Evaluation of Six Maize (*Zea Mays L.*) Varieties for Drought Tolerances Using Biochemical Indices Associated With Drought Tolerance

By

Dale Abo Bushero

A Thesis Submitted the Department of Applied Biology Program in Partial Fulfillment of the Requirement of the Degree of Master of Science in Biology (Biotechnology)

School of Applied Natural Science
Office of Graduate Studies
Adama Science and Technology University

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Adama, Ethiopia

December, 2018

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Approval boards of examiners

Adama Science and Technology University

School of Applied Natural sciences

Applied Biology Program

We, undersigned, members of the board of examiners of final open defense by Dale Abo Bushero have read and evaluated his thesis entitled "**Evaluation of Six Maize (*Zea Mays L.*) Varieties for Drought Tolerances Using Biochemical Indices Associated with Drought Tolerance"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the requirement of the Degree of MSc in Biotechnology.

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Declaration

This thesis is my original work and has not been presented for award of a degree in any other university.

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Acknowledgments

My deepest gratitude goes to my advisor Dr. Leta Tullu and Dr. Mulugeta Kebede for their valuable comments, supporting ideas and advices. Secondly I would like to thank ASTU for full sponsorship of education and financial support. I would like to thank, Mogos Tasfaye, Doni Hinsene, Diriba Guta and Biadgilign for the guidance and assistance you gave on laboratory work. And also I would like to thank Adaba Tilahun for the relevant SAS program and codes you gave me, the critique you made on my thesis. My kind appreciation also goes to National Agricultural Biotechnology Research Center (NABRC) and melkasa Agricultural Research Center for providing materials help. My unending thank to my family who were always with me through all times and seasons.

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List of acronyms

CSA	Central Statistical Agency
DT	Drought Tolerant
CRD	Complete randomized design
SLA	Specific Leaf Area
SLW	Specific Leaf Weight
WS	Water Stressed
WW	Well-Watered
ANOVA	Analysis Of Variance
LSD	List Significance Difference
SAS	Statistical Analysis System
FAO	Food and Agricultural Organization
CIMMT	International Maize and Wheat Improvement Center
SLN	Specific Leaf Nitrogen
LNC	Leaf Nitrogen Content
ROS	Reactive Oxygen Species
RWC	Relative Water Content
SS	Soluble Sugar
LEA	Late Embryogenesis Abundant
EIAR	Ethiopian Institute of Agricultural Research
DDW	Double Distilled Water

Abstract

Drought is one of major stress factor, which results in a profound effect on plant growth and yield. The effect becomes severe mainly where rain fed agriculture is practiced. Having this thought, assessing of variety that can better tolerate such condition can help for screening and are important in reducing associated yield loss. Maize (Zea mays L.) variety has been hypothesized to be of different genetic make-ups; hence this study was conducted to evaluate drought-tolerance of maize variety under drought stress and normal conditions. The study was carried out in greenhouse using completely Randomized Design (CRD) with three replications. Six maize variety MH-140, Melkassa-2 (MH-2), Melkassa-3 (MH-3), Melkassa-4 (MH-4), Melkasa-6Q and MHQ138 were used and grown under well-watered and water stress condition. Data were collected for growth parameters of specific leaf area (SLA) and specific leaf weight (SLW), Biochemical parameters such as leaf proline, chlorophyll content, soluble sugar content, relative water content, leaf nitrogen (N) content and protein content. All data were subjected to analysis of variance (ANOVA) using SAS 9.4. Mean comparisons were performed using LSD test at $P < 0.05$. The result was interpreted with table. Based on SLA and SLW value, Melkassa-4 (MH-4) was observed to be drought tolerant whereas, MHQ138 was drought sensitive variety. Leaf proline, chlorophyll content, soluble sugar content, relative water content, leaf nitrogen (N) content and protein content, value had revealed Melkassa-4 drought tolerant variety whereas MHQ138 was drought sensitive variety.

Key words: Biochemical parameters; Drought tolerance; DT maize genotypes; Growth parameters;

1. INTRODUCTION

1.1. Background

Maize or corn (*Zea mays* L.) is a cereal crop which belongs to the family of grasses (Poaceae). It is domesticated crop originated in the America and is one of the most widely distributed of the world's food crops. Corn is used as livestock feed, as human food, as biofuel, and as raw material in industry. It is a versatile crop grown over a range of agro climatic zones. In fact, the suitability of maize to diverse environments is unmatched by any other crop. It is grown from 58^oN to 40^oS, from below sea level to altitudes higher than 3000 m and in areas with 250 mm to more than 5000 mm of rainfall per year (Prasad, 2014). Maize being the third most important cereal crop in the world after wheat and rice contributes substantially to world economy as a trade, food, feed, and industrial grain crop (Ongeti, 2012).

Maize is one of Ethiopia's major and strategic cereal crops that have important role in the country's food security and farmers' livelihood. It is grown in 13 agro-ecological zones on about 2 million ha (16.08%) and 39% of the total maize area in Ethiopia is now planted with improved varieties (Eyob Bezabeh, 2015). Among all cereals, maize is second to tef (*Eragrostis tef*) in area coverage but first in productivity and total production (CSA, 2014). Millions of people in Ethiopia depend on maize for their daily food especially where maize is the major crop. Maize has a significant importance in the diets of rural Ethiopia and gradually penetrated into urban centers. Green maize being sold at road sides throughout the country as a hunger-breaking food available during the months of February to May annually. Maize on the other hand can be eaten green right in the field even before roasting or cooking if it becomes very necessary.

Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair plant growth and productivity worldwide. Drought is one of the major abiotic stresses that severely affect and reduce the yield and productivity of food crops worldwide up to 70% but, plants response to drought stress is complex and involves changes in their morphology, physiology and metabolism (Lum *et al*., 2014). Drought is the most important problem of dry land regions and leads to poverty, food insecurity and malnutrition in sub Saharan African countries like Ethiopia.

Water is one of the crucial requirements for plants to survive, grow and develop. The shortage of water in the soil reserve results in limitation of available water in the plant cell and leads to loss of cell turgidity and closure of stomata. This condition limits CO_2 assimilation and nutrient uptake which is driven by transpiration and causes lower chemical energy production. Food productivity is decreasing due to effects of various biotic and abiotic stresses (Ahmadizadeh *et al*., 2011). Drought has been highlighted as one of the major causes of reduced maize production and food insecurity in Ethiopia where agriculture production is largely rainfed (Shiferaw et al., 2011). Therefore, minimizing these losses is a major area of concern to ensure food security under changing climate.

Therefore, the importance of characterizing crop varieties for their responses to drought as a strategy to face the challenges emanating from climate change is to select suitable genotypes to drought prone areas.

1.2. Statement of the problem and justification of the study

In sub Saharan Africa country, many maize varieties have been developed and released but there has been low adoption of these varieties in drought prone marginal areas where the majority of the resource poor farmers live (Mhike, 2013). In Ethiopia also there are many maize verities have been developed and released to local country farmers.

These maize varieties have been hypothesized to be of different genetic make-ups. However, some of these maize varieties have not been evaluated under drought stressed and normal condition.

In this thesis, effort was made to establish the rationale behind the low adoption of those varieties through evaluation of their responses to drought under controlled watering management and to identify key indices for drought tolerance which was considered in future variety development programs targeting drought prone areas of Ethiopia.

1.3. Objectives of the study

1.3.1. General objective

- To evaluate six maize varieties using different, plant growth and biochemical parameters under each treatment.

1.3.2. Specific objectives

- To estimate the amount of free proline of each variety under each treatment in response to water stress.
- To estimate the relative susceptibility of maize variety to water stress in each variety under each treatment.
- To examine the leaf nitrogen content and protein content in response to drought stress.
- To examine the relative water content and chlorophyll content in each variety under each treatments.
- To estimate the amount of total soluble sugars of each variety under each treatment.
- To identify the best drought indices which will help to select variety that can be grow under drought prone areas.

2. LITERATURE REVIEW

Maize is important to about 300 million people who depend on it in Africa (CIMMYT, 2010). The biggest threat to maize production is drought, which is likely to increase under climate change. In response to this threat, drought-tolerant (DT) maize varieties have been developed with an aim to ensure maize production under mild drought conditions. Thus, since the late 1990s, DT maize varieties have been viewed as part of the solution to sustaining maize production mainly under smallholder farming conditions. A DT maize variety is defined as a variety that can produce approximately 30% of its potential yield (1–3 t /ha) after suffering water stress for six weeks before and during flowering and grain-filling (Lunduka *et al*., 2017). Climate models predict that by 2050, many varieties of maize now under cultivation will no longer be viable due to the long-term effects of increasing global warming (Mhike, 2013).

Screening of reliable drought tolerance indicators is an important tool in the selection of tolerant genotypes (Fita et al., 2015). Plant responses to drought are evident on multiple levels, including morphological, physiological and biochemical traits. The screening of these traits in genetically different materials may be a valuable tool in the selection of drought tolerant genotypes.

In drought tolerant plants, there are many defense mechanisms such as osmoregulation, antioxidant and hormonal systems, helping plants to stay alive and develop earlier to their reproductive stages (Ashraf, 2010). Physiological and morphological characteristics such as osmotic adjustment, stomatal behavior, chloroplast activity, leaf water potential, root volume, root weight, leaf area and dry matter production are found different in maize cultivars grown under limited water supply (Mian *et al.,* 1993).

The environmental stresses such as drought, high temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production because they affect almost all plant functions (Lawlor and Cornic, 2002). Drought is a major abiotic factor that limits agricultural crop production (Golbashy *et al*., 2010).

2.1. Effect of drought on plant growth

Plant growth is mainly accomplished by cell division, enlargement, and differentiation. Drought impairs mitosis and cell elongation which results in poor growth (Fahad *et al*, 2017). The effect of water stress starts from plant cells. Water availability is the major factor that affects plant growth and development (Waseem *et al*., 2011) and every process within a plant cell can be affected by water stress. Water deficit is a condition at which plants suffer from lack of enough water in the soil which is reduced as a result of limited precipitation.

All stages of plant growth from seed germination to plant maturation are dependent on water. Drought, similar to other stresses, causes an imbalance in mineral nutrition, alteration of membrane permeability and cellular osmotic balance, and generation of oxidative stress (Kumar *et al*., 2017). The decline in plant growth is the most outcome of water unavailability in the plant and the drop of water potential results in retardation of leaf, inhibition of cell expansion, Turgor loss, inhibit leaf expansion, inhibit root elongation and stem elongation (Taiz and Zeiger, 2006).

Drought has adverse effects on maize life cycle; particularly reproductive growth phase is most susceptible to drought stress. Translocation of photosynthetic assimilates to the reproductive parts is most probable reason for more susceptibility of maize plant during reproductive growth stage under drought stress (Taiz and Zeiger, 2006). Pollen and silk development, pollination, embryo development, endosperm development and kernel development are the different component phases of reproductive growth stage which are severely threatened by drought stress.

2.1.1. Specific leaf area (SLA)

Specific leaf area can be used to estimate the reproductive strategy of a particular plant based upon light and moisture (humidity) levels, among other factors (Milla and Reich*, 2008*). Specific leaf area is one of the most widely accepted key leaf characteristics used during the study of leaf traits (Freschet *et al*, 2011). Water stresses have varying effects on specific leaf area. In a variety of species, drought decreases specific leaf area (Laureano *et al.,* 2008). Casper et al*.* *(*2001) stated that under drought conditions, leaves were smaller than leaves on control plants

SLA is a sign of leaf thickness, and usually decreased under drought stress (Marcelis *et al*., 1998). Decreasing pattern of SLA under drought situation is due to the different sensitivity of photosynthesis and leaf area expansion to soil drying. Leaf area is one of the factors that can determine CO_2 absorption. Concentration of more nutrients especially nitrogen is important to resist drought stress. SLA shows the thickness of the leaf and the extent of photosynthetic machine potential for greater assimilation (Painawadee *et al*., 2009). Reduced nutrient availability is one of the most important factors limiting plant growth and nutrient supply strongly affects crop productivity and the main importance of nitrogen on plant growth under drought conditions are to increase water use efficiency, stomatal conductance, photosynthesis and ATPase activity, higher cell membrane stability and improved osmotic adjustment (Studer *et al*., 2017). Plants with higher SLA have higher metabolic activity than those with lower SLA. More leaf area might cause more water losses due to more evapotranspiration from the leaf surface. Plant with Lower SLA value indicates the higher drought resistant genotype and the plant with higher SLA value indicates the less drought tolerant genotype (Painawadee *et al*., 2009).

2.1.2. Specific leaf weight (SLW)

Specific leaf weight indicates leaf dry mass per area. It has been widely used as a reliable morpho-physiological marker contributing to drought tolerance for various crop plants (Mohammadreza *et al*., 2014). Specific leaf weight has a great association with photosynthesis products. Eyerusalem (2015) found that SLW associates with specific leaf nitrogen (SLN) and leaf nitrogen concentration (LNC) and three variables are interdependent to each other. A small remobilization of assimilates out of leaf in stay-green promotes relatively thicker leaf than senescent hybrids. Higher specific leaf weight helps for the selection of the genotypes with higher grain yields under water stressed condition (Ali *et al*., 2009) and higher SLW can help in selection of drought tolerant accessions (Eyerusalem Arusi, 2015). Pawar (2007) stated that sorghum genotypes with greater SLW was more drought tolerant than genotype with lower SLW.

2.2. Effect of drought on plant physiology

Understanding physiological behavior of plants under drought conditions may result in predicting drought tolerant varieties of crops (Kerepesi *et al*., 2000). In many plant species under drought condition the alteration in different metabolic activities (Lawlor and Cornic, 2002), the reduction of relative water content in plants and decrease in plant vigor (Halder and Burrage, 2003) was observed. According to Olaoye (2009) maize seedling response varies with respect to its growth rate at different field capacities of soil and accumulates a variety of compatible solutes such as proline and betaine, an adaptive mechanism of tolerance to salinity and drought (Farooq *et al*., 2009).

A crucial aspect to drought tolerance is the assessment of the degree of drought tolerance of different genotypes. In many studies the identification of tolerant and susceptible cultivars is based on few physiological measures related to drought response. The difficulty in identifying a physiological parameter as a reliable indicator of yield in dry conditions has suggested that yield performance over a range of environments should be used as the main indicator for drought tolerance (Voltas *et al*., 2005).

Drought causes harmful effects on plant life. In terms of plant physiology water stress cause stress in plant growth, yield reduction, increased respiration, increased photosynthesis and enzyme activity in the plant due to high evapotranspiration and temperature (Fathi and Tari, 2016). Growth and physiological activity (photosynthesis) are the two processes most critical for crop yield and are among the first plant functions to be inhibited by water deficit.

Photosynthesis has a great role in determining crop productivity in all species. Water stress leads to closure of stomata and reduced transpiration rates, a decrease in the water potential of plant tissues, decrease in photosynthesis (expressed by reduction in growth and productivity) and growth inhibition (Zlatev and Lidon, 2012). Severity of drought stress determines the level of physiological effects on the plant. Photosynthesis is less sensitive to mild water stress than leaf expansion. Water deficit leads to turgor loss of guard cells and closure of stomata and it consequences for limitation of CO_2 intake and reduction of internal CO_2 concentration, finally reduce photosynthesis product. It also results in damage on chloroplast as a result of excess excitation energy which is not quenched by chloroplast and results for production of reactive oxygen species (ROS) (Waseem *et al*., 2011).

Delay of leaf senescence increases the total photosynthesis within a life of annual plant by extending active photosynthesis, as a result it can increase carbon assimilation and maximize mass per grain (Eyerusalem Arusi, 2015) and also, photosynthetic rate of single leaf is affected by different leaf characters such as leaf thickness or specific leaf weight, leaf nitrogen content, stomatal density, carboxylating enzymes, inter veinal distance and leaf vein frequency. Therefore, Plant growth and the existing physiology can be assessed by studying traits such as SLA and SLW.

2.2.1. Biochemical response of plants to drought

Osmotic adjustment is an important physiological adaptation for drought tolerance and it is an accumulation of solutes in the cell in order to decrease water potential and maintain cell turgor and volume. Accumulation of solutes such as sugar, organic acids, amino acids (proline) and inorganic ions occur when a decline in water potential occur in the root (Eyerusalem Arusi, 2015). Osmotic adjustment plays a significant role in maintaining turgor potential and other associated processes such as stomatal opening, photosynthesis, shoot growth and growth of roots to deeper soil layers which assist in supply water to plants. Osmotic adjustment had a significant role in maintaining stomatal conductance and accumulation of solutes that extend time for CO_2 assimilation and increase the net assimilation rate under drought condition. Results of study show that grain yield from genotypes that have higher osmotic adjustment ability are less affected than those with lower ability of osmotic adjustment (Pawar, 2007).

2.2.1.1. RELATIVE WATER CONTENT (RWC)

The relative water content (RWC) of a tissue is the fraction of turgid weight that is remaining in the tissue. Tissue water content can vary greatly among organs, developmental stages, seasons, habitats, and species. However, a relative degree of tissue hydration can be calculated by comparing the current hydration of a tissue to its maximum potential hydration. RWC represents a useful indicator of the state of water balance of a plant, essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. Drought stress results in decreased RWC (Shaw *et al*., 2002). Bahadur *et al*. (2010) noticed significant reduction in leaf RWC in okra and tomato, respectively with imposing drought tolerance. Higher RWC is a good indicator of drought stress tolerance (Shaw *et al*., 2002).

2.2.1.2. SOLUBLE SUGAR CONTENT

Soluble sugars are considered as important metabolites in plants under drought stress. Soluble sugars are among compatible metabolites and osmolyte which are increased by increasing drought stress and Reduction of the Soil Water Content and protect plant against water shortage and damages resulting from drought stress on cells membrane (Yuan *et al*., 2009, Ji *et al*., 2009). The increase and accumulation of soluble sugars maintain the turgidity in leafs under tension and they prevent from dehydration of proteins and cell membranes (Arabzadeh, 2012). During drought, soluble sugars function as a typical osmoprotectant as their hydroxyl groups substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration (Homayouni and Khazarian, 2014). Enhanced accumulation of soluble sugars in stressed tissues corresponds to a reduction of starch concentration and it is closely associated with drought tolerance capacity of plants (Hoekstra and Buitink, 2001). Drought-induced reduced concentration of starch was also reported in corn and wheat genotypes (Saeedipour and Moradi, 2011). Alteration of the content of soluble sugars and other carbohydrates and solutes in the leaves of water stressed plants may act as metabolic signals in the response to stress (Chaves *et al*., 2003).

2.2.1.3. Proline

Throughout their life cycle, plants are subjected to various types of environmental stresses, which include salinity, water deficit, temperature extremes, toxic metal ion concentration and UV radiations. These environmental factors limit the growth and productivity of plants to varying degrees, depending upon severity of stress. Water stress affects morphological, physiological, enzyme production and biochemical contents of plants. Osmotic adjustment is a mechanism employed by plants to maintain plant water relations under osmotic stresses. It involves the accumulation of a wide range of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, glycine betaine, organic acids, calcium, potassium, chloride ions, (Farooq *et al*., 2009). Plant responses to abiotic stresses (salinity, heat and drought) are complex involving signal reception and transduction followed by genetic and physiological responses. One mechanisms utilized by the plants for overcome the water stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugars. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm (Anjorin et al., 2016). Thus, proline can be used as a metabolic marker in relation to stress.

Proline production caused by various types of stresses and it play a great role in drought tolerance but, the accumulation depends on the type and intensity of the stress. Proline is thought to play adaptive roles in inducing osmotic adjustment and protecting subcellular structures in stressed plants. In response to water stress, proline accumulation generally occurs in the cytosol where it plays significant role in cytoplasmic osmotic adjustment (Ahmadizadeh *et al*., 2011). Proline in drought-stressed plants is not only used for osmotic adjustment but also for scavenging, detoxifying oxidants, for preventing membrane damage, protein denaturation and as a metabolic marker of drought stress (Aminatun *et al*., 2013).

Moreover, genotypes differ in their level of proline production. Drought tolerant genotypes produce higher yield and proline than drought susceptible genotypes (kanani *et al.,* 2013).

2.2.1.4. Protein

Many specified proteins synthesized under water scarcity such as dehydrins (polypeptide), LEAs (late embryogenesis abundant), RABs (responsive to ABA), storage proteins (in vegetative tissues). Dehydrins are among the accumulated proteins in response to loss of water and increased in barley, maize, pea, and Arabidopsis and under water stress LEA proteins plays important role as protection of plants and Osmotin is also accumulated protein under water stress in several plant species such as tobacco, triplex, tomato and maize (Aklncl and Lösel, 2012).

Plant response to water stress depends mainly on the severity and duration of the stress and the growth stage of the plant. Different physiological and biochemical processes are altered by drought, such as water relation, gas exchange, photosynthesis and the metabolism of carbohydrates, protein, amino acids and other organic compounds (Silva *et al*., 2009). Quantitative increases in protein suggest that, plant adapts to water stress by altering synthesis of proteins, amino acids, and sugars to maintain its osmotic balance (Ali-Ahmad and Basha, 1995). The rate and levels of accumulation of these metabolites may determine the ability of a genotype to withstand the level of water stress.

The change in protein synthesis or degradation is one of the main metabolic mechanisms, which may substantially affect drought tolerance of plants (Ahmadizadeh *et al*., 2011). The initial increase in total soluble proteins during drought stress could be due to the expression of new stress proteins, but the decrease could be due to a severe decrease in photosynthesis. Several proteins were reduced by stress in maize mesocotyls (Aklncl and Lösel, 2012).

2.2.1.5. Chlorophyll and nitrogen content

Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. Decreasing chlorophyll levels is considered as a symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Fathi and Tari, 2016). Chlorophyll content represents one of the most frequently used tools for evaluating the severity of drought stress (Efeoglu *et al*., 2009). When comparing different genotypes, higher content of chlorophyll was generally associated with higher tolerance to stress (Li *et al*., 2006).

Drought stress caused a large decline in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll content in different sunflower varieties (Anjum *et al*., 2011). Exposure of two olive cultivars to reduced irrigation led to lower Chl (a + b) contents. Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis. Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation, and the appearance of lipid droplets (Anjum *et al*., 2011).

Water shortage stress is one of the most important abiotic stress factors which seriously limit plant growth and field crops production, more than any other environmental stresses. Since the plant leaf is the one of the most responsive organs to environmental conditions, the symptoms of environmental stress are exhibited more clearly than in the case of the stem and the roots. Considering that the chlorophyll content of leaf is a primary requisite for plant photosynthesizes (Arjenaki *et al.*, 2012), consequently, its shortage can result in many crises, such as chlorosis, reduction in growth rate and crop yield.

Water shortage stress is one of the most important abiotic stress factors which seriously limit plant growth and field crops production, more than any other environmental stresses. Plant leaf is the one of the most responsive organs to environmental conditions, the symptoms of environmental stress are exhibited more clearly than in the case of the stem and the roots. Mineral nutrients are essential chemical elements for plant growth and reproduction, acquired primarily in the form of inorganic ions from the soil (Taiz and Zeiger, 2006). All macronutrients are integrated into important organic compounds, such as amino acids and proteins (N and S), nucleic acids (N and P), phospholipids (P) and chlorophyll (Mg). Under conditions of water stress, roots are unable to take up many nutrients from the soil due to a lack of root activity as well as slow ion diffusion and water movement rates.

Nitrogen (N) is present in the atmosphere (the main reservoir), lithosphere and hydrosphere. It is required in large amounts and constitutes many biologically important organic molecules in plant cell components, including proteins, nucleic acids, purines, pyrimidines, coenzymes (vitamins) and many other compounds.

Water stress and insufficient N supply in the soil limit plant productivity in most natural and agricultural ecosystems (Taiz and Zeiger, 2006). The nitrogen status of a plant has a significant influence over its water relation, as nitrogen and water often interact. When the soil faces a prolonged period of drought, nitrogen mobility is severely restricted by the dehydrated soil. Thus, when a plant faces water deficit, nitrogen deficiency occurs, which rapidly inhibits plant growth and leads to chlorosis (yellowing of the leaves), as observed by Mahieu *et al*. (2009) in pea where water stress strongly affected dry weight and N accumulation.

Nitrogen deficiency reduces the ability of a crop to convert available water into yield. The early uptake of nitrogen under conditions of low moisture enhances both shoot and root development, which is critical to the final yield (Jonhston, 2002).

2.3. Types of drought and their effects on maize production

2.3.1. Agricultural drought

Agricultural drought refers to shortage of sufficient water available for a crop at any given stage of its development resulting in limits plant growth and productivity, devastating socio- economic impacts and ultimately reduced crop yields (Mhike, 2013). It is a major cause of food insecurity for many households as it has been estimated to cause annual maize yield loss of 24 million tons in the developing world. Breeding for drought tolerance mainly focuses on this type of drought.

2.3.2. Hydrological drought

Hydrological drought refers to the reduction in precipitation on surface and sub-surface water resources which affects water-reliant activities like irrigation, hydro-electricity generation, fishing and recreational activities such as water rafting. This also affects the availability of groundwater inducing water stress on communities relying on groundwater for their household needs. River basins often face the brunt of hydrological droughts, though such droughts rarely coincide with meteorological and agricultural droughts. Hydrological drought may be the result of long term meteorological droughts that results in the drying up of reservoirs, lakes, streams, rivers and a decline in groundwater levels (sai *et al*., 2016).

2.3.3. Meteorological drought

Meteorological drought occurs when there is a reduction in expected rainfall supply over a particular period. Meteorological drought is based on the degree of dryness and the length of the dry period. The meteorological definitions are site or region-specific, and measure dryness and rainfall received comparing this against a given baseline (Mhike, 2013).

2.4. Effects of drought on maize plant physiology

Drought causes harmful effects on plant life. Reduction of growth occurs as a result of various physiological responses including change in ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon assimilation and utilization (Eyerusalem Arusi, 2015). Maize is particularly sensitive to water stress occurring just before and during flowering when the crops yield potential is defined. The flowering period is the most crucial stage in terms of negative effects of drought on yield.

2.5. Effect of drought on crop yield

Maize is the principal staple food crop produced and consumed by most households in eastern, central and southern Africa. Drought is major causes of food insecurity for many households as it has been estimated to cause annual maize yield loss of 24 million tons in the developing world (Tongoona *et al*., 2003). Yield of the plant depends on the amount of photosynthetic carbon assimilation. The effect of drought on photosynthetic activity is ultimately expressed by crop yield and productivity.

When maize plants were exposed to drought stress at tease ling stage, it led to substantial reduction in yield and yield components such as kernel rows/cob, kernel number/row, 100 kernels weight, kernels/cob, grain yield/plant, biological yield/plant and harvest index (Ahmadizadeh *et al*., 2011).

2.6. Mechanism of drought tolerance

There are several mechanisms of drought and stress tolerance in plants and research is currently underway to explain the mechanisms by which plants can survive during periods of water deficit. Several drought tolerant plants occur naturally. Molecular biology tools can assist in the screening and identification of new drought tolerant plants and varieties.

Plants respond and adapt to and survive under drought stress by the induction of various morphological, biochemical and physiological responses. Drought tolerance is defined as the ability to grow, flower and display economic yield under suboptimal water supply. Drought stress affects the water relations of plants at cellular, tissue and organ levels, causing specific as well as unspecific reactions, damage and adaptation reactions and tolerant plants initiate defense mechanisms against this effect (Addis Tadesse and Melkam Anteneh, 2016).

2.6.1. Drought escape

Escape from drought is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape (Farooq *et al*., 2009). Crop duration is interactively determined by genotype and the environment and determines the ability of the crop to escape from climatic stresses including drought.

Drought escape can be expressed by consistence of crop cycle along with water availability due to genotypic variation in phenology. Drought escaper's plants have the ability to complete their life cycle before the onset of drought condition. This mechanism achieved by early maturity and plants having high degree of developmental elasticity. Drought escape mechanism depends on the success of reproduction before the occurrence of drought stress through adjusted growth and development.

2.6.2. Drought avoidance

By definition drought avoidance is the ability of the plant to maintain relatively high-water content under soil or atmospheric drought stress. Avoidance is achieved either by reducing amount of water loss or by increasing the capacity of water uptake of roots. Drought avoidance consists of mechanisms that reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system (Addis Tadesse and Melkam Anteneh, 2016). Drought avoidance refers to the condition where the plant reduces water loss to prevent dehydration (Kooyers, 2015) Mechanism of avoidance involves: Stomatal closure, increase in root to shoot ratio, solute accumulation.

The root characters such as biomass, length, density and depth are the main drought avoidance traits that contribute to final yield under terminal drought environments and a deep and thick root system is helpful for extracting water from considerable depths (Farooq *et al*., 2009).

2.6.3. Drought tolerance

Drought tolerance is the ability of plant to maintain tissue turgidity and extending plant growth under severe drought condition (Oliveira *et al*., 2012). Tolerance achieved through osmotic adjustment (accumulation of solutes, free amino acids in the roots and shoots of the plant to maintain cellular turgidity. Drought tolerant plants produce solutes, protective proteins and active defense mechanisms for oxidative damage and these enables the plants to maintain low water potential in the leaf and enhance turgidity and water potential gradient that enables the plant to extract more water from the soil. These all result in a progress in stomatal conductance, photosynthesis, cell elongation and finally growth at low water available condition (Eyerusalem Arusi, 2015).

2.7. Origin and geographic distribution of maize

Maize originated in Central America and was introduced to West Africa in the early 1500s by the Portuguese traders and it was introduced to Ethiopia during the 1600s to 1700s (Da Fonseca *et al*., 2014). Today, maize is one of the most important food crops world-wide. It has the highest average yield per hectare and is third after wheat and rice in area and total production in the world. It is grown in most parts of the world over a wide range of environmental conditions, ranging between 50° latitude north and south of the equator. It also grows from sea level to over 3000 meters above sea level (Mandefro *et al*., 2002).

In Ethiopia, maize grows from moisture stress areas to high rainfall areas and from lowlands to the highlands. The total annual production and productivity exceed all other cereal crops, though it is surpassed by teff in area coverage (CSA, 2014). Therefore, considering its importance in terms of wide adaptation, total production and productivity, maize is one of the high priority crops to feed the increasing population of the country.

2.8. Maize Production

Among the maize production, constraints drought is the single most common cause of severe

food shortage in developing countries and climate change is predicted to further exacerbate its impact (Sivakumar *et al*., 2005). In maize, moisture stress is most devastating when it occurs at flowering with causing yield losses of 45-60% (Campos *et al*., 2004).

Private farmers mainly produce maize during the main long rainy season from May to September. In some areas, a small amount is produced in the short rainy period from February to May. Farmers in the western region also plant maize on bottom lands using residual moisture in January and harvest in June/July. This mainly solves the food shortage in the main season (Girma Aboma *et al*., 2001).

In 2015/2016, maize area was 21.17% of the total area under cereals in the country while grain production of maize accounted for 30.9% of total cereal production (CSA, 2018). In the 1980s, the total production within a year was 20 million tonnes and maize production area exceeded slightly 1 million hectares only in 1987, 1988 and 1989 (Kebede *et al*., 1993). However, in the 1990s, maize production in Ethiopia increased: the total area and production remained over 1.3 million hectares and 23.4 million tonnes from 1996-2000, respectively. The yield per hectare also increased slightly in the late 1990s. From 1995-2000, growth rate per year for yield per hectare, maize area and total production was 3.1%, 7.1% and 11.3%, respectively.

Table 1: Estimates of area, production and yield of cereal crops for private peasant holdings in 2015/16

Crop types	Area in (million ha)	Total production in million tonnes	Yield (t/ha)
Cereals	9,974,316.28	231,287,970.83	23.18
Maize	2,111,518.23	71,508,354.11	33.86
Teff	2,866,052.99	44,713,786.91	15.60
Barley	944,401.34	18,567,042.76	19.66
Wheat	1,664,564.62	42,192,572.23	25.34
Sorghum	1,854,710.93	43,232,997.52	23.30

Source: CSA, 2018

Maize has spread as an economic crop to almost all parts of the world including Ethiopia. Although maize is grown all over the country, its production has reached the highest level in the high potential areas (mid-altitude and sub-humid zones) of the country through the use of improved packages generated by the National Maize Research Team. The high potential area covers about 60% in hectarage and contributes over 80% to the total maize production in the country (Mandefro *et al*., 2002). On the other hand, the drought stressed maize growing areas occupy about 40% of the maize growing area, but contribute less than 20% to the total maize production.

There were many drought maize varieties released for drought-stressed areas: Melkasa-2, Melkasa-3, MH140, Melkasa-6Q and MHQ138. Other varieties being grown under rain fed conditions include A-511 and local varieties such as Sheye, Hararghe, Bukuri, Limat and China. The agronomic characteristics of these varieties are described in Table 2.

Table 2: Description of varieties recommended and grown in the drought stressed areas of Ethiopia

Crop Variety	Year released	Altitude (m)	Rainfall (mm)	Days to Maturity	Seed Color	Research Station Yield (q/ha)	Farmers' Field Yield (q/ha)
BH660	1993	1600-2200	1000-1500	160	White	90-120	60-80
BH540	1995	1000-2000	1000-1200	145	White	80-90	50-65
BH543	2005	1000-2000	1000-1200	148	White	85-110	55-65
Melkasa-1	2000	LMS	600-1000	90	Yellow	35-45	25-35
Melkasa-2	2004	LMS	600-1000	130	White	55-65	45-55
Melkasa-3	2004	LMS	600-1000	125	White	50-60	45-50
Melkasa-4	2006	LMS	600-1000	105	White	35-45	30-35
Melkasa-5	2008	LMS	600-1000	125	White	40-50	35-40
Melkasa-6Q	2008	LMS	600-1000	120	White	45-55	30-40
Melkasa-7	2008	LMS	600-1000	115	Yellow	45-55	30-40
MH140	2013	LMS	1000-1800	140	White	85-95	65-75
MH-138Q	2012	LMS	1000-1800	140	White	75-80	55-65

Note: BH = Bako hybrid, MH = Melkasa hybrid, LMS = Low Moisture Stress

Source: EIAR 2018

3. MATERIALS AND METHODS

3.1. Description of growth conditions

The experiment was conducted in green house found at the National Agricultural Biotechnology Research Center located at Holeta from March 2018 to August 2018. Holeta is located in the Oromia special zone surrounding Finfinne (Addis Ababa). It has a latitude and longitude of $9^0$3′N $38^0$3′E and an altitude of 2391m above sea level. All genotypes were grown in the green house under water controlled condition. A soil for growing maize variety was prepared by mixing clay soil (4.6 Kg) with manure in the ratio of 3:1 and was filled into pot. During the experiment, temperature and relative humidity were measured by hanging thermo-hydrometers in the glasshouse. The average minimum and maximum temperatures recorded were 11.4^0C and 34.7^0C, respectively. The maximum and minimum relative humidity during the experiment was 11.8 % and 73 % respectively.

3.2. Experimental design and treatments

The experiment was carried out using Completely Randomized Design (CRD) with three replications. Maize varieties used in the study were MH-140, Melkassa-2, Melkassa-3, Melkassa-4, MHQ138 and Melkassa-6Q (variety from Melkassa agricultural research center). Each variety was grown under the same water levels until imposed to drought stress and treatments. Sowing was done on March 28, 2018 each pot was planted with 2 seeds and only one healthy seedling per pot was allowed to grow, so as to avoid competition. The soil was then watered fully up to complete saturation with 2 litters of water.

3.3. Water level determination

In three pots 7 kg of soil was filled. Three liters of water were added slowly and uniformly on top of the soil in each of three pots until the soil was completely saturated. The pots having the saturated soil were then kept on a water collecting tray for 48 hours to collect trickling water until all downward movement of excess water had ceased completely. After 48 hours, the volume of the water collected in the tray was measured using a measuring cylinder and the same amount was deducted from the 3 L volume of water used to saturate the soil. This observation was repeated three times and the average was then taken as the volume of water that should be applied to this soil to keep the soil moisture at a level available for optimum plant growth. Through this method it was established that 2 litter of water was the optimum volume needed to be given to each plant/pot to keep the soil moisture near the upper limit of field capacity. Plants were watered with this amount of water weekly (when maize needs water) from planting to physiological maturity unless otherwise watering was discontinued to impose drought stress (Eyerusalem Arusi et al., 2015).

Well-watered (WW) =100% = FC (field capacity). = 2L

Water stressed (WS) = watering was stopped for three week after flowering.

3.4. Data collection

3.4.1. Growth parameters

3.4.1.1. Specific leaf area (SLA)

Data about specific leaf area was taken at post-flowering stage when maximum growth (after 120 days after planting) was attained. Two fully expanded leaves were selected randomly from the middle part of a single plot and a total of 6 leaves were taken from the 3 replication. Leaf area was determined by leaf area meter (CI-202 area meter) (figure) as cited in Zhao *et al*. (2016).

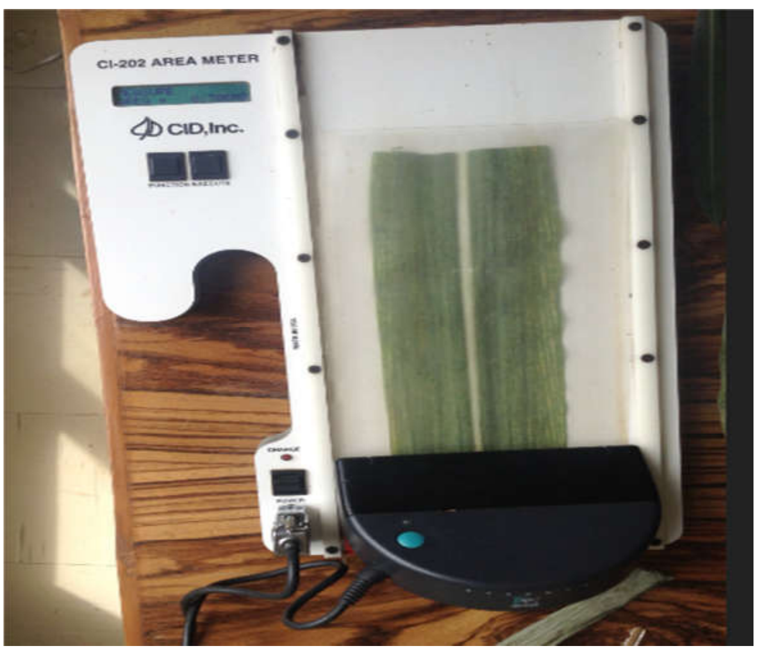


Figure 1: CI-202 area meter used in the leaf area measurement

Then, dry weight determination was done by digital sensitive balance after leaf samples were oven dried for 24 hours at 70^0C. Finally, specific leaf area (SLA) was calculated by the formula below.

SLA = leaf area (cm^2)/Leaf dry weight (g)

3.4.1.2. Specific leaf weight (SLW)

Data about specific leaf weight was taken at post-flowering stage when maximum growth (after 120 days after planting) was attained. Two fully expanded leaves were selected randomly from the middle part of a single plot and a total of 6 leaves were taken from the 3 replicas. Leaf area was determined by leave area meter (CI-202 area meter). Then, dry weight determination was done by sensitive balance after leaf samples were oven dried for 24 hours at 70^0C. Finally, specific leaf weight (SLW) was calculated by the formula below.

SLW = Leaf dry weight (g)/Leaf area (cm^2)

3.4.3. Biochemical parameters

3.4.3.1. Measurement of chlorophyll content

Chlorophyll content was measured in leaf collected at 2 to 3 pm, when plants were expected to experience the most drought stress as result of high temperature and soil moisture deficit. The Minolta SPAD-502 chlorophyll meter was used to acquire a rapid estimate of leaf Chlorophyll content (Van den Berg and Parkins, 2004). The measurements were taken on the flag leaf. Three measurements were taken per pot (five measurements per plant). The results were then averaged to result in a single value to represent the plot.

3.4.3.2. Relative water content

Relative water content (RWC) was measured in leaf samples collected at 2 to 3 pm, when plants were expected to experience the most drought stress as result of high temperature and soil moisture deficit, and fresh weight was recorded immediately using sensitive balance. The samples were then immersed in distilled water and kept in darkness at 4°C overnight to minimize respiration losses and turgid weight was recorded after blotting the adhering water using blotting paper. The samples were then dried in a dry oven set at 70°C for 24 h and dry weight was recorded. RWC% was calculated according to Bedada et al. (2016) as:

RWC% = (FW-DW/TW-DW) ×100

Where, FW is fresh weight, DW is dry weight and TW is turgid weight of leaf samples.

3.4.3.3. Proline determination

Analysis of proline content in the leaves was measured following method of Bates *et al*. (1973). Reagents used in this analysis were; aqueous sulfo salicylic acid, toluene, glacial acetic acid, acid ninhydrin (prepared by warming 1.25 g ninhydrin, 30 ml glacial acetic acid and 20 ml of 6M phosphoric acid). Agitating until dissolved and was kept in refrigerator at 4 ^{O}C (the reagents remained stable for 24 hours).

As proline production occurs in response to stress. Proline analysis of leaf samples was done at post-flowering stage after drought stress was induced. Fresh plant sample (0.5 g) take from leaf was homogenized with 10 ml of 3 % aqueous sulfo salicylic acid and the homogenate was centrifuged at 12000g for 10 minutes. The homogenate was used for proline concentration determination. Two ml of the homogenized tissues was reacts with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube and allowed to react for 1 hour in a water bath at 100^{0}C. It was then cooled, producing radish color. The reaction mixture was extracted with 4 ml of toluene, mixed vigorously in a test tube and stirred for 15-20 second until separate layers was formed. The content from each tube was transferred to test tube. Chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction was separated from aqueous phase (figure 2) to other test tube and warmed at room temperature and absorbance was read at 520 nm by spectrophotometer.

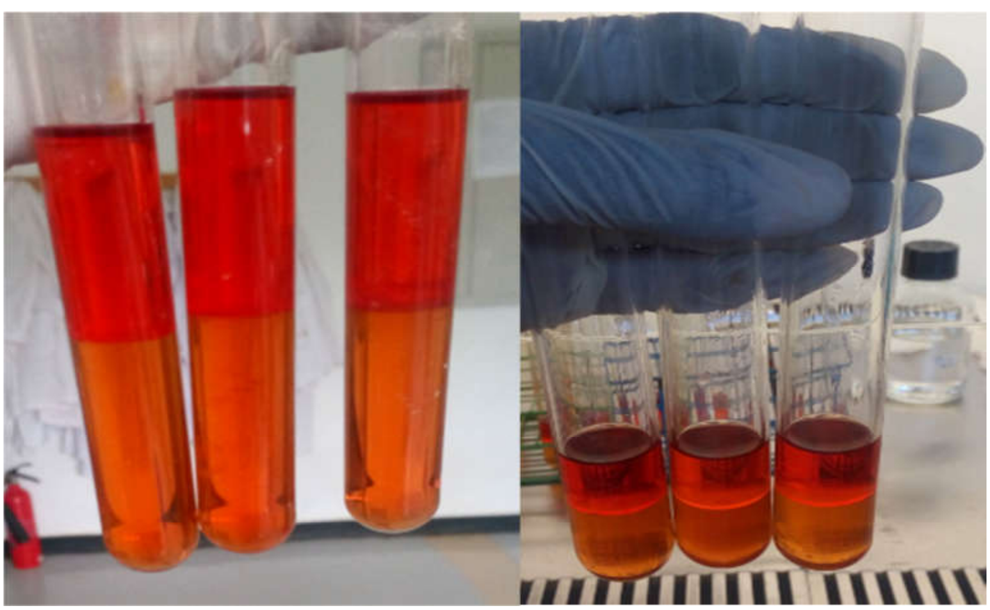


Figure 2: Chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction

The proline concentration was determined from standard curve constructed from known concentration of proline and expressed in moles of proline per gram fresh weight of the leaf.

3.4.3.4. Leaf nitrogen and protein content determination

The Kjeldahl method has been used for the determination of nitrogen in a wide range of samples. The determination of Kjeldahl nitrogen is made in foods and drinks, meat, feeds, cereals and forages for the calculation of the protein content. Also the Kjeldahl method is used for the nitrogen determination in wastewaters, soils and other samples (Pawar, 2007).

Leaf nitrogen content was determined using modified Kjeldahl procedure used by Pawar (2007). Reagents required for nitrogen analysis was; standard H_2SO_4 (0.1N %), NaOH (40 %), Boric acid (2 %), salt mixture (prepared from 250 g of K_2SO_4 mixed with 50 g of $CuSO_4$ and 50 g of metallic selenium) and mixed indicator (prepared by dissolving 0.3 g of Bromo cresol green and 0.2 g of methyl red in 400 ml of 90 % ethanol. The procedure involves three major steps: digestion, distillation and titration.

Digestion

Dried leaf sample (0.5 g) was mixed in Kjeldahl tubes with 7 ml of concentrated H_2SO_4, 3 ml of 30 % H_2O_2 and 2 mg of salt mixture. The content was digested for 1 hour and 50 minutes until a clear solution was obtained. The solution was cooled and taken to the distillation process. Dried leaf sample (0.5 g) was added into Kjeldahl tubes and digested in boiling 7 ml of concentrated sulfuric acid, with the addition of a catalyst, until complete dissolution and oxidation. The nitrogen contained in the sample becomes Ammonium Sulfate.

Distillation

The digested solution was taken to the distillation apparatus (Figure 8). Ten ml of 40 % NaOH was added and made to distill. Along with this, 20 ml of 2 % boric acid was placed at the outlet of condenser. Steam from the boiler was passed through sample. The ammonia released from the solution was captured by boric acid.

The process was continued for 5 minutes and followed by Titration.

Titration

The content was titrated against the standard HCL (0.1N) until bluish green color was turned to pink. The results can be expressed in % N, % NH_3 or protein (%N x factor).

Figure 3: Distillation and titration of nitrogen from leaf

The N content (N in percentage) in the sample was calculated using the following formula:

$$N (\%) = \frac{(\text{Sample titre value} - \text{Blank titre value}) * \text{N of Hcl} * 14 * 100}{\text{Sample weight (g)} * 1000}$$

Protein (%) = % Nitrogen x conversion factor (6.25)

3.4.3.5. Soluble sugar estimation

The soluble sugar was estimated following the method of Dey (1990)

Reagents used in this analysis was 5% phenol (phenol solution was prepared by mixing 5 g of phenol in 95 ml of DDW (double distilled water), 90% ethanol (was prepared by mixing 90 ml of ethanol and 10 ml of DDW). 0.5 g of fresh leaf material was kept in 10 ml of 90% ethanol for 1 hr. at 60^0c in water bath. The extract was then filtrated using filter paper into volumetric flask and the residue re-extracted. Final volume was made up to 25ml by adding 90% ethanol.

Figure 4: Soluble sugar extracted using 90% ethanol

One milliliter of the aliquot was transferred to test tube and 1 ml of 5% phenol was added to it and mixed thoroughly, later 5 ml of sulfuric acid was added to it and mixed thoroughly, by vertical agitation. For exothermic reaction the test tube was cooled in the air.

Absorbance was recorded at 490 nm on spectrophotometer. Soluble sugar was calculated as follows:

$$\text{Soluble sugar} = \frac{\text{Absorbance of Sample x K value x Dilution Factor}}{\text{Weight of Fresh Tissue}}$$

Where, k value = OD value x 1000/2

Dilution factor = vf/vi

Where Vf = V aliquot + V diluent

V Diluent = V of phenol + V of sulfuric acid.

3.5. Data Analysis

Data was subjected to analysis of variance (ANOVA) using SAS 9.4 Mean comparisons was performed using LSD test at $P < 0.05$ and correlation analysis. Correlation analysis was carried out to study the nature and degree of relationship between growth parameters (SLA and SLW) and biochemical parameters, following the method of Pawar (2007).

4. RESULTS AND DISCUSSION

4.1 Specific Leaf Area and Specific Leaf Weight

Analysis of variance revealed (ANOVA) that SLA was significantly affected by water stress, maize variety and the interaction between them at $P < 0.018$. The results of mean comparison showed that the water stress treatment had the lowest levels of SLA and well watered treatment had the highest amount of SLA (Table 3).

SLA obtained from Melkasa-4 was significantly different under water stress condition. However, Melkasa-2, MHQ138 and Melkasa-3 showed non-significant difference in their SLA under water stress conditions. Under water stressed condition, MH140 and MHQ138 was with the highest SLA. Significantly Melkasa-4 was with lower SLA when compared with other variety (Table 3). On the other hand analyses of variance (ANOVA) showed that SLW was significantly affected by water stress, maize variety and interaction between them at $P < 0.018$. The results of mean comparison showed that the water stress treatment had the lowest levels of SLW and well watered treatment had the highest amount of SLW (Table 3). SLW obtained from Melkasa-4 was significant under water stress condition. However, MH140, Melkasa-3 and Melkasa-6Q showed non-significant difference in their SLW under water stress conditions. Under water stressed condition, MHQ138 was with the lower SLW. Significantly Melkasa-4 was with higher SLW when compared with other variety (Table 3).

Table 3: SLA and SLW of maize variety under well watered and water stressed.

Variety	Mean SLA		Mean SLW	
	WS	WW	WS	WW
MH140	45.1^{ba}	47.1^{aa}	0.022^{bbc}	0.048^{abc}
Melkasa-2	38.2^{bc}	48^{ac}	0.026^{ba}	0.060^{aa}
Melkasa-3	38.6^{ba}	45.1^{aa}	0.021^{bc}	0.035^{ac}
Melkasa-4	33.6^{bba}	48.7^{aba}	0.03^{bbc}	0.045^{abc}
Melkasa-6Q	44.8^{ba}	49.9^{aa}	0.022^{bc}	0.040^{ac}
MHQ138	48.9^{bbc}	44.4^{abc}	0.02^{bba}	0.038^{aba}
CV %			15.9	19.3

Means assigned with the same later have no significant difference at $P < 0.01$. WW= well water, WS= water stress, CV= coefficient of variation, SLA = Specific leaf area and SLW= specific leaf weight.

It is a well-documented fact that SLA is an important growth parameter in response to favorable and unfavorable condition (Campos *et al*., 2004), associated with physiological activity (photo assimilation) (Pawar, 2007). SLA indirectly tells the leaf thickness. The lower SLA value obtained in the present study may indicate a reduction of growth in response to water stress and it is one of the tolerance mechanisms under water stressed condition (Anjum *et al*., 2011). Decrease in SLA could be due to the impact of the developing moisture stress on cell elongation, expansion and the reduction in cell number, which ultimately reduced the leaf area. Plants with higher SLA have higher metabolic activity than those with lower SLA. More leaf area might cause more water losses due to more evapotranspiration from the leaf surface. Plant with Lower SLA value indicates the higher drought resistant genotype and the plant with higher SLA value indicates the less drought tolerant genotype (Painawadee *et al*., 2009).

Specific leaf weight is the proportion of leaf dry weight to unit leaf area. Specific leaf weight differs significantly in maize variety (Table 3). In maize, it has been shown that specific leaf weight decreases in water stress, and this is associated with reduced assimilate formation in leaves and leaf growth rate (Mehmood-Ul-Hassan *et al*., 2013). A survey of literature revealed that morpho-physiological traits such as specific leaf weight, leaf dry matter (Misra, 1995) had been widely used as selection parameters contributing towards drought tolerance for various crop plants in addition to grain yield. The increase in SLW also indicates a reduction in leaf area per unit dry weight. maize variety Melkasa-4 has lower SLA and higher SLW than other variety used in the present study so, this variety is more drought tolerant than other variety, while MH140 and MHQ138 maize variety has higher SLA and lower SLW than other genotypes and are more drought susceptible than other. Similar to this finding, Ali *et al*. (2009) reported that Lower specific leaf weight help for the selection of the genotypes with higher grain yields under water stressed condition. The SLW changes observed in all variety in response to drought stress indicated that the MHQ138 variety is the most sensitive variety to drought stress while, variety Melkasa-4 is more drought tolerant than the variety used in the present study.

4.2. Relative Water Content (%)

Relative water content of the leaf is the relative proportion of the fresh weight, dry weight and the turgid weight of the stressed plant. The relative water content of the maize variety differs significantly (Table 4). The results of mean comparison showed that the water stress treatment had the lowest levels of RWC and well watered treatment had the highest amount of RWC (Table 4). Similar to this finding, Decov *et al*. (2000) described that relative water content and water potential in leaves of maize decreases significantly when leaves are subjected to drought, which reduces the leaf growth rate and ultimately its cell wall plasticity. And also, Adejare and Umebese (2007) reported that RWC values were significantly reduced under water stress treatments at flowering stages in the bread and durum wheat plants.

The RWC values of all the variety were differed under well-watered conditions and under drought stress condition (Table 4). Under water stress condition the variety Melkasa-4 had the maximum relative water content followed by Melkasa-3. The lowest relative water content was recorded in MHQ138 followed by Melkasa-2. It is evident from the present study that the relative water content (RWC) of the six maize varieties decreased significantly under drought stress. The reason behind is Water deficit has exert a negative effect on RWC.

The possible reason for decreasing RWC in maize variety leaves could be the limitation of soluble sugar content supply caused by water stress (Lawlor and Cornic, 2002). Preservation of higher RWC by the varieties play a role in its tolerance under receding soil moistures mentioned in Shivalli (2000).

Table 4: Relative water content of maize variety under well watered and water stressed condition.

Variety	Mean of Relative water content	
	WS	WW
MH140	60.5^{bd}	85.6^{ad}
Melkassa-2	59.6^{bd}	85.4^{ad}
Melkassa-3	61.9^{bb}	91.4^{ab}
Melkassa-4	64.3^{ba}	94.7^{aa}
Melkassa-6Q	60.1^{bb}	93.3^{ab}
MHQ138	58.6^{bc}	90.9^{ac}
Cv %		1.02

Means assigned with the same later have no significant difference at $P < 0.0001$. Bars represent mean. Where, RWC= Relative water content WW= well water, WS= water stress

Relative water content (RWC) is a key indicator of the degree of cell and tissue hydration, which is crucial for optimum physiological functioning and growth processes. It has been recommended as selection criterion in breeding programs aimed at increasing the drought tolerance in crop plants (Balota, 1995). Studies have shown that maintenance of a relatively high RWC during mild drought is indicative of drought tolerance (Colom and Vazzana, 2003). From the present study Melkasa-4 demonstrated the highest RWC followed by Melkasa-3 which in turn revealed the significance of these variety for water stress while, MHQ138 variety was with lowest RWC (Table 4). Similar to this finding, Ramos et al. (2003) state that RWC of bean leaves under drought stress significantly was lesser than the control (under well water condition) and higher RWC is a good indicator of drought stress tolerance (Shaw *et al*., 2002). In the present study, water stress decreased the RWC in all variety, but this reduction was larger in drought sensitive variety (MHQ138) and (Melkasa-4) has higher RWC. Similar to this finding, Hassanzadeh *et al*. (2009) showed that sesame cultivars having high RWC are more resistant against drought stress.

4.2. Chlorophyll Content (SPAD Reading)

Analysis of variance (ANOVA) showed that chlorophyll content was extremely significantly affected by water stress, maize variety and interaction between them at $P < 0.0001$. The data on SPAD readings indicated in all the six variety (Table 5). The results of mean comparison showed that the water stress treatment had the lowest levels of chlorophyll content and well watered treatment had the highest amount of chlorophyll content. The reason for decrease in chlorophyll content was, water deficit can destroy the chlorophyll synthesis and prevent plants from making it (Lessani and Mojtahedi, 2002). The variety Melkasa-4 was with the maximum SPAD reading and followed by Melkasa-3 While, the variety MHQ138 was with the minimum SPAD value. Similar to this finding, Hassanzadeh *et al*. (2009) state that chlorophyll content of sesame was higher in irrigation treatment than stress treatment.

Table 5: Leaf Chlorophyll content of six maize varieties under well watered and water stressed condition.

Variety	Mean of chlorophyll	
	WS	WW
MH140	50.6^{bb}	82.0^{ab}
Melkassa-2	48.2^{bc}	82.8^{abc}
Melkassa-3	60.9^{ba}	83.1^{aa}
Melkassa-4	65.1^{ba}	84.4^{aa}
Melkassa-6Q	54.1^{bb}	82.5^{ab}
MHQ138	44.6^{bc}	81.5^{ac}
Cv %		3.78

Means followed by the same letter within a column are not significantly different according Fisher's protected LSD test at $P < 0.0001$. WW= well water, WS= Water stress.

The other reason for chlorophyll content as affected by water deficit is that drought stress produce reactive oxygen species (ROS) that lead to chlorophyll destruction according to Hassanzadeh *et al*. (2009). Homayoun *et al*. (2011) stated that in the plants under the drought stress the chlorophyll in leaves of resistant cultivars are showing the highest content of chlorophyll. Abdellah *et al*. (2011) recorded the highest reduction in the chlorophyll content in susceptible wheat cultivar under water stress of 30% FC. In the present study the maize variety Melkasa-4 with higher chlorophyll content which is the most stress better tolerant variety, while MHQ138 variety with lower chlorophyll content is more stress less tolerant than other variety under water stressed treatment. Similar to this finding, Ghaffari *et al*. (2012) stated that the tolerant sunflower line had higher chlorophyll than the susceptible line under drought stress condition. The high chlorophyll indicates that genotype has more efficient in protecting their photosynthetic apparatus under drought stress, while the lower chlorophyll content indicate less efficient in protecting their photosynthetic apparatus under drought stress.

4.3. Nitrogen Content

Analysis of variance (ANOVA) revealed that Nitrogen content was extremely significantly affected by water stress, maize variety and interaction between them at $P < 0.018$ and $p < 0.029$ respectively. The results of mean comparison showed that the water stress treatment had the lowest levels of Nitrogen content and well watered treatment had the highest amount of nitrogen content. Nitrogen content in leaves recorded in maize variety presented in (Table 6) indicated that all maize variety differed significantly. Similar to this finding, DaMatta *et al*. (2002) reported that when a plant faces water deficit, nitrogen deficiency occurs. Also Mahieu *et al*. (2009) reported that water stress reduces the nitrogen and dry weight accumulation in all plant parts. Maximum nitrogen content was observed in Melkasa-4 and lowest was recorded in MHQ138 followed by Melkasa-2. In the present study, drought decreased the N accumulation in all variety, but this reduction was larger in less tolerant variety. In a study of semiarid grasslands of Inner Mongolia, China, plants from irrigated plots had higher leaf N concentration than plants from un-watered control plots (Lu and Han 2010). In the present study the maize variety Melkasa-4 with higher nitrogen content which is the most stress tolerant variety, while MHQ138 variety with lower nitrogen content is less stress tolerant than other variety under water stressed treatment. Similar to this finding, Lu and Han (2010) revealed that drought tolerant plant have higher nitrogen content and susceptible plant have lower nitrogen content. De Souza *et al*. (1997) studied the effect of water deficit on leaf characteristics and concluded that severe drought stress accelerated leaf senescence by reducing leaf nitrogen (N) and chlorophyll contents.

Table 6: Leaf N content of six maize varieties under well watered and water stressed condition.

Variety	Mean of nitrogen content	
	WS	WW
MH140	2.4^{bbac}	5.5^{abac}
Melkassa-2	2.3^{bdc}	5.3^{adc}
Melkassa-3	2.4^{bbdc}	5.3^{abdc}
Melkassa-4	2.5^{bba}	5.4^{aba}
Melkassa-6Q	2.4^{ba}	5.6^{aa}
MHQ138	2.2^{bd}	5.4^{ad}
CV %		2.9

Means followed by the same letter within a column are not significantly different according Fisher's protected LSD test at $P < 0.018$ and $p < 0.029$.WW= well water, WS= water stress.

4.4. Soluble proteins

Analysis of variance (ANOVA) showed that the effect of drought stress on soluble protein trait, there was a significant difference at the $P < 0.018$ and $p < 0.029$ respectively (Table 7). The results of mean comparison showed that the water stress treatment had the lowest levels of soluble protein and well watered treatment had the highest amount of soluble protein (Table 7). The present study results are consistent with those of Mohammadkhani and Heidari (2008) described that under drought stress total soluble protein content in the roots and leaves of both maize varieties decrease. The reason for this is that drought stress induced changes in protein synthesis in maize. Also the reduction of soluble sugar under water stress condition may be due to the generation of reactive oxygen species (ROS) which causes fragmentation of protein molecules ensuring disruption in protein synthesis as mentioned in Ghaffari *et al*. (2012).

The soluble protein content was maximum in variety Melkasa-4 followed by Melkasa-3 While, the lowest protein content was recorded in MHQ138 in water stressed condition. Ghanbari *et al*. (2007) reported that concentrations of soluble proteins at different levels of drought stress in the tolerant variety of alfalfa is almost constant that seemingly has been favorable maintaining the structure of the plant and plant activities, while in sensitive genotype with increasing stress, the concentration of soluble proteins decreased.

Salekdeh *et al*. (2002) revealed how the leaves in which the protein level was higher exhibited higher leaf water potentials, leaf growth rate, and maintenance of cell wall plasticity when compared to the leaves of cultivars having lower leaf protein contents. The present results of study are consistent with those of Salekdeh *et al*. (2002), and reveal significant variation among the six genotypes tested in this study. Surendar et al. (2013) observed that the tolerant cultivars experienced lesser reduction of soluble protein than the susceptible ones. According to the results of this study in the maize variety Melkasa-4 with higher soluble protein content which were the most stress tolerant variety, while MHQ138 variety with lower soluble protein content is less tolerant than other varieties. In the present study, drought decreased the soluble protein content in all genotypes, but this reduction was larger in less tolerant variety.

Table 7: Leaf protein content of six of maize variety under well watered and water stressed condition.

Variety	Mean of soluble protein	
	WS	WW
MH140	$14.7^{\mathbf{bbac}}$	$34.6^{\mathbf{abac}}$
Melkassa-2	$14.6^{\mathbf{bdc}}$	$33.1^{\mathbf{adc}}$
Melkassa-3	$15.1^{\mathbf{bbdc}}$	$33.2^{\mathbf{abdc}}$
Melkassa-4	$15.8^{\mathbf{bba}}$	$33.9^{\mathbf{aba}}$
Melkassa-6Q	$14.9^{\mathbf{ba}}$	$35.1^{\mathbf{aa}}$
MHQ138	$13.7^{\mathbf{bd}}$	$33.7^{\mathbf{ad}}$
CV %		**2.88**

Means followed by the same letter within a column are not significantly different according Fisher’s protected LSD test at $P < 0.018$ and $p < 0.029$. WW= well water WS= water stress.

Among the incredible responses of plants to abiotic stresses like drought and salinity, is an enhanced production and accumulation of compatible solutes to osmotically adjust themselves (Serraj and Sinclair, 2002). Among such compatible solutes the impact of soluble proteins is quite patent. The maximum proteins content in leaves were recorded at well watered (control) and decreased with the increased in water stress period in all the variety. The water stress injury causes damage to protein synthesizing mechanism. The possible reason for decreased protein content under water stress may be due to increased activity of protease (seems amino acid like proline are less incorporated for protein synthesis during water stress condition) and also it may be due to proteolysis or decreased synthesis or both. Leaf proteins undergo accelerated hydrolysis as water stress develops.

4.5. Soluble Sugar Content

Total soluble sugar content is an important physiological indicator of a plants ability to survive under drought stress. The results obtained from the analysis of variance (ANOVA) for soluble sugar (Table 8) revealed that maize variety, drought stress treatment and their interaction was significant ($P < 0.0001$). The results of mean comparison showed that the water stress treatment had the highest levels of soluble sugar (SS) and well watered treatment had the lowest content of soluble sugar (SS) (Table 8). Significantly higher soluble sugars were noticed in Melkasa-4 as compared to all other variety used in the present study. However, the variety MH140 and MHQ138 recorded lower sugar content over all other variety used in the present study. This difference in response is also likely to be due to the genetic differences among the variety used in the present study. An increase in soluble sugar content as the water level decreases is well documented and agreed with the findings of Mohammadkhani and Heidari (2008). These results indicate that the soluble sugar in the leaves was significantly ($P < 0.0001$) increased due to the increase in the level of drought stress. The differences in the responses to drought stress among the six selected maize variety show that each variety has different ability to synthesis soluble sugar with an increase in drought stress treatment.

Soluble sugar content is an important physiological indicator of plants ability to survive under drought stress. Trouverie *et al*. (2003) reported that accumulation of simple sugars such as glucose and fructose could be associated with an increase in the leaf cell wall plasticity and membrane turgidity.

Anjorin *et al*. (2016) attributed the increased in soluble sugars to decreased translocation of assimilate from the leaves to other organs in plant under water stress. Sugars were reported to be responsible for protecting the cells during drought by mechanisms which involve substitution of the hydroxyl groups of sugar for water to maintain Hydrophilic interactions in membranes and proteins during dehydration as sugars interact with proteins and membranes through hydrogen bonding, thereby preventing protein denaturation and Sugars also contribute majorly to verification, which is the formation of a biological glass in the cytoplasm of dehydrated cells (Anjorin *et al*., 2016). The data on sugars indicated significant differences among the genotypes (Table 8).

Table 8: Soluble content of maize variety under well watered and water stressed condition.

Variety	Mean of Soluble Sugar	
	WS	WW
MH140	$6.3^{\mathbf{ac}}$	$1.6^{\mathbf{bc}}$
Melkasa-2	$6.7^{\mathbf{ac}}$	$1.5^{\mathbf{bc}}$
Melkasa-3	$7.5^{\mathbf{ab}}$	$1.4^{\mathbf{bb}}$
Melkasa-4	$8.3^{\mathbf{aa}}$	$2.0^{\mathbf{ba}}$
Melkasa-6Q	$6.5^{\mathbf{ac}}$	$1.6^{\mathbf{bc}}$
MHQ138	$6.4^{\mathbf{ac}}$	$1.5^{\mathbf{bc}}$
CV %		**5.9**

Means assigned with the same later have no significant difference at P < 0.0001. Bars represent mean. Where, SS= soluble sugar WW= well watered, WS= water stressed.

4.6. Proline content

The results on free proline content revealed significant differences among the variety (Table 9). These results indicated that the proline in the leaves was significantly (P < 0.0001) increased due to the increase in the level of drought stress. The differences in the responses to drought stress among the six selected maize variety showed that each variety has different ability to synthesis proline with an increase in drought stress treatment. Aminatun *et al*. (2013) found that drought-susceptible cultivars of Zea mays had higher proline contents than drought-resistant ones. According to the results of this study among the variety, Melkasa-4 had the maximum proline content which was superior over all other variety and is more drought tolerant than other variety. The minimum proline accumulation was recorded in MHQ138 and it is less drought tolerant than other variety. This difference in response is also likely to be due to the genetic differences among the variety used in the present study. Also Sharada and Naik (2011) all found higher concentrations of proline in drought-tolerant types.

Naser *et al*. (2010) suggested that proline accumulation could be used as a marker of drought tolerance. According to these statement maize variety Melkasa-4 is more drought tolerant than other variety and MHQ138 variety is less drought tolerant than others (Table 9). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). Accumulation of proline has been advocated as a parameter of selection for stress tolerance (Jaleel *et al*., 2007).

Table 9: Proline concentration of leaf under well watered and water stressed condition.

Variety	Mean of proline content	
	WS	WW
MH140	10.6^{ac}	2.1^{bc}
Melkasa-2	10.9^{adc}	1.7^{bdc}
Melkasa-3	12.5^{aba}	1.8^{bba}
Melkasa-4	13.6^{aa}	1.6^{ba}
Melkasa-6Q	11.1^{abc}	2.1^{bbc}
MHQ138	9.8^{ad}	1.8^{bd}
CV %		**6.7**

Means assigned with the same later have no significant difference at $P < 0.0001$. WW= well water, WS= water stress.

4.4. Correlation analysis

4.4.1. Relationship among parameters

In order to analyze how each parameter interrelated to a given condition, correlation analyses were performed for each water level. Under water stressed condition, all parameters showed positively strong correlation with each other except with SLA which showed negative correlation with other biochemical parameters. SLA showed a strong negative correlation with all other parameters (Table 10). SS showed strong correlation with all the parameters. SLW showed a weak positive correlation with all other parameters. However, under well watered condition, all parameters showed weak correlation with each other. Among these, SLW showed a negative correlation with all biochemical parameters except with chlorophyll which was positively correlated (Table 11). Protein showed a positively strong correlation with nitrogen (N) under both condition.

Table 10: Simple correlation of physiological parameters under water stressed condition

	SS	PROT	RWC	N	PROL	CHL	SLW	SLA
SS	1	0.811	0.925	0.811	0.960	0.890	0.711	-0.874
PROT		1	0.927	1	0.933	0.937	0.686	-0.834
RWC			1	0.927	0.967	0.952	0.689	-0.814
N				1	0.933	0.937	0.686	-0.834
PROL					1	0.974	0.673	-0.877
CHLO						1	0.528	-0.767
SLW							1	-0.826
SLA								1

Key: PROT= protein, PROL= proline SLA= specific leaf area, SLW= specific leaf weight, SS= soluble sugar, N= nitrogen, CHLO= chlorophyll, RWC= relative water content.

Table 11: Simple correlation of parameters under well watered condition.

	SS	PROT	RWC	N	PROL	CHL	SLW	SLA
SS	1	0.315	0.593	0.315	-0.208	0.637	-0.010	0.495
PROT		1	0.223	1	0.828	-0.218	-0.225	0.543
RWC			1	0.223	-0.171	0.486	-0.676	0.166
N				1	0.828	-0.218	-0.225	0.543

PROL					1	-0.549	-0.236	0.197
CHLO						1	0.111	0.450
SLW							1	0.404
SLA								1

Key: SLA= specific leaf area, SLW= specific leaf weight, SS= soluble sugar, N= nitrogen, CHLO= chlorophyll, RWC= relative water content.

5. CONCLUSIONS and RECOMMENDATIONS

Drought tolerant maize genotypes maintain their growth and development along with economical grain yield under drought stress. On the other hand, drought susceptible maize genotypes may be lacking any one of these adaptive mechanisms and facing severe damage in terms of growth, development and grain yield. In the present study all maize variety are not equally affected by drought stress due to high level of variability in genetic background of those crops. The differences exist was due to the ability of different maize variety to tolerate water stressed conditions. In the drought stress condition, highest level of proline content, TSS content, N content, Protein content, SLW, RWC content, Chlorophyll content and lower SLA was obtained in the maize variety Melkasa-4, while for lower proline content, SS content, N content, Protein content, SLW, RWC content, Chlorophyll content and higher SLA at the same drought stress treatment, the lowest level was obtained in the MHQ138.

Therefore, higher proline content, SS content, N content, Protein content, SLW, RWC content, Chlorophyll content and lower SLA can help in selection of maize variety.

Based on the present study, the following conclusions have been drawn.

1. Drought stress decreased the nitrogen content, protein content, SLA, SLW, relative water content, chlorophyll content of the variety studied.
2. Soluble sugar content and proline content were increased in the study of variety under drought stress.
3. The differences in proline content, SS content, N content, Protein content, SLW, RWC content, Chlorophyll content and lower SLA among maize variety can be used as a suitable selection (marker) indicator for drought tolerance of maize variety.
4. Maize variety Melkasa-4 is drought tolerant than other maize variety under present study, while maize variety MHQ138 is less tolerant to water stress than maize variety under present study.
5. Among the parameters, the present findings revealed that the soluble sugar content and proline content to the drought stressed variety have great impact on the morphology and physiological status of maize variety.
6. Soluble sugar content and proline content can be used as potential biochemical to select maize variety.

Further work on the molecular identification of genes that code for increased accumulation of these metabolites under drought stressed condition would assist rapid development of maize variety and further investigation on the resistance mechanisms in maize variety at the molecular level is required. Since the current research was done under controlled condition, the consistency of observed traits should be studied under field condition.

6. REFERENCES

Abdellah, A., Boutraa, T. and Alhejely, A. (2011). Therates of photosynthesis, chlorophyll content, dark respiration, proline andabscicic acid (ABA) in wheat (*Triticumdurum*) under water deficit conditions.*Int. J. Agric. Biol,* **13**: 215-221.

Addis, Tadesse. and Melkam, Abera. (2016). Drought Tolerance Mechanisms in Field Crops. *World Journal of Biology and Medical Sciences,* **3***:* 15-39.

Adejare, F. and Umebese, C. (2007). Stomatal resistance to low leaf water potential at different growth stages affects plantbiomass in Glycine max L. *American Journal of Agricultural and Biological Sciences,* **2**: 136 -141.

Ahmadizadeh, M., M. Valizadeh., Zaefizadeh, M and Shahbazi, H. (2011). Antioxidative protection and electrolyte leakage in durum wheat under drought stress condition. *J. Appl. Sci. Res*, **7**: 236-246.

Akıncı, S. and Lösel, M. (2012). Plant Water-Stress Response Mechanisms, Water Stress. *Open access peer-reviewed chapter,* ISBN: 978-953-307-963-9 Publisher InTech.

Al Hakimi, A., Monneveux, P. and Galiba, G. (1995). Soluble sugars, proline and relative water content (RWC) as traits for improving drought tolerance and divergent selection for RWC from T. polonicum into T. durum. *Journal of Genetics and Breeding,* **49**: 237–244.

AL-Ghamdi, A. (2009). Evaluationof oxidative stress tolerance in two wheat (*Triticum aestivum*) cultivars in response to drought. *Int. J. Agric. and Biol,* **11**: 7-12.

Ali, M., Abbas, A., Niaz, S., Zulkiffal, M. and Ali, S. (2009). Morpho-physiological criteria for drought tolerance in sorghum (Sorghum bicolor) at seedling and post-anthesis stages. *Int. J. Agric. Biol,* **11**: 674-680.

Ali-Ahmad. M. and Basha. S. (1995). Effect of Water Stress on Composition of Peanut Leaves. *Peanut Science*, **25**: 31-34.

Aliakbari, M., Saed-Moucheshi, A., Hasheminasab, H. and Pirasteh-Anosheh, H. (2013). Suitable stress indices for screening resistant wheat genotypes under water deficit conditions. *Int. J. Agron. Plant Prod,* **4**: 2665-2672.

Aminatun, M., Endang, S., Paul, Holford. and Sismindari, A. (2013). Tolerance of Accessions of Glagah (Saccharum spontaneum) to Drought Stress and Their Accumulation of Proline. *American Journal of Agricultural and Biological Sciences,* **8**: 1-11.

Anjorin, F., Adejumo, S., Agboola. L. and Samuel, Y. (2016). Proline, soluble sugar, leaf starch and relative water contents of four maize varieties in response to different watering regimes. *Cercetări Agronomice în Moldova*, **3**: 51-62.

Anjum, Sh., Muhammad, S., Long-chang, W., Muhammad, B. and Asif, S. (2012). Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system. *Australian Journal of Crop Science*, **6**: 45-51.

Anjum. Sh., Xiao-yu, X., Long-chang, W., Muhammad, S., Chen, M. and Wang L. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, **6**: 2026-2032.

Arjenaki, F., Jabbari, R. and Morshedi, A. (2012). Evaluation of drought stress on relative water content, chlorophyll content and mineral elements of wheat (Triticum aestivum L.) varieties. *Int. J. Agric. Crop. Sci,* **4**: 726-729.

Arabzadeh, N. (2012). The Effect of Drought Stress on Soluble Carbohydrates (Sugars) in Two Species of *Haloxylon persicum* and *Haloxylon aphyllum*. *Asian Journal of Plant Sciences,* **11:** 44-51.

Ashraf, M. (2010). Inducing drought tolerance in plants: some recent advances. *Biotechnol. Adv.*, **28**: 169-183.

Bahadur, A., Kumar, R., Mishra, U., Rai, A., & Singh, M. (2010). Physiological approaches for screening of tomato genotypes for moisture stress tolerance. *National Conference of Plant Physiology*, 142.

Balota, M. (1995). Excised-leaf water status in Romanian and foreign winter wheat cultivars andthe physiological and environmental effects on excised-leaf water loss. *Romanian. Agric. Res,* **3**: 69–76.

Bates, L., Waldren, R. and. Teare, L. (1973). Rapid determination of free proline for water stress studies. *Plant Soil,* **39**: 205-207.

Bedada, Leta., Miccah, S., Steven, M., Wondyifraw, Teffera., Charless, M., Clet, W., Richard, O., Eduardo, B.and Francis, W. (2016). Drought tolerant tropical maize (*Zea mays* L.) developed through genetic transformation with isopentenyltransferase gene. *African Journal of Biotechnology*, **15**: 2447-2464.

Bekele Shiferaw, Boddupalli P, Jonathan, H. and Marianne, B. (2011). Past successes and future challenges to the role played by maize in global food security. *Food security*, **3**: 307.

Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential. *Australian Journal of Agricultural Research,* **56**: 1159–1168.

Campos, H., Cooper, M., Habben, J., Edmeades, G. and Schussler, J. (2004). Improving drought tolerance in maize. *Field Crop Res,* **90**: 19–34.

Casper, B., Forseth, I., Kempenich, H., Seltzer, S. and Xavier, K. (2001). Drought prolongs leaf life span in the herbaceous desert perennial Cryptantha flava. *Functional Ecology,* **15**: 740–747.

Chaves, M., Maroco, J. and Pereira J. (2003). Understanding plant response to drought: from genes to the whole plant. *Functional Plant Biology,* **30**: 239-264.

CIMMYT. (2010). Global Alliance for Improving Food Security and the Livelihoods of the Resource-poor in the Developing Wood. Draft Proposal submitted by CIMMYT and IITA to the CGIAR Consortium Board.

Colom, M. and Vazzana, C. (2003). Photosynthesis and PSII functionality of drought-resistant and drought sensitive weeping lovegrass plants. *Environ. Exp. Bot,* **49**: 135–144.

CSA (Central Statistics Agency). (2014). Agricultural Sample Survey 2013/2014 (2006 E.C.). Volume I. Report on Area and Production of Major Crops (Private Peasant Holdings, Meher Season). *Aust. J. Agric. Res,* **56**: 1159–1168.

Da Fonseca, R., Bruce, S., Nathan, W., Enrico, C., Pontus, S., Matteo, Fumagalli., José, Samaniego., Christian, Carøe., María, Ávila-Arcos., David, H., Thorfinn, K., Filipe, V., Mattias, J., Bernardo, A., Eske, W., Rasmus, N., Matthew, H., Anders, A., Jeffrey, I. and Gilbert, M. (2014). The origin and evolution of maize in the Southwestern United States. *Nature Plants*, **3:** 34-35.

DaMatta, F., Loos, R., Silva, E., Loureiro, M. and Ducatti, C. (2002). Effects of soil water déficit and nitrogen nutrition on water relations and photosynthesis of pot-grown Coffea canephora Pierra. *Trees,* **16**: 555-558.

De Souza, P., Egli, D. and Bruening, W. (1997). Water stress during seed filling and leaf senescence in soybean. *Agron J,* **89:** 807-812.

Decov, I., Tsonev, T. and Yordanov, I. (2000). Effects of water stress and high-temperature stress on the structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus. Photosynthetica*, **38**: 361-366.

Dey, P. (1990). Methods in plant biochemistry. Vol. 2.Carbohydrates. Academic Press. London (1990). Duxbury, A.C. and D.S.

Efeoglu, B., Ekmekci, Y. and Cicek, N. (2009). Physiological responses of three maize cultivars to drought stress and recovery. *S. Afr. J. Bot,* **75**: 34–42.

Eyerusalem, Arusi Morka. (2015). Physiological Indices for Drought Tolerance in Stay-green Sorghum (Sorghum bicolor L. Moench) Accessions. MSc Thesis, Addis Ababa University.

Eyob Bezabeh (2015). National government vs Cimmt investment trends in maize research. *Journal of Agriculture and Environmental Management,* **4**: 192-196.

Fahad, Sh, Ali, B., Usman, N., Shakeel, A., Ayesha, F., Ali, Z., Sehrish, S., Wajid, N., Steve, A., Shah, S., Muhammad, I., Hesham, A., Chao, W., Depeng, W. and Jianliang, H. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Front Plant Sci,* **8**: 1147.

FAO. (2009). FOASTAT, FAO Statistics Division, Food and Agricultural Organization.

Farooq, M., Wahid, N., Kobayashi, F. and Basra, S. (2009). Plant drought stress effects, mechanisms and management. *Agron. Sustain. De,* **29**: 185–212.

Fatemeh, H., Shahattary, A. and Mansourifar, C. (2017). The effect of drought stress on morphological and physiological traits and essence percentage of medicinal plant, Nigella sativa. *Biosci.Biotech. Res,* **1**: 298-305.

Fathi, A. and Tari, D. (2016). Effect of drought stress and its mechanism in plants. *Int. J. Life Sci,* **10**: 1–6.

Fernandez, J. (1992). Effective selection criteria for assessing plant stress tolerance. In proceedings of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress. *Agricultural Sciences,* **5**: 257-270.

Fita, A., Burruezo, A., Boscaiu, M., Prohens, J. and Vicente, O. (2015). Breeding and domesticating crops adapted to drought and salinity. *Front. Plant Sci,* **6**: 47-49.

Freschet, G., Dias, A., Ackerly D., Aerts, R., van Bodegom, P., Cornwell, W., Dong M., Kurokawa, H., Liu, G., Onipchenko, V., Ordoñez, J., Peltzer, D., Richardson, S., Shidakov, I., Soudzilovskaia, N., Tao, J. and Cornelissen, J. (2011). Global to community scale differences in the prevalence of convergent over divergent leaf trait distributions in plant assemblages. *Global Ecology and Biogeography*, **5**: 67-51.

Ghaffari, M, Toorchi, M., Valizadeh, M. and Shakiba, M. (2012). Morpho-physiologicalscreening of sunflower inbred lines under drought stress condition. *Turk. J. Field Crops,* **17**: 185-190.

Ghanbari, F., Nadjaf, S., Shabahang, S. and Ghanbari, A. (2007). Effects of irrigation regimes and row arrangement on yield, yield components and seed quality of pumpkin (Cucurbita pepo L.). *Asian Journal of Plant Science,* **6**: 1072-1079.

Girma, Aboma., Mosisa, Worku., Dereje, Bacha., Abdissa, Gemeda. and Demissew, Kitaw. (2001). Participatory analysis of off-season maize production in Eastern and Western Wollega zones Ethiopian. *Institute of agricultural research,* **37:** 43-52.

Golbashy, M., Mohsen, Ebrahimi., Saeid, Khorasani. and Rajab Choukan. (2010). Evaluation of drought tolerance of some corn (Zea mays L.) hybrids in Iran. *African Journal of Agricultural Research,* **5**: 2714-2719.

Halder, K. and Burrage, S. (2003). Drought stress effects on water relations of rice grown in nutrient film technique. *Pakistan J. Biol. Sci.,* **6**: 441–444.

Hare, P. and Cress, W. (1997). Metabolic implications of stress-induced proline accumulation
in plants. *Plant Growth Regulation,* **21**: 79–102.

Hoekstra, F. and Buitink, J. (2001). Mechanisms of plant desiccation tolerance. *Trends Plant Sci,* **8**: 431- 438.

Homayoun, H, Daliri, M. and Mehrabi, P. (2011). Effect of Drought Stress on Leaf Chlorophyll in Corn Cultivars (*Zea mays*). *Middle-East Journal of Scientific Research*, **9**: 418-420.

Homayouni, H., and Khazarian, V. (2014). Effects of deficit irrigation on soluble sugars, starch and proline in three corn hybrids. *Indian J. Sci. Res*, **7:** 910–917.

Morgan, J. (1992). Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. *Austral Journal of Plant Physiology*, **19**: 67–76.

Jaleel, C., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R. and Panneerselvam, R. (2007). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus rosesseedlings under salt stress. *South Afr. J. Bot*. **73**: 190–195.

Ji, Z., Wang, J., Li, J., Xue, S. and Zhang, M. (2009). Dynamic changes of soluble sugar in the seedlings of Robinia pseudoacacia under drought stress and re-watering in different seasons.*Acta Bot. Boreali-Occidentalia*, **5**: 65-78.

Jonhston, A. (2002). Optimizing plant nutrition to minimize crop stress.*Agri- Briefs Agronomic*, **6**: 122-126.

Kanani, Mesfin., Kasraie, P. and Abdi, Haji. (2013). Effects of late season drought stress on grain yield, protein, proline and ABA of bread wheat varieties. *Intl. J. Agron. Plant Prod*, **5**: 2943-2952.

Kebede, Mulatu., Gezahegne, Bogale., Benti, Tolessa., Mosisa, Worku., Yigzaw, Desalegne. and Assefa, Afeta. (1993). Maize production trends and research in Ethiopia. pp. 4-12. In Benti Tolessa and J.K. Ransom (eds.). Proceedings of the First National Maize Workshop of Ethiopia. IAR/CIMMYT, Addis Ababa, Ethiopia.

Kerepesi, I. and Galiba, G. (2000). Osmotic and salt stress indicated alteration in soluble carbohydrate contents in wheat seedlings. *Crop Sci,* **40**: 482–487.

Kooyers, J. (2015). The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Science,* **234**: 155–162.

Kumar, D., Al Hassan, M., Naranjo, A., Agrawal, V., Boscaiu, M. and Vicente, O. (2017). Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander L.*). PLoS ONE 2017, 12, e0185017.

Laureano, R., Lazo, Y., Linares, J., Luque, A., Martínez, F., Seco, I. and Merino, J. (2008).The cost of stress resistance: construction and maintenance costs of leaves and roots in two populations of Quercus ilex. *Tree physiology,* **28**: 1721–1728.

Lawlor, W. and Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Cell Environ,* **25**: 275–295.

Lessani, H. and Mojtahedi, M. (2002). Introduction to Plant Physiology (Translation). 6thEdn.,Tehran University press, Iran, ISBN: 964-03-3568-1.

Li, R., Guo, P., Baum, M., Grando, S. and Ceccarelli, S. (2006). Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agric. Sci*, **5**: 751–757.

Low, P. (1985). Molecular basis of the biological compatibility of nature's osmolytes. In Gilles R, Gilles-Baillien M (eds) Transport Processes and Osmoregulation. 469-477, Springer-Verlag, Berlin.

Lu, T. and Han, G. (2010). Nutrient re-sorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. *Plant Soil,* **327**: 481-491.

Lum, M., Hanafi, M., Rafii, Y. and Akmar, A. (2014). Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *The Journal of Animal & Plant Sciences*, **24:** 1487-1493.

Lunduka, R., K, Mateva., C, Magorokosho. and P, Manjeru.(2017). Impact of adoption of drought-tolerant maize varieties on total maize production in south Eastern Zimbabwe. *Journal Climate and Development,* **4**: 65-68.

Magorokosho, K., Pixley, K. and Tongoona, P. (2003). Selection for drought tolerance in two tropical maize populations. *African Crop Science Journal*, **11**: 151-161.

Mahieu, S., Germon F., Aveline, A., Hauggaard-Nielsen, H, Ambus, P. and Jensen, S. (2009). The influence of water stress on biomass and N accumulation, N portioning between above and below ground parts and on N rhizo deposition during reproductive growth of pea (Pisum sativum L.). *Soil Biology and Bio-chemistry*, **41**: 380-387.

Mandefro, Nigussie., Tanner, D. and Twumasi-Afriyie, S. (2002). Enhancing the Contribution of Maize to Food Security in Ethiopia: Proceedings of the Second National Maize Workshop of Ethiopia, 12-16 November 2001, Addis Ababa, Ethiopia. Addis Ababa, Ethiopia. *Ethiopian Agricultural Research Organization (EARO) and International Maize and Wheat Improvement Center (CIMMYT).*

Marcelis, L. F. M., Heuvelink, E., and Goudriaan, J. (1998). Modeling biomass production and yield of horticultural crops a review.*Sci. Hort,* **74**: 83–111.

Mehmood-ul-Hassan, A., Abdul, Q., Abdul, R., Muhammad, A., Imran, M., Sami, Ullah, K. and Matthew, J. (2013). Evaluation of Maize Cultivars for Drought Tolerance Based on Physiological Traits Associated with Cell Wall Plasticity. *Jokull. Journal,* **63:** 45-53**.**

Mhike, X,.(2013). Use of morpho-physiological traits to enhance breeding for drought tolerance in tropical maize. Ph.D. Thesis Submitted to Makerere University.

Mian, M., E,Nafziger., F, Kolb. and R, Teyker. (1993). Root growth of wheat genotypes in hydroponic culture and in the greenhouse under different soil moisture regimes. *Crop Sci,* **33**: 283-286.

Milla, R. and Reich, B. (2008). Environmental and developmental controls on specific leaf area are little modified by leaf allometry. *Functional Ecology,* **22***:*565–576*.*

Misra, N. (1995). Assimilate partitioning in pearl millet (*Pennisetum glaucum* L.R.Br.). *Acta Physiol Plant,* **17**: 41-46.

Mohammadkhani, N. and Heidari, R. (2008). Drought-induced Accumulation of Soluble Sugars and Proline in Two Maize Varieties. *World Applied Sciences Journal*, **3**: 448-453.

Mohammadreza, H., Zokaee, Khosroshahi., Mahmoud, Esna-Ashari., Ahmad, Ershadi. and Ali, Imani. (2014). Morphological Changes in Response to Drought Stress in Cultivated and Wild Almond Species. *International Journal of Horticultural Science and Technology,* **1**: 79-92.

Naser, L., Kourosh, K., Bahman. and Reza, A. (2010). Soluble sugars and proline accumulation play a role as effective indices for drought tolerance screening in Persian walnut (*Juglans regia* L.) during germination. *Fruits,* **65**: 97-112.

Neisiani, F. (2009). Effect of foliar application of pyridoxine on antioxidant enzyme activity, proline accumulation and lipid peroxidation of maize (*Zeamays* L.) under water deficit. *Not. Bot. Horti. Agrobot (Notulae Botanicae Horti Agrobotanici),* **37**: 116-121.

Olaoye, L. (2009). Screening for moisture deficit tolerance in four maize (*Zea mays* L.) populations derived from drought tolerant inbred x adapted cultivar crosses. *Tropic.Subtropi. Agroecol,* **10:** 237–251.

Oliveira, A., Alencar, M. and Gomes-Filho, E. (2012). Physiological and biochemical responses of semiarid plants subjected to water stress. *Water Stress*, **1**: 1-16.

Ongeti. J. (2012). Breeding maize for early maturity and drought tolerance in Kenya using anthesis to silking interval. MSc Thesis University of Nairobi.

Painawadee, M., Jogloy, S., Kesmala, T., Akkasaeng, C. and Patanothai, A. (2009). Identification of traits related to drought resistance in Peanut (Arachis hypogaea L.). *Asian J. Plant Sci.* **8**: 120-128.

Pawar, N. (2007). Physiological indices for drought Tolerance in rabi sorghum. Ph.D. Dissertation submitted to Dharwad University, 26-38.

Pimratch, S., Jogloy, S., Vorasoot, N.,Toomsan, B., Patanothai, A. and Holbrook, C. (2008). Relationship between biomass production and nitrogen fixation under drought-stress conditions in peanut genotypes with different levels of drought resistance. *Journal of Agronomy and Crop Science,* **194**: 15-25.

Prasad, G. (2014). Performance of maize (Zea mays L.) hybrids under different sowing dates in kharif season. I.M.M. ed.) InTech. M. Sc. Dissertations. Chaudhary Charan Singh Haryana Agricultural University, Indian.

Ramagopal, S. (1993). Advances in understanding the molecular biology of drought and salinity tolerance in plants- the first decade. *Adv. Pl. Biotech. Biochem,* **8**: 123-125.

Ramos,M., Richard, P., Janet, S. and Euan, J. (2003). Effect of water stress on nitrogen fixation and nodule structure of common bean.*Pesq. Agropec.Bras. Brasilia,* **38**: 339-347.

Saeedipour, S., and Moradi, F. (2011). Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling and impact of invertase activity on carbon metabolism during kernel development. *J. Agric. Sci,* **3**: 32-44.

Sai, S., Murthy, K., Chandrasekar, A., Jeyaseelan, P., Diwakar, M. and Dadhwal, V. (2016). Agricultural drought assessment & monitoring. *Mausam*, **67**: 131-142.

Salekdeh G, Siopongco J, Wade L, Gareyazie B. and Bennett, J. (2002). Proteomic analysis of rice leaves during drought stress and recovery. *Proteomics*, **2**: 1131-1145.

Schwacke, R., Grallath, S., Breitkreuz, K., Stransky, H., Frommer, W. and Rentsch, D. (1999).
LeProT1, a transporter for proline, glycine betaine, and γ -amino butyric acid in tomato pollen. *Plant Cell,* **11**: 377–391.

Serraj, R. and Sinclair, T. (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions. *Plant Cell Environ*, **25**: 333-341.

Sharada, P. and Naik, G. (2011). Physiological and biochemical responses of groundnut genotypes to drought stress. *World J. Sci. Technol,* **1**: 60-66.

Shaw, B., Thomas, H. and Cooke, T. (2002). Responses of sugar beet (Beta vulgaris L.) to drought and nutrient deficiency stress. *Plant Growth Regulation,* **37**: 77–83.

Shirinzadeh, A., Zarghami, R., Azghandi, V., Shiri, R. and Mirabdulbaghi, M. (2010). Evaluation of drought tolerance in mid and late mature corn hybrids using stress toleranceindices. *Asia. J. Plant. Sci*, **9**: 67-73.

Shivalli, S. (2000). Characterization of morpho-physiological traits for higher productivity in rabi Sorghum. M. Sc. Thesis, University of Agricultural Sciences, Dharwad.

Silva, E., Nogueira, R., Vale, F., Melo, N. and Araujo, F. (2009). Water relations and organic solutes production in four umbu tree (Spondias tuberosa) genotypes under intermittent drought. *Brazilian Journal of Plant Physiology,* **21**: 43-53.

Songsri, P., Jogloy, S., Holbrook, C., Kesmala, T., Vorasoot, N., Akkasaeng, C. and Patanothai, A. (2009). Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. *Agricultural Water Management*, 96, 790-798.

Studer, C., Yuncai, H.and Schmidhalter, U. (2017). Interactive Effects of N-, P- and K-Nutrition and Drought Stress on the Development of Maize Seedlings. *Agriculture*, **7**: 90.

Kadam, S, Yogesh Shukla., Narayan, Subhash., Chandrakant, Singh.and Kiran, Suthar. (2017). Screening of Wheat Genotypes (Triticum durum L.) in Response to Drought Stress by Some Physiological and Biochemical Indices. *Int. J. Pure App. Biosci,* **5**: 969-977.

Taiz, L. and Zeiger, E. (2006). Plant Physiology (4th Edn), Sinauer Associates, Massachusetts, 690.

Trouverie, J., C. Thevenot, J., Rocher, B. and Sottaand, J. (2003). The role of abscisic acid in the response of a specific vacuolar invertase to water stress in the adult maize leaf. *J. Exp. Botany*, **54**: 2177- 2186.

Verbruggen, N. and Hermans, C. (2008). Proline accumulation in plants*.* *Amino Acids,* **35**: 753- 759.

Van den Berg, A. and Perkins, T. (2004). Evaluation of a portable chlorophyll meter to estimate chlorophyll and nitrogen contents in sugar maple (Acer saccharum Marsh.) leaves. *Forest Ecology and Management*, **200**: 113–117.

Voltas, J., Lopez, C. and Borras, G. (2005). Use of bioplot analysis and factorial regretion for the investigation of superior genotypes in multi-environmental trails. *European, journal of agronomy*, **22:** 309-324.

Waseem, M., Ali, A., Tahir, M., Nadeem, M.A., Ayub, M., Tanveer, A., Ahmad, R. and Hussain, M. (2011). Mechanism of drought tolerance in plant and its management through different methods. *Cont. J. Agric. Sci*, **5**: 10-25.

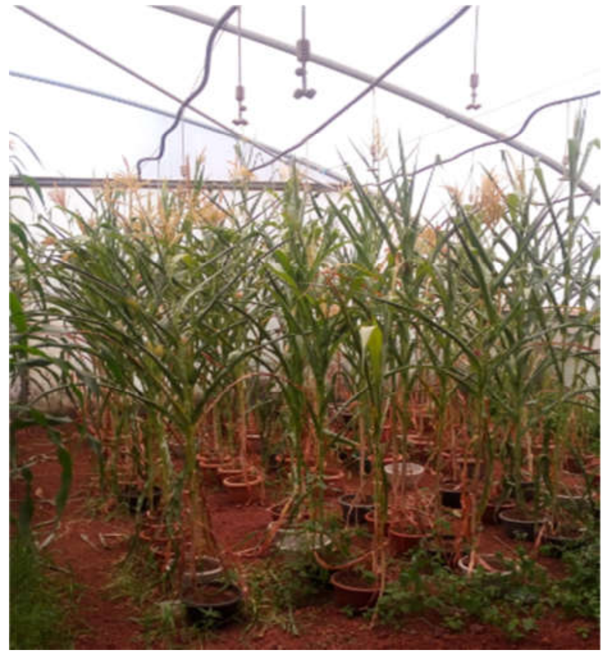
Yuan, Y., G, Lue., M. Xu. and E, Wang. (2009). Analysis on the physiological characteristic of *Populus euphratica* with different DBH under drought stress. *Xinjiang, Agric. Sci,* **46**: 299-305.

Zhao,Ch., Heli, L., Pingheng, L., Guijun, Y., Xiaohe, G. and Yubin, L. (2016). Effect of vertical distribution of crop structure and biochemical parameters of winter wheat on canopy reflectance characteristics and spectral indices.

Zlatev, Z. and Lidon, F. (2012). An overview on drought induced changes in plant growth, water relations and photosynthesis. *J. Food Agric,* **24**: 57-72.

APPENDICES

Appendix 1: Partial view of the greenhouse maize genotypes after water stress induced during flowering

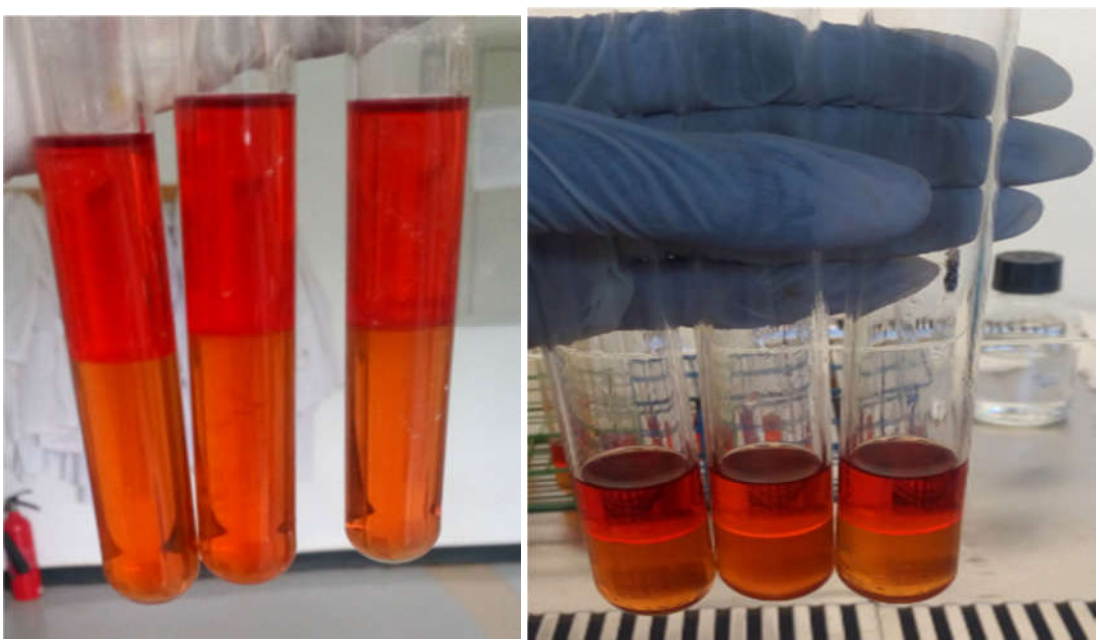


Appendix 2. Raw data for biochemical parameters

Genotypes	REP	Treatment	PROTEIN	CH	PRO	N%	RWC	TSS
MH140	1	WS	14.20264	50.2	10.7	2.272422	60.9756	6.22667
Melkasa-2	1	WS	14.1501	48.3	10.9	2.264016	59.8086	7.38479
Melkasa-3	1	WS	15.2709	61.0	12.5	2.443344	62.6749	7.84196
Melkasa-4	1	WS	16.77698	65.0	13.7	2.684316	64.6288	8.42317
Melkasa	1	WS	15.21836	48.5	12.4	2.434938	60.0518	6.62059
6Q MHQ138	1	WS	13.4496	44.8	9.8	2.151936	58.4415	6.28903
MH140	2	WS	15.21836	50.2	10.6	2.434938	60.5714	6.25231
Melkasa-2	2	WS	14.5704	48.6	10.9	2.331264	59.7849	6.44444

Melkasa-3	2	WS	14.86811	60.9	12.5	2.378898	61.0588	7.97392
Melkasa-4	2	WS	15.28841	65.0	13.7	2.446146	63.8850	8.39765
Melkasa 6Q	2	WS	14.97319	49.7	10.5	2.39571	59.5406	6.45561
MHQ138	2	WS	13.79985	45.0	9.9	2.207976	58.9136	6.46679
MH140	3	WS	14.72801	51.5	10.6	2.356482	60.0448	6.49666
Melkasa-2	3	WS	15.1308	47.7	10.9	2.420928	59.3137	6.48172
Melkasa-3	3	WS	15.28841	61.0	12.5	2.446146	61.9791	6.78002
Melkasa-4	3	WS	15.28841	65.4	13.6	2.446146	64.4385	8.35945
Melkasa 6Q	3	WS	14.58791	64.2	10.5	2.334066	60.7495	6.50414
MHQ138	3	WS	13.97498	44.2	9.9	2.235996	58.6410	6.47425
MH140	1	WW	33.7641	82.3	2.1	5.402256	86.9109	1.76952
Melkasa-2	1	WW	32.71335	82.3	1.8	5.234136	85.9296	1.56511
Melkasa-3	1	WW	33.23873	83.8	1.8	5.318196	91.5151	1.43405
Melkasa-4	1	WW	33.15116	85.5	2.1	5.304186	94.3310	2.01135
Melkasa 6Q	1	WW	34.67475	83.1	2.9	5.54796	92.8382	1.67523
MHQ138	1	WW	32.62579	81.4	1.7	5.220126	91.4046	1.58351
MH140	2	WW	35.69048	82.1	1.8	5.710476	83.4693	1.47473
Melkasa-2	2	WW	33.92171	82.7	1.6	5.427474	85.0197	1.47473
Melkasa-3	2	WW	32.71335	83.0	2.2	5.234136	91.6334	1.47473
Melkasa-4	2	WW	33.46639	84.4	1.0	5.354622	95.8904	2.01135
Melkasa 6Q	2	WW	36.07575	82.9	1.6	5.77212	93.6263	1.68093

MHQ138	2	WW	34.72729	81.6	1.6	5.556366	90.1041	1.49259
MH140	3	WW	34.28948	81.7	2.6	5.486	86.614 1	1.615045
Melkasa-2	3	WW	32.71335	83.5	1.7	5.234	85.317 4	1.494387
Melkasa-3	3	WW	33.7641	82.5	1.5	5.402	91.297 2	1.487225
Melkasa-4	3	WW	34.98998	83.5	1.9	5.598	94.090 2	2.005121
Melkasa 6Q	3	WW	34.67475	81.7	1.9	5.547	93.591 2	1.665755
MHQ138	3	WW	33.85166	81.6	2.2	5.416	91.463 4	1.715328



Appendix 3. Chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction.