIC$| \leq 7$ have substantially less support, and models having $|\Delta$AIC$| > 10$ don't have any support (Burnham & Anderson, 2004). The Q-Q-plot as well as plots of residuals vs fitted values were used to visually check the assumption of normality and homogeneity. The p-values were however obtained using the (nlme) function with the full model.

3. Results.

3.1. Temperature and moisture effect.

Temperature (T) and moisture (M) had strong effects on growth and photosynthesis, with the two varieties (V) responding similarly to temperature but with some differences in response to moisture (Table 3.1).

The two varieties differed significantly in the overall growth (biomass) and WUE. There was a strong and consistent effect of temperature on biomass and all gas exchange parameters with a strong main effect of drought stress on biomass only. The significant V x M interactions indicate that the photosynthetic responses to moisture differed between the two varieties. The maximum quantum yield (F_v/F_m) was greater than 0.80 indicating that the PSII efficiency was maintained under both drought and heat stress (Table 3.1, Figure 3.1).

For gas exchange parameters (A, E, g_s, WUE), the general (mean) response under the treatments are shown (Table 3.1, Figure 3.1), while the effect of measuring temperature is discussed separately (Table 3.2, Figure 3.2).

TABLE 3.1. Combined 3-way factorial AVOVA.

A summary table of the p-values from the analysis of the various parameters.
Red ($p \leq 0.001$), Blue ($p \leq 0.01$), Green ($p \leq 0.05$)

RESPONSE	DRY WEIGHT	A	E	g_s	WUE	FV/FM
VARIETY	$p < 0.001$	0.883	0.4118	0.362	$p < 0.001$	0.765
TEMP	$p < 0.001$	$p < 0.001$	$p<0.001$	$p < 0.001$	$p < 0.001$	0.259
MOIST	$p < 0.001$	0.553	0.430	0.380	0.513	0.275
VAR:TEMP	0.117	0.261	0.192	0.207	$p < 0.001$	0.889
VAR:MOIST	0.430	0.042	0.018	0.022	0.903	0.173
TEMP:MOIST	0.079	0.553	0.924	0.984	0.026	0.889
VAR:TEMP:MOIST	0.570	0.669	0.430	0.429	0.059	0.010

3.2. Parameter responses under temperature and moisture treatments.

Temperature and moisture affected dry weight after 14 days as well as the physiological responses after 7 days of treatment of both maize varieties (Figure 3.1)
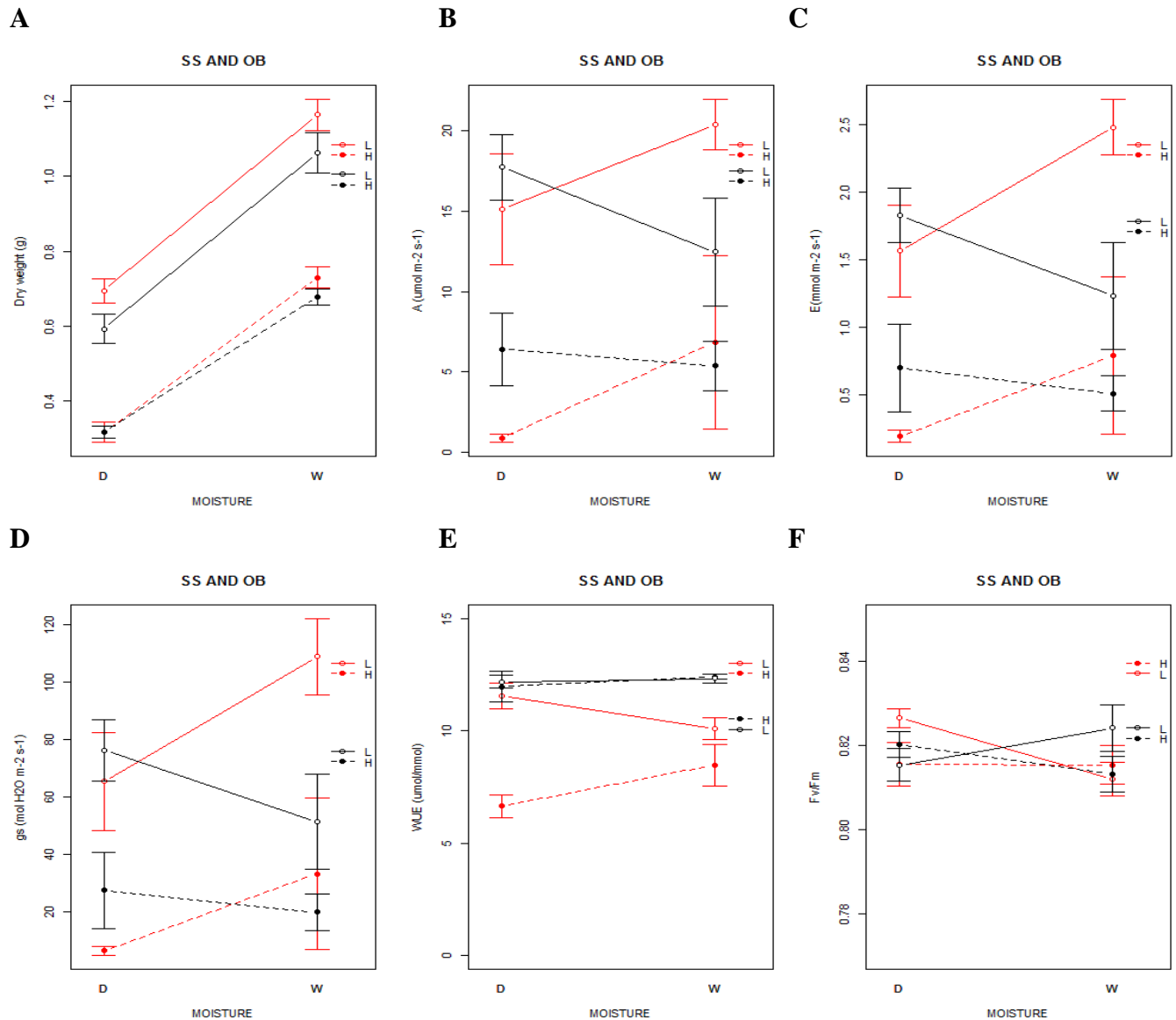
Figure 3.1. Responses of two maize varieties (**Obaatampa (red symbols and lines**) and **Sun Sweet (black symbols and lines)**) grown under different combination of High (**H**) and low (**L**) temperature (35 /25 °C) and moist/ wet (**W**) and dry (**D**) conditions for 7 days. Fig. **A.** Above ground biomass (**dry weight**), Fig. **B.** Photosynthetic rate/ Assimilation (A)**,** Fig. **C.** Transpiration (E), Fig. **D.** Stomatal conductance (g_s), Fig. **E.** Water Use Efficiency (WUE) and Fig. **F.** Maximum quantum efficiency of photosynthesis (F_v/F_m).

3.2.1. Dry weight.

Drought stress caused a significant decrease in dry weight by about 0.40 g per plant at both temperatures and in both varieties (Figure 3.1A)

Both varieties grew significantly better at 25˚C, with OB gaining more biomass than SS under both moisture conditions levels ($p<0.001$). At high temperature, dry weight decreased significantly by about 0.40g in both dry and watered plants and in both varieties. This growth reduction was slightly stronger in OB, which grew a little better at low temperatures, however, the V x T interaction was not significant.

3.2.2. Photosynthetic parameters

Photosynthetic rates (A) of the two varieties responded differently to drought stress with OB increasing under watered conditions whiles SS decreased (Figure 3.1B). The V x M interaction was significant at $p=0.04$. OB had a significantly higher photosynthetic rate (A) as compared to SS under moist (wet) condition but a lower A in dry conditions irrespective of temperature. Overall, A was roughly 10 µmol m^{-2} s^{-1} higher at low temperature than high temperature in both varieties (Table 1A, Appendix 1)

Transpiration (E) responded similarly to A with significant V x M interaction and temperature effects. OB shows significantly higher rate to E as compared to SS in the moist condition (Figure 3.1C, Table 1.1). E decreased in response to drought stress in OB, while that of SS increased slightly. The temperature effect was roughly 0.7 mmol m^{-2} s^{-1}, with low temperature being higher as compared to high temperature (Table 1A, Appendix 1).

Stomatal conductance (g_s) also showed a similar response pattern to A and E with significant V x M interaction and temperature effects. g_s decreased in response to drought stress in OB, but increased slightly in SS which was not significantly different (Figure 3.1 D, Table 1.1). Both varieties respond differently with a significant interaction. The strong similarity in the response of g_s as compared to A and E indicates that, the photosynthesis and transpiration responses are largely controlled by stomatal conductance rather than biochemical or photochemical processes.

In all, g_s was roughly 30 mol m^{-2} s^{-1} higher at low temperature than at high temperature (Table 1A, Appendix 1).

Water Use Efficiency (WUE) was significantly higher in SS as compared to OB. However, the significant temperature effect, V x T and T x M interactions showed that, though the effect of temperature was different for each variety, their response also differed under the various moisture conditions at both temperatures. While temperature had no effect on the WUE of SS, that of OB decreased at high temperatures. The effect of moisture treatments was not significant for SS both temperatures, however, OB showed an increase at low temperature but decrease at high temperature in response to drought (Figure. E, Table 1.1).

3.2.3. Maximum photochemical efficiency of photosystem II (PSII) F_v/F_m.

The significant three-way interaction reveals that, the maize varieties responded differently under the different treatments. However, the maximum photochemical efficiency of photosystem II F_v/F_m above 0.80 indicates that, the PSII of the maize plants remained in a good working condition. (Figure. F, Table 1.1)

Although there was a significant interaction, the light reactions were not hindered by the treatments, but rather changes in the other response parameters.

3.3. Effect of Measurement temperature

Measurement temperature (MT) had a significant effect on E as well as WUE but not g_s and A. In the 4-way analysis, temperature had a strong and consistent effect on all the parameters while moisture didn't. Using the Akaike Information Criterion (AIC) for discrimination between candidate models, the second to best models were found to explain the data best with fewer interactions AIC<2 (Table 2 A, B, C, Appendix 2).

Though the varieties differed significantly under WUE, they weren't under E. There was also significant T x M and V x MT interaction for WUE whiles both V x M and T x MT interactions were significant for transpiration (E). (Figure 3.2, Table 3.2).

TABEL 3.2. COMBINED 4-WAY FACTORIAL ANOVA.

Summary table of the p-values from the analysis of the various parameters.
Red (p ≤ 0.001), Blue (p ≤ 0.01), Green (p ≤ 0.05)

RESPONSE	A	E	g_s	WUE
VARIETY	0.883	0.412	0.362	p<.0.001
TEMP	p<.0.001	p<.0.001	p<.0.001	p<.0.001
MOIST	0.553	0.430	0.380	0.522
MEASTMP	0.095	p<.0001	0.063	p<.0.001
VAR:TEMP	0.261	0.192	0.207	p<.0.001
VAR:MOIST	0.042	0.018	0.022	0.905
TEMP:MOIST	0.553	0.924	0.984	0.029
VAR:MEASTMP	0.338	0.398	0.740	0.006
TEMP:MEASTMP	0.230	p<.0.001	0.247	0.786
MOIST:MEASTMP	0.743	0.682	0.979	0.912
VAR:TEMP:MOIST	0.668	0.430	0.429	0.064
VAR:TEMP:MEASTMP	0.947	0.364	0.865	0.752
VAR:MOIST:MEASTMP	0.049	0.014	0.103	0.126
TEMP:MOIST:MEASTMP	0.647	0.932	0.740	0.886
VAR:TEMP:MOIST:MEASTMP	0.616	0.404	0.674	0.861

TRANSPIRATION (E)

A

B

C

WATER USE EFFICIENCY (WUE)

D

E

F
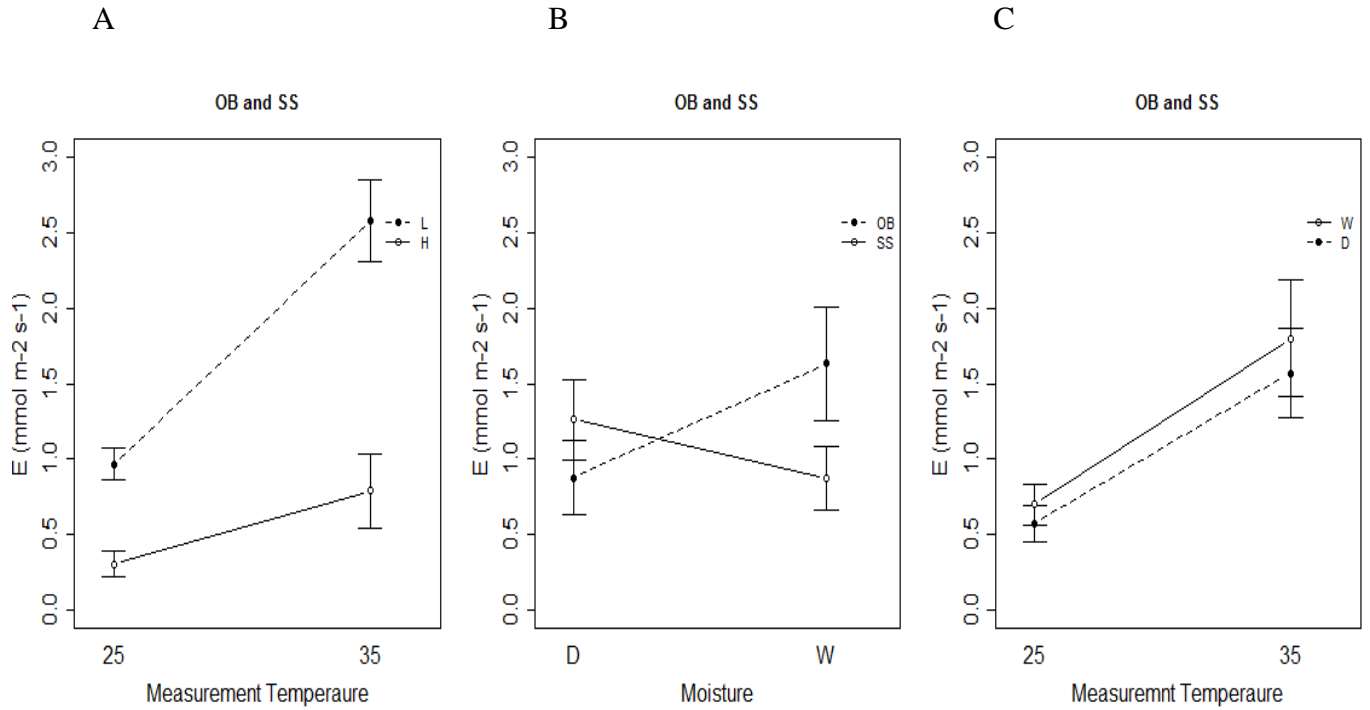
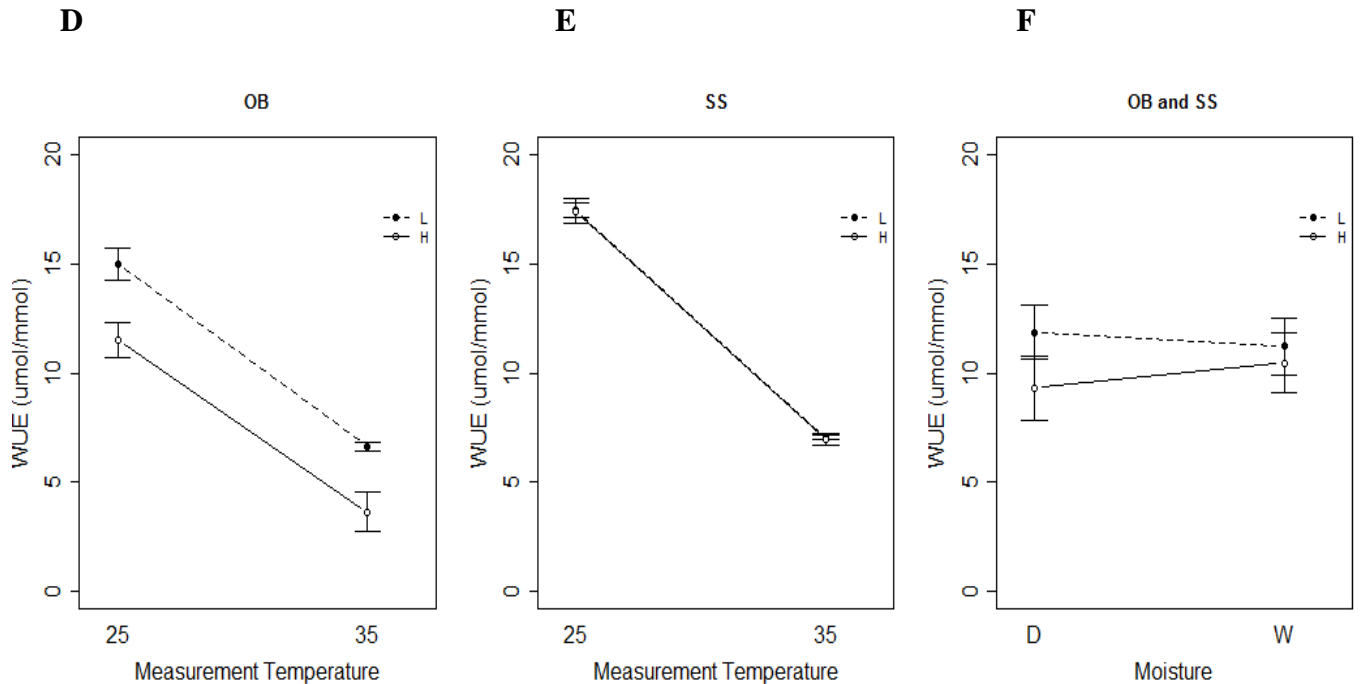**Figure 3.2.** Responses of two maize varieties (**Obaatampa and Sun Sweet**) to measurement temperature under the temperature and moisture combinations for 7 days. Transpiration (Fig. A, B, C) and WUE (Fig. D, E, F).

Different measurement temperatures significantly affected the Transpiration (E) with a significant effect of temperature (T) and T x MT interaction with no varietal difference. At higher measurement temperature, there was a greater increase in transpiration for plants under low treatment temperature as compared to higher treatment temperature for both varieties (1.61 mmol $m^{-2} s^{-1}$ and 0.49 mmol $m^{-2} s^{-1}$) respectively (Figure 3.2. A, Table 1B(appendix)). Though moisture treatments were not significantly different (Figure 3.2. C), both varieties responded differently due to the significant V x M interaction with higher transpiration in the watered (moist) conditioned plant for OB whiles that of SS was higher for drought conditioned plants irrespective of temperature (Figure 3.2. B).

Though the varieties weren't significantly different, at higher measuring temperature, the transpiration rate increased by (1.83 mmol $m^{-2} s^{-1}$ and 1.39 mmol $m^{-2} s^{-1}$) in OB and SS at low temperature treatments respectively with only a (0.48 mmol $m^{-2} s^{-1}$ and 0.50 mmol $m^{-2} s^{-1}$) increase at high treatment temperatures (Figure 3.2. A, Table 1B, Appendix 2).

Water use efficiency (WUE) was affected by the measuring temperature in both varieties with OB showing more variation as compare to SS due to the significant variety and V x MT interaction (Figure D and E, Table 3.2).

The significant temperature, T x MT and T x M interactions reveal that, higher measuring temperature decreased the WUE in SS irrespective of treatment temperature by (10.46 µmol/mmol), whiles OB showed a (8.37 µmol/mmol and 7.91 µmol/mmol) decrease at low and high treatment temperature respectively (Figure D, E, F, Table 1B). Though SS was better than OB, the WUE efficiency of OB was significantly better at low temperature treatments compared to high temperature treatments with a (3 µmol/mmol) difference in WUE (Figure D and E, Table 1B). SS was however, better than OB under low temperature treatment at low measurement temperature (Figure D and E, Table 1B).

4. Discussion

The aim of the study was to investigate both the single and combined effect of the temperature and moisture on growth (biomass), photosynthetic parameters and chlorophyll fluorescence in young maize leaves of two maize varieties.

A factorial experiment revealed that, although the plants maintained photochemical efficiency in all treatments with a significant V x T x M interaction, temperature and moisture strongly affected growth (biomass), while temperature had a significant main effect on the photosynthetic responses (A, E, g_s, WUE). The two varieties differed significantly in the overall growth (biomass) and WUE whiles the other photosynthetic responses (A, E, g_s) showed significant V x M interaction indicating that the varieties differed in response to the moisture treatments. WUE further showed a significant V x T and T x M interaction indicating the variation in varietal response at different temperatures as well as the different effect of temperature under the moisture conditions. In all, results also showed that, high temperature and drought mostly affected physiological responses negatively.

Under the different measurement temperatures, two photosynthetic parameters (E and WUE) were affected significantly while A and g_s were not. Though the varieties differed significantly under WUE, they weren't under E. However, the significant T x M and V x MT interaction for WUE indicates different temperature-moisture response while measurement temperature affected both varieties differently. The significant V x M and T x MT of transpiration (E) also showed the varied response of the plant under different moisture conditions as well as the effect of measurement temperature at the various treatment temperatures. The results indicate that, higher measurement temperature increased transpiration while decreasing WUE with variation under treatment conditions for each variety.

4.1. High temperature and low moisture generally negatively affect physiological responses

From the factorial experiment, both high temperature and low moisture (drought) have a significant negative effect on the growth (biomass) and the photosynthetic parameters, but has no significant effect on the maximum photochemical efficiency of PSII in maize. However, the effect of temperature was shown to be much stronger than that of moisture generally.

4.1.1. Biomass

The combined effect of heat and drought stress caused a 0.80 g decrease in the biomass of both varieties with heat and drought stress contributing a 0.40 g biomass reduction each. This indicates that, both varieties performed better under low temperatures and moist/ well-watered (W) conditions with OB performing significantly better than SS at low temperature but not much at high temperature. However, OB was expected to grow much better than SS under high temperature since OB was a tropical variety which should possess better tolerance to high temperatures.

Other findings indicated that, temperature and drought reduce growth due to the allocation of materials to either adapt or cope with the adverse environmental effect (Crafts-Brandner & Salvucci, 2002; Vitale *et al.*, 2007; Wahid *et al.*, 2007; Wang *et al.*, 2004). According to Karim *et al.,* (2000), maize varieties grown at high temperature exhibited marked decrease in growth parameters with high variation in variety. Drought stress for 12 days retarded the growth and reduced the dry weight of maize, but th plants recovered after watering for 6 days (Efeoğlu *et al.*, 2009).

During stress, the cost of acclimatization entails the allocation of material such as compatible solutes including proteins and non-protein amino acids (i.e. proline and glycine betaine, respectively), carbohydrates (i.e. sucrose and raffinose) and protective proteins such as Heat shock proteins (HSPs) and dehydrins to survive (Bita & Gerats, 2013; Hale & Orcutt, 1987; Kenneth, 2017; Wang *et al.*, 2004).

The synergistic effects of temperature and drought stress aggravate the reduced growth in plants (Bita & Gerats, 2013; Hale & Orcutt, 1987). The findings of Shah (2003) although in wheat indicated that, temperature and drought have a synergistic effect on the biomass of plants. However, this study showed that, although both high temperature and drought significantly affected maize biomass, there with no significant interaction between the two factors, but the low p-value ($p=0.079$), gives a small indication of some synergistic interaction since both factors negatively affected growth.

Plants acclimatized or adapted to harsh environmental condition have better mechanisms of survival as compared to the unadapted varieties, however, the inherent stress tolerance comes a cost during plant growth (Hale & Orcutt, 1987; Karim *et al.*, 2000). The experiment revealed that, though high temperature adversely affects growth in both varieties, OB, a tropical variety surviving

drought and heat stress performed better than SS at low temperature and slightly better at high temperature under moist conditions with both having different mechanisms for dealing with moisture stress (A, E, g_s). The reduction in biomass could be attributed to metabolic costs, reduced photosynthetic system as well as reduced turgor at low water potential (Bita & Gerats, 2013; Hale & Orcutt, 1987; Taiz *et al.*, 2015).

This confirms that, although the plants have similar growth response to stress, the environmental or growth conditions of plants contribute to their acclimatization and adaptation making them have different mechanisms of survival to perform better under any growth conditions (Hu *et al.*, 2010; Wang *et al.*, 2004). Based on the observed similarity in growth (biomass) of both varieties at high temperature, the temperate variety (SS) could perform quite well as OB in tropical environment during the early stage of seedling stage, however, the vegetative stage of (SS) might respond differently since the window of sensitivity varies with plant type, duration and dose of existing stress factors (Hale & Orcutt, 1987).

4.1.2. Photosynthetic response

The photosynthetic parameters were significantly affected by temperature with a better performance at low temperature. However, WUE showed several variations due to the significant varietal difference, V x T and T x M interactions. The significant V x M interaction of the other photosynthetic parameters (A, E and g_s) led to the observation of unique trends in response to both varieties under the moisture levels. Though the WUE was better for SS compared to OB even at low temperature, the other parameters (A, E and g_s) showed otherwise, with a general increase in response to moisture in OB while SS decreased. This revealed that, SS performed better as compared to OB under dry conditions whiles under well-watered conditions, OB performed better. It was expected that, OB would perform better under both conditions since it was adapted to harsh weather conditions in the tropics.

The observed response (trend) in this study could be attributed to both physiological as well as genetic mechanisms to survive stress conditions per variety (Hale & Orcutt, 1987; Li *et al.*, 2015; Taiz *et al.*, 2015). According to Benešová *et al.*, (2012), the opening and closure of the stomata of

maize plant are dependent on their sensitivity to stress. Their findings showed that, susceptible varieties of maize tend to close their stomata to conserve water whiles tolerant varieties maintain an opened stomata even under dehydration conditions. Crafts-Brandner & Salvucci, (2002) also showed that during the periods of heat stress, maize plants open their stomata to increase transpiration in order to cool down.

Under high temperatures as well as drought conditions, variation in plant sensitivity results in either the partially or completely closure of their stomata to prevent excessive water loss resulting which contributes to stomatal inhibition of photosynthesis (Hale & Orcutt, 1987; Lehmann & Or, 2015; Taiz *et al.*, 2015). Since the stomata of the plant represent the main pathway for the exchange of gases (CO_2 and O_2) as well as water (transpiration) (Lehmann & Or, 2015), stomatal conductance (g_s) can regulate the photosynthetic rate (A) as well as transpiration (E). The strongly similar trends observed for A, E, and gs in this study indicates that, this is the main mechanism by which drought and heat stress affect photosynthesis.

Furthermore, the observed increase in the stomatal conductance (opened stomatal aperture) of OB resulted in an increase A for growth while resulting in the loss of water through E due to vapor pressure deficit at high temperature to cool plant with enough moisture available. On the other hand, SS does otherwise than the expected with increased stomatal conductance during water stress (drought) conditions whiles it decreased stomatal conductance during moist conditions contrary to OB. Although g_s, A as well as E responses clearly support the negative effect of high temperature, it is observed that, the response of plant to temperature is strongly dependent on the variety as well as water availability which could an adapted survival quality (Crafts-Brandner & Salvucci, 2002; Li *et al.*, 2015; Taiz *et al.*, 2015).

The water use efficiency (WUE) of C4 species decreases as stomatal resistance increases (Sinclair *et al.*, 1975), however, there are differences in plants response to the use of water (Taiz *et al.*, 2015). In this study, WUE didn't follow the same visible trend as the other photosynthetic responses (Figure 3.1). In Comparing OB and SS, the varietal difference was evident with SS having a relatively better water use efficiency and not affected by temperature or moisture stress. The variation in OB, on the other hand shows that, although moisture wasn't significant, temperature had a stronger effect than moisture since OB responded differently at both temperatures under the moisture treatment due to the T x M interaction (TABLE 3.1). At higher

temperatures, OB had a better WUE under well-watered conditions but a decrease at low temperatures. It could be inferred that, high temperatures caused a closure of the stomata (Taiz *et al.*, 2015), resulting in a better WUE; making watered well (wet/well-watered) plants have a much higher water use efficiency. The contrary observation at low temperatures could indicate that, the stomata opening and closure were regulated by the moisture level. Also, plants under more favorable temperatures and moisture conditions efficiently use water by the closing of the stomata while well-watered plants had their stomata open thereby losing water and eventually having a low water use efficiency. This observation goes against the findings of Sinclair *et al.*, (1975), since the WUE rather increased as the stomatal resistance increased.

Moreover, although different varieties had different water use efficiencies, in the absence of high unfavorable temperatures, stomata are regulated by moisture, but regulated strongly by temperature under heat stress / high temperatures conditions.

The exposure of maize plants grown at a low temperature (25˚C) to higher temperatures (35˚C) led to the transient inhibition (approximately 50%) of photosynthesis that recovered after 4 hours, but, exposure temperature above 45˚C led to a permanent change in the plant. However, plants grown at higher temperatures (41˚C) showed no damage at a much higher exposed temperature (50˚C) (Sinsawat *et al.*, 2004).

This study showed that, measurement temperature only affected E and WUE amongst the photosynthetic parameters. Both varieties showed a general increase in E whiles the WUE differed for both varieties with a decreased at high measuring temperatures. Plants under low temperature treatments (25˚C) were more adverse affected than the plants grown at high temperature (35˚C) which exhibited a much less effect of higher measuring temperature. These observations go in accordance to the observation of Sinsawat *et al.*, (2004), who indicated that, the plants grown at high temperatures (41˚C) were not damaged by the sudden change in measurements.

However, the significant T x M interaction for WUE indicates that, although moisture doesn't significantly affect the variety, it plays a vital role in the mechanism for survival under different temperatures. However, the significant V x M as well as T x MT interactions of E indicate that, the temperature change is the main trigger for transpiration to cool down plant temperatures (Taiz *et al.*, 2015). This indicates that, although E and WUE are affected by temperature, the level of moisture in the environment affects WUE.

The reduction in the WUE could be attributed to more increase in E than A at higher temperatures with different varieties responding differently under different conditions. The reduction in the WUE due to the reduction in A could however be attributed to the inhibiting effect of higher temperature to the light and dark reaction (Bita & Gerats, 2013; Taiz *et al.*, 2015).

4.1.3. Chlorophyll fluorescence

The adverse effect of stress reduces the functioning efficiency of the PSII thereby affecting the production of ATP and NADPH in the light reactions which are necessary for the carbon reactions (Hale & Orcutt, 1987; Taiz *et al.*, 2015). According to the finding of Efeoğlu *et al.*, (2009), the exposure of maize plants to drought stress for a period of 12 days caused a decrease in the F_v/F_m of the plant but plants speedily recovered during the watering period of 6 days). Crafts-Brandner & Salvucci, (2002) also recorded that, temperature below 30°C resulted in the activation of NPQ with plants still maintaining a high F_v/F_m, however, temperature above 42.5°C led to the decrease in F_v/F_m below 0.80 indicating the adverse effect it had on the function of PSII.

This study showed that, the exposure of the plant to the stress condition didn't affect photosystem II (PSII) function, with both varieties having a maximum quantum yield (F_v/F_m) above 0.80. Although the heat dissipation capacity was not tested, it can be said that PSII was in good working condition since single factors, as well as the interaction of both stress factors, did not hinder the channeling of the light energy for photochemical reaction due to the undamaged photosynthetic system. These observations go in accordance to the findings of Crafts-Brandner & Salvucci, (2002) and Efeoğlu *et al.*, (2009) with the temperature (35°C) and drought stress (35ml per week) within their experimental range.

This observation could be attributed to the fact that, the period of stress exposure was too short to cause damage to the health of the plant although longer duration of a stress factor could result in chronic stress which negatively affects plants (Taiz *et al.*, 2015).

Furthermore, it could be inferred that, the dark adaptation period (12 hours) was enough to restore the plants to the ready state for photochemical reaction (Taiz *et al.*, 2015). The light intensity (400 $\mu mol\ m^{-2}s^{-1}$) on the other hand could also be too low to trigger the damage to photochemical

reactions since maize is highly tolerant to adverse effect (Hale & Orcutt, 1987; Karim *et al.*, 2000; Taiz *et al.*, 2015) although stress factors increase the susceptibility of photosynthetic damage (Taiz *et al.*, 2015).

However, the reduction in other plant growth responses could be attributed to changes in other processes such as the Carbon cycle reactions due to the limitation imposed by stomatal conductance or effects on enzyme function (Taiz *et al.*, 2015).

5. Conclusion

In conclusion, maize varieties exhibit variation in physiological response to stress (moisture and temperature) at the early stage of growth. The regulation of the stomata under certain conditions are dependent on the variety. While drought stress causes limited gaseous exchange through stomata closure under normal conditions (low favorable temperatures) in OB, SS does otherwise. However, higher temperatures strongly reduce the stomatal conductance of both varieties due to plants response to prevent excess water loss through E.

Furthermore, both varieties had a maximum photochemical efficiency above 0.80 indicating that, both varieties were healthy to perform photochemical reactions effectively after the prolonged (7 days) exposure to heat and drought stress.

Since the photochemical reactions were functioning effectively, the reduction in the growth (biomass) of the seedlings show that, both heat and drought stress seems to affect photosynthesis via the stomatal conductance thereby which limit Calvin cycle reaction of the plant which in the long run reduce the growth of the plant.

Lastly, although it was expected that the tropical variety (OB) would grow much better than the temperate variety (SS) at high temperature, both showed similar growth with OB just slightly better at high temperature under moist conditions. Both varieties however showed different mechanisms of coping with stress.

6. Limitations

Treatments were not independently replicated since only two growth chamber were available for the experiment.

Though CO_2 response data was taken, the unavailability of a CO_2 response software and poor data quality for C4 plants hindered the analysis of the data.

7. Recommendations

The maintenance of a constant soil water potential such as the use of neutral osmoticum like polyethylene glycol should be considered in future experiments. This is because, high temperature drives higher rates of transpiration, so that some of the observed temperature effects may be due to drought.

Multiple varieties from both locations should be considered in further experiment to ascertain whether the differences in response are either due to their environmental adaptation or just varietal based.

Also, though this project failed to observe the plant response to stress for an extend periods of time due to chamber size and plant death, it would be beneficial to obtained insight in the effect of prolonged period of stress on seedlings to understand survival mechanism adapted by the seedlings.

8. References

Adams III, W. W., & Demmig-Adams, B. (2004). Chlorophyll fluorescence as a tool to monitor plant response to the environment *Chlorophyll a Fluorescence* (pp. 583-604): Springer.

Adams III, W. W., Zarter, C. R., Mueh, K. E.*, et al.* (2008). Energy dissipation and photoinhibition: a continuum of photoprotection *Photoprotection, photoinhibition, gene regulation, and environment* (pp. 49-64): Springer.

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

Bagley, J., Rosenthal, D. M., Ruiz-Vera, U. M.*, et al.* (2015). The influence of photosynthetic acclimation to rising CO2 and warmer temperatures on leaf and canopy photosynthesis models. *Global Biogeochemical Cycles, 29*(2), 194-206. doi:10.1002/2014gb004848

Baker, N. R. (2008). Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annual Review of Plant Biology, 59*, 89-113. doi:10.1146/annurev.arplant.59.032607.092759

Battisti, D. S., & Naylor, R. L. (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. *Science, 323*(5911), 240-244.

Benešová, M., Holá, D., Fischer, L.*, et al.* (2012). The Physiology and Proteomics of Drought Tolerance in Maize: Early Stomatal Closure.

Bita, C., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science, 4*, 273.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference understanding AIC and BIC in model selection. *Sociological methods & research, 33*(2), 261-304.

Close, T. J. (1996). Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiologia Plantarum, 97*(4), 795-803.

Correia, M. J., Osorio, M. L., Osorio, J.*, et al.* (2006). Influence of transient shade periods on the effects of drought on photosynthesis, carbohydrate accumulation and lipid peroxidation in sunflower leaves. *Environmental and Experimental Botany, 58*(1-3), 75-84. doi:10.1016/j.envexpbot.2005.06.015

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

de Carvalho, R. C., Cunha, A., & da Silva, J. M. (2011). Photosynthesis by six Portuguese maize cultivars during drought stress and recovery. *Acta Physiologiae Plantarum, 33*(2), 359-374.

De Groote, H., Dema, G., Sonda, G. B.*, et al.* (2013). Maize for food and feed in East Africa—The farmers' perspective. *Field Crops Research, 153*, 22-36.

Demmig-Adams, B., Muller, O., Stewart, J. J.*, et al.* (2015). Chloroplast thylakoid structure in evergreen leaves employing strong thermal energy dissipation. *Journal of Photochemistry and Photobiology B-Biology, 152*, 357-366. doi:10.1016/j.jphotobiol.2015.03.014

Efeoğlu, B., Ekmekci, Y., & Cicek, N. (2009). Physiological responses of three maize cultivars to drought stress and recovery. *South African Journal of Botany, 75*(1), 34-42.

Hale, M. G., & Orcutt, D. M. (1987). *The physiology of plants under stress*: John Wiley & Sons.

Hu, X., Li, Y., Li, C.*, et al.* (2010). Characterization of small heat shock proteins associated with maize tolerance to combined drought and heat stress. *Journal of plant growth regulation, 29*(4), 455-464.

Karim, M., Fracheboud, Y., & Stamp, P. (2000). Effect of high temperature on seedling growth and photosynthesis of tropical maize genotypes. *Journal of Agronomy and Crop Science, 184*(4), 217-223.

Kenneth, A. (2017). Dehydrins and Metabolites expressed in maize seedlings under heat and drought stress.

Lazar, D. (2015). Parameters of photosynthetic energy partitioning. *Journal of Plant Physiology, 175*, 131-147. doi:10.1016/j.jplph.2014.10.021

Lehmann, P., & Or, D. (2015). Effects of stomata clustering on leaf gas exchange. *New Phytologist, 207*(4), 1015-1025. doi:10.1111/nph.13442

Li, C. F., Tao, Z. Q., Liu, P*., et al.* (2015). Increased grain yield with improved photosynthetic characters in modern maize parental lines. *Journal of Integrative Agriculture, 14*(9), 1735-1744. doi:10.1016/S2095-3119(14)60959-X

Liu, S., Hao, Z., Weng, J*., et al.* (2015). Identification of two functional markers associated with drought resistance in maize. *Molecular breeding, 35*(1), 53.

Liu, Z., Sun, N., Yang, S. J*., et al.* (2013). Evolutionary transition from C-3 to C-4 photosynthesis and the route to C-4 rice. *Biologia, 68*(4), 577-586. doi:10.2478/s11756-013-0191-5

MASSAD, R. S., Tuzet, A., & Bethenod, O. (2007). The effect of temperature on C4-type leaf photosynthesis parameters. *Plant, cell & environment, 30*(9), 1191-1204.

Pfundel, E. E. (2003). Action of UV and visible radiation on chlorophyll fluorescence from dark-adapted grape leaves (Vitis vinifera L.). *Photosynthesis Research, 75*(1), 29-39. doi:Doi 10.1023/A:1022486925516

Shah, N., & Paulsen, G. (2003). Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant and Soil, 257*(1), 219-226.

Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D*., et al.* (2007). Fitting photosynthetic carbon dioxide response curves for C3 leaves. *Plant, cell & environment, 30*(9), 1035-1040.

Shiferaw, B., Prasanna, B. M., Hellin, J*., et al.* (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security, 3*(3), 307.

Sinclair, T. R., Bingham, G. E., Lemon, E. R*., et al.* (1975). Water use efficiency of field-grown maize during moisture stress. *Plant Physiology, 56*(2), 245-249.

Sinsawat, V., Leipner, J., Stamp, P*., et al.* (2004). Effect of heat stress on the photosynthetic apparatus in maize (Zea mays L.) grown at control or high temperature. *Environmental and Experimental Botany, 52*(2), 123-129. doi:10.1016/j.envexpbot.2004.01.010

Systems, P. (2014). CIRAS-3 Portable Photosynthsis System Operation Manual Version 1.06 PP Systems, Amesbury, MA, USA.

Taiz, L., Zeiger, E., Møller, I. M*., et al.* (2015). *Plant physiology and development*: Sinauer Associates, Incorporated.

Tardy, F., & Havaux, M. (1996). Photosynthesis, chlorophyll fluorescence, light-harvesting system and photoinhibition resistance of a zeaxanthin-accumulating mutant of Arabidopsis thaliana. *Journal of Photochemistry and Photobiology B-Biology, 34*(1), 87-94. doi:Doi 10.1016/1011-1344(95)07272-1

Vitale, L., Di Tommasi, P., Arena, C*., et al.* (2007). Effects of water stress on gas exchange of field grown Zea mays L. in Southern Italy: an analysis at canopy and leaf level. *Acta Physiologiae Plantarum, 29*(4), 317-326. doi:10.1007/s11738-007-0041-6

Wahid, A., Gelani, S., Ashraf, M*., et al.* (2007). Heat tolerance in plants: an overview. *Environmental and Experimental Botany, 61*(3), 199-223.

Wang, W., Vinocur, B., Shoseyov, O*., et al.* (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in plant science, 9*(5), 244-252.

9. Appendix 1

Table 1A **Means and standard error.**

Parameter estimates of the various parameters under temperature and moisture treatments combinations for 2 maize varieties.

	DRY WEIGHT (g)		PHOTOSYNTHETIC RATE (A) (μmol m^{-2} s^{-1})		TRANSPIRATION (E) (mmol m^{-2} s^{-1})		STOMATAL CONDUCTANCE (mol m^{-2} s^{-1})		WATER USE EFFICIENCY (WUE) (μmol/mmol)		FV/FM	
	MEAN	± SE	MEAN	± SE	MEAN	± SE	MEAN	± SE	MEAN	± SE	MEAN	± SE
H D												
OB	0.316	0.027	0.875	0.263	0.193	0.045	6.375	1.663	6.648	0.493	0.816	0.005
SS	0.316	0.016	6.412	2.256	0.700	0.325	27.500	13.303	11.978	0.695	0.820	0.003
H W												
OB	0.730	0.028	6.825	5.391	0.789	0.581	33.250	26.295	8.490	0.917	0.815	0.005
SS	0.678	0.022	5.375	1.527	0.510	0.127	19.875	6.280	12.420	0.116	0.812	0.004
L D												
OB	0.694	0.033	15.113	3.472	1.563	0.341	65.500	17.114	11.548	0.576	0.827	0.002
SS	0.592	0.039	17.725	2.048	1.826	0.201	76.250	10.798	12.188	0.288	0.817	0.004
L W												
OB	1.165	0.041	20.375	1.568	2.478	0.206	108.875	13.266	10.113	0.483	0.809	0.004
SS	1.063	0.053	12.463	3.359	1.230	0.396	51.250	16.569	12.338	0.207	0.824	0.006

Table 1B.   **Means and standard error.**

Measuring temperature affected the Transpiration (E) and Water use efficiency (WUE) of both varieties under temperature and moisture treatments combinations

TEMP.	TRANSPIRATION (E) (mmol m^{-2} s^{-1})				WATER USE EFFICIENCY (WUE) (µmol/mmol)			
	H		**L**		**H**		**L**	
	MEAN	**± SE**	**MEAN**	**± SE**	**MEAN**	**± SE**	**MEAN**	**± SE**
OB								
D								
25	0.095	0.033	0.883	0.263	10.990	1.383	15.995	1.074
35	0.290	0.064	2.243	0.428	2.305	0.628	7.100	0.084
W								
25	0.408	0.312	1.325	0.178	12.055	0.997	14.030	0.848
35	1.170	0.852	3.630	0.265	4.925	1.510	6.195	0.163
SS								
D								
25	0.373	0.130	0.935	0.165	16.930	1.091	17.135	0.500
35	1.028	0.537	2.718	0.253	7.025	0.538	7.240	0.118
W								
25	0.343	0.139	0.730	0.117	17.953	0.300	17.840	0.337
35	0.678	0.163	1.730	0.696	6.888	0.139	6.835	0.114

10. Appendix 2

Table 2A: Factors included in candidate linear mixed models fitted by maximum likelihood. Comparisons of Akaike Information Criterion (AIC) for each model were performed to select the best model. In all models, Plant (plnt) was included as a random factor (1| plnt).

<u>Four main effects</u>
1. var * temp * moist * meastmp +(1|plnt)
2. var * temp * moist + meastmp +(1|plnt)
3. var * temp + moist + meastmp +(1| plnt)
4. var + temp + moist + meastmp +(1| plnt)
5. var + temp + moist *meastmp +(1| plnt)
6. var + temp * moist * meastmp +(1| plnt)
7. moist + temp + plnt * meastmp+(1|plnt)
8. var + moist + temp * meastmp +(1|plnt)
9. moist + var * temp * meastmp +(1plnt)
10. var * moist + temp * meastmp +(1plnt)
11. meastmp + temp + var * moist+(1|plnt)
12. var * temp * meastmp + moist +(1|plnt)

<u>Three main effects.</u>
13. var * temp * moist   +(1| plnt)
14. var * temp * meastmp +(1| plnt)
15. temp* moist* meastmp +(1| plnt)
16. var * moist* meastmp +(1| plnt)
17. var * temp * moist+(1| plnt)
18. var *temp + meastmp +(1| plnt)
19. temp* moist + meastmp +(1| plnt)
20. var * moist + meastmp +(1| plnt)
21. var * temp + moist +(1| plnt)
22. temp + moist + meastmp +(1|plnt)
23. var + moist + meastmp +(1|plnt)
24. var + temp + moist+(1|plnt)
25. var + temp + meastmp +(1|plnt)
26. var + moist + meastmp +(1|plnt)
27. temp + moist + meastmp +(1|plnt)
28. var + temp + moist +(1|plnt)
29. var + temp * meastmp +(1|plnt)
30. var + moist * meastmp +(1|plnt)
31. temp + moist * meastmp +(1|plnt)
32. var + temp * moist +(1|plnt)
33. var + temp * meastmp +(1| plnt)
34. var * moist*meastmp +(1| plnt)
35. temp * moist * meastmp +(1| plnt)

Table 2B. Best selected model for (E) using AIC.

	K	AICc	DeltaAICc	AICcWt	Cum.Wt	LL
Cm10	9	146.74	0.00	0.43	0.43	-62.70
Cm29	6	147.15	0.41	0.35	0.79	-66.84
Cm33	7	149.02	2.28	0.14	0.92	-66.51
Cm 8	8	151.02	4.28	0.05	0.98	-66.20

Table 2C. Best selected model selection for (WUE)

	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
cm14	10	256.75	0.00	0.40	0.40	-116.30
cm 18	7	258.08	1.33	0.20	0.60	-121.04
cm 12	11	259.25	2.51	0.11	0.72	-116.09
cm 2	11	259.66	2.92	0.09	0.92	-116.29