Some Morphological, Physiological and Biochemical Characterization of Drought Tolerant Wheat (*Triticum* spp.) Varieties

By: Alemayehu Tefera

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree

Masters of science in Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

September, 2019

Some Morphological, Physiological and Biochemical Characterization of Drought Tolerant Wheat (Triticum spp.) Varieties

By: Alemayehu Tefera

Advisor: Dr. Mulugeta Kebede

Co-advisor: Mr. Kassu Tadesse

A thesis Submitted to

The Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree

Master of Science in Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

September, 2019

Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Alemayehu Tefera have read and evaluated his thesis entitled "Some Morphological, Physiological and Bio-chemical Characterization of Drought Tolerant Wheat (*Triticum* spp.) Varieties" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree Master of Science in Biology (Biotechnology).

Name of Student	Signature	Date
______________________	______________________	______________________
Advisor	Signature	Date
______________________	______________________	______________________
External Examiner	Signature	Date
______________________	______________________	______________________
Internal Examiner	Signature	Date
______________________	______________________	______________________
Department head	Signature	Date
______________________	______________________	______________________
School Dean	Signature	Date
______________________	______________________	______________________
Postgraduate Dean	Signature	Date
______________________	______________________	______________________

Declaration

I hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other university and all sources of material used for this thesis have been duly acknowledged.

Name: ____

Signature: ____

This MSc thesis has been submitted for examination with my approval as thesis advisor.

Name: ____

Signature: ____

Date of submission: ____

Advisor's approval sheet

To: Applied biology department

Subject: Thesis Submission

This is to certify that the thesis entitled **"Some Morphological, Physiological and Biochemical Characterization of Drought Tolerant Wheat (*Triticum* spp.) Varieties"** submitted in partial fulfillment of the requirements for the Degree Master of Science in Biology (Biotechnology), the Graduate Program to the Department of Applied Biology and have been carried out by **Alemayehu Tefera ID No: A/PR15348/10**, under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

______________________________	_____________	_________________
Name of major Advisor	Signature	Date

Acknowledgments

First of all, I thank God's blessings in guiding, enriching to undertake and accomplish this research project.

I would like to express my gratitude to my Major Advisor, Dr. Mulugeta Kebede, for providing encouragement, guidance and unwavering support academically, professionally and personally throughout my studies which made my MSc programme, a satisfying experience. I would like to express my deep and sincere gratitude to Mr. Kasu Tadesse and Mr. Tsegaye Kebede for their idea, kindness, admirable supervision, direct guidance and fruitful and interesting discussions throughout this work. They assisted in all matters, provided solutions to different problems and always gave input even at times of personal anxieties. Both Mr. Kasu Tadesse and Mr. Tsegaye Kebede supported and helped me from beginning till the end of the study. I owe my sincere thanks to them, for their valuable suggestions, constant encouragement and timely help.

I would also like to extend my heartfelt appreciation to the Department of Applied Biology, Adama Science and Technology University (ASTU) for writing me letters so I could get all facilities outside of ASTU. Kulumsa Agricultural Research Center (KARC), Debre Zeit Agricultural Research Center (DZARC) and Melkassa Agricultural Research Center (MARC) management and the technical staff for all the assistance I obtained from them.

I take this humble opportunity to extent my respectful thanks and warm regards to Dr. Birhanu Fenta for his willing to help whenever approached. I owe my sincere thanks to him for being kind and affectionate in every form of assistance.

I am grateful to Dr. Tilahun Hordofa and Mr. Mekuria Temtim for their consistent and invaluable inspirations throughout the course of this study. Their assistance in preparation of thesis was indispensable indeed. They always encouraged and helped me in this research project. I do not have words to acknowledge them but I wish them all success in their career.

I offer my sincere thanks and profound regards with deep sense of gratitude to all, who have helped me to accomplish this destination. Everyone is not adverted in this epistle but none is forgotten.

Table of Contents

Contents **pages**

List of tables

List of Figures

List of Appendices

List of Acronyms and Abbreviations

CWR	Crop Water Requirement
DDW	Double Distilled Water
EIAR	Ethiopian Institute of Agricultural Research
ETo	Evapotranspiration
FC	Field Capacity
FW	Fresh Weight
KARC	Kulumsa Agricultural Research Center
MARC	Melkassa Agricultural Research Center
PRO	Proline content
CC	Chlorophyll Content
ROS	Reactive Oxygen Species
RuBisCO	ribulose-1, 5-bisphosphate carboxylase/oxygenase enzyme
RWC	Relative Water Content
SPL	Spike length
SPS	Spikelet Per Spike
TSS	Total Soluble Sugar
WS	Water Stressed
WW	Well Watered
TKW	Total Kernel Weight

Abstract

Wheat is an important food for more than 35% of the world population and widely cultivated in wide range of altitudes in Ethiopia. However, recently, it is being affected by water stress throughout the country. Having this thought, considering the study of morphological, physiological and biochemical parameters ware important to understand the effect of water stress. The study was carried out at Kulumsa Agricultural Research Center (KARC), in lath house using randomized complete block design (RCBD) with three replications comprising two water regimes of well-water (100% FC) and water stressed (30% FC). Twelve wheat varieties, six from bread wheat Wane, Danka, Kakaba, Ogolcho, Paven-76 and Kingbird and six from durum wheat Donmatia, Mangudo, Utuba, Alemtena, Ude and Mukuye were planted in pots. In this study, a total of seven traits plant height (PH), spike length (SPL), spikes per plant (SPP), chlorophyll content(CC), relative water content (RWC), Proline (PRO) and total soluble sugar (TSS) under water stress were considered. In this study, the results showed that all the seven traits were significantly affected (p<0.05). Nevertheless, under water stress a non-significant difference was observed on the plant height and relative water content among the varieties. The longest and superior SPL (6.9 cm) was recorded from Ogolcho variety under water stress condition. Compared to Ogolcho, the SPL of Mukuye, Ude, Alemtena, Utuba, Mangudo and Wane varieties were found shorter than by 39%, 37%, 36%, 35%, 32% and 29%, respectively. Alemtena and Utuba contained a high amount of proline content (4.78µmol/g and 4.65µmol/g) and total soluble sugar (51.05mg/g and 49.6 mg/g) respectively. In a nutshell, the differences in the synthesis of proline and total soluble sugar with an increase in water stressed among the selected wheat varieties showed that each variety had different ability to cope up with water stresses. This difference in the responses is also likely to be due to the genetic differences among the varieties.

Key words: Field capacity; Proline; *Triticum durum* desf.; *Triticum aestivum* L.; Water stress.

1. INTRODUCTION

1.1 Background of the study

The initial domestication of wheat (*Triticum species)* occurred 10,000 years ago (Tanno and Willcox, 2006). The history of wheat domestication saw the selection for an indehiscent ear and a naked grain (to ease harvesting and improve flour quality), increased yield, more and larger seeds, the adaptation to a wide range of climatic and farming regimes (Gustafson *et al*., 2009) and the development of polyploidy. There are six types of *Triticum* species of which *Triticum aestivum* and *Triticum durum* are the most dominantly grown species in Ethiopia. Based on the number of chromosomes, wheat is usually classified into three groups: diploid (2x = 2n = 14, where n = 7), tetraploid (2x = 4n = 28), and hexaploid (2x = 6n = 42). Wheat gives more protein and calories to the diet of the world than any of other cereal crops (Abd-El-Haleem *et al.,* 2009).

Wheat is one of the most important small cereal crops in Ethiopia widely cultivated in wide range of altitudes. Most wheat producing area in Ethiopia lie between 6^o and 16^o N latitude and 35^o and 42^o E longitudes of an altitude range from 1500 to 3000 meters above sea level (masl). However, the most suitable agro-ecological zones fall in between 1900 to 2700 masl (Bekele *et al*., 2000). Agriculture in Ethiopia is predominantly traditional and thus mainly landraces are grown. This is mainly attributed to problems of adaptability and stability of improved varieties to the adverse farming conditions that prevail on small peasant farms and also due to the unavailability of seeds of improved varieties to farmers in sufficient quantity. Phenomenon encountered by wheat crop and long duration of water stress lead to severe reduction in overall production (Nezhadahmadi *et al*., 2013).

Drought induces significant alterations in plant physiology and biochemistry. Some plants have a set of physiological adaptations that allow them to tolerate water stress conditions. The degree of adaptations to the decrease of water potential caused by drought may vary considerably among species. (var) Plant response to water stress include morphological and biochemical changes and later as water stress become more severe to functional damage and loss of plant parts (Sangtarash, 2010).

The stress factors especially drought, negatively affect plant growth and development and causes a sharp decrease of plants productivity (Pan *et al.,* 2002). It can have a substantial impact on the agriculture of the affected region as its short intense can cause significant damage and harm the economy. In addition to the complexity of drought itself, plant responses to water scarcity are complex due to unpredictable factors in the environment and the interaction with other abiotic and biotic factors. 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 water 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-Sahara African countries like Ethiopia (FAO, 2009). The response of plants to water stress depends on several factors such as development stage, severity and duration of stress and cultivar genetics (Beltrano and Marta, 2008).

Development of drought tolerant varieties has been the approach used to alleviate the negative effects of water stress on crops and crop yields. To date conventional plant breeding techniques have allowed the development of high yielding, drought tolerant crop varieties. However, the shortcomings of this approach are that it is time consuming, labor intensive, may lead to the loss of other desirable traits from the host's gene pool (Ashraf, 2007). Therefore, the objective of this research was to study the effect of soil water stress on some morphological, physiological and biochemical parameters in durum wheat and bread wheat varieties and to determine the traits which can be used for identification tolerant wheat varieties under water stress conditions.

1.2. Statement of the problem

Wheat is one of the most important cereal crops worldwide. It is currently grown on more than 215 million hectares annually and its production is more than 725 million metric tonnes in 2014, the third highest cereal production after maize (1021 million metric tonnes) and rice (740 million metric tonnes) (FAO, 2015). A key factor in the success of wheat as a global food crop is its adaptation to a wide range of climat (IWGSC, 2014).

Wheat is one of the most important cereal crops in Ethiopia extensively cultivated in wide range of altitudes and it ranks fourth next to Teff (*Eragrostis tef*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) in area coverage and third in total production (CSA, 2018). Even though the country is having the potential climate for wheat culture and being the center of diversity for wheat, the average national yield is low (on average 2.1 t/ha) well below the experimental yield of above 5 t/ha (MOA, 2012). Ethiopian farmers rely almost entirely on rain-fed agriculture and seasonal rainfall is volatile in large parts of the country. Over the last fifty years, Ethiopia has experienced 15 drought seasons. Drought has a direct effect on poverty and to attain national food self-sufficiency and rural poverty reduction (Dercon, 2004).

Therefore, it is important to identify drought tolerant wheat varieties which are grown under water stress condition. This study used some morphological, physiological, and biochemical traits under well watered and water stressed condition to help in the selection of drought tolerant varieties which will be better than other varieties under water stress condition.

1.3. Objective of the study

1.3.1. General objective

The general objectives of this study was to assess the effect of soil water stress on some morphological, physiological and biochemical characteristics of durum wheat (*Triticum durum* desf.) and bread wheat (*Triticum aestivum* L.) varieties for drought resistance.

1.3.2. Specific objectives

- To investigate morphological traits (plant height, the spikes length and number of spikelet per spikes) of durum wheat (*Triticum durum* desf.) and bread wheat (*Triticum aestivum* L.) varieties under well watered and water stress condition.
- To determine chlorophyll content and relative water content in leaves of durum wheat (*Triticum durum* desf.) and bread wheat (*Triticum aestivum* L.) varieties under well watered and water stress condition.
- To determine proline and total soluble sugar content in the leaves of durum wheat (*Triticum durum* desf.) and bread wheat (*Triticum aestivum* L.) varieties under well watered and water stress condition.

1.4. Significance of the study

World population is expected to increase over the next years. Ethiopia is one of the countries predicted to account for increases world population. Therefore, there is a pressing need for a significant increase in food production to feed this population. Hence, this study on wheat response to water stress will become increasingly important, as most climate change scenarios suggest an increase in aridity in many areas of the globe. Therefore, outperforming varieties under drought stress conditions can be useful in future wheat breeding programs, and early selection for based on morphological, physiological, and biochemical traits recommended in this study will be important for developing drought-tolerant wheat varieties.

2. LITERATURE REVIEW

2.1. Effects of drought on wheat

The effects of drought range from morphological to molecular levels and are manifested at all phenological stages of plant growth. Environmental stresses trigger a wide variety of plant responses, ranging from changed gene expression and cellular metabolism to changes in growth and productivity. Technological developments have led to climate change resulting in a number of biotic and abiotic stress factors with severe disturbance to ecosystems. Among abiotic stresses, water stress, or water deficiency is the major factor, which causes severe damage to plants. Global warming, less precipitation, low ground water table, and reduction in the level of soil water are the main factors responsible for the development of drought condition in field crops (Mishra and Cherkauer, 2010).

Drought limits plant growth and field crops production more than any other environmental stresses (Almeselmani *et al*., 2011). It remains an ever-growing problem that severely limits crop production worldwide and causes important agricultural losses particularly in arid and semiarid areas. Demirevska *et al.* (2008) concluded that drought affects morphological, physiological and biochemical processes in plants resulting in growth inhibition, stomatal closure with reduction in transpiration, decrease in chlorophyll content and inhibition of photosynthesis. Beltrano and Marta (2008) found that response of plants to water stress depends on several factors such as developmental stage, severity and duration of stress and cultivar. Buchanan *et al.* (2000) explained that during water stress, water is lost from the tissue of the plant which affects the membrane structure and function.

Drought stress is characterized by reduction of water content that diminish relative water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant. The problem is particularly very serious in arid and semi-arid regions of many developing and under developed countries. In these regions, water potential in the rhizospheres more reduced during water stress period which decreases water availability for plant growth and development (Jaleel *et al.*, 2009). All stages of plant growth from seed germination to plant maturation are dependent on water (Manivannan *et al.*, 2008). The decline in plant growth is the most outcome of water unavailability in the plant and the drop of water potential results for retardation of leaf and stem elongation. The inhibition of cell expansion due to turgor loss of cells and reduction of carbon assimilation are the major reasons for reduction in growth as a consequence of drought. Turgor loss due to water stress inhibits turgor dependent activities such as leaf expansion and root elongation (Taiz and Zeiger, 2006).

Wheat plants may be affected by drought at any stage of life, but certain stages such as germination and anthesis growth stages are very critical (Dhanda *et al.*, 2004). Wheat breeders have been successful in increasing yield under productive conditions, but achievement has been uncertain in production regions where environmental stresses occur. Shao *et al.* (2009) explored physiological mechanisms of water stress tolerance for different wheat genotypes as it is essential to find out new drought resistant gene resources. Water is needed by wheat crop for the whole growth duration, but there are some stages which are more liable to water deficiency. Dencic *et al.* (2000) found that water stress during stages before anthesis can reduce number of ear heads and kernels per ear. When water stress occurs during later stages, it causes a reduction in number of kernels/ears and kernel weight (Gupta *et al* 2014). Baser *et al.* (2004) reported the effect of water stress on the yield and yield components of winter wheat and obtained a reduction of about 40% in yield under water stressed conditions as compared to the control.

2.2. Morphological traits of wheat influenced under water stress

Wheat yield under drought stress suffer serious moisture deficit throughout its growth period from seedling to full maturity (Bilal *et al*., 2015). Under drought condition decreasing pattern was experienced in morphological yield contributing characters like plant height (PH), grains per spike (GPS), spikes per plant (SPP), 1000- grain weight (TGW) in wheat. Blum & Pnuel (1990) reported that yield and yield contributing traits of wheat crop were drastically decreased under least annual precipitation. Drought stress leads to reduction in number of fertile tillers per plant, grains per spike and 1000-grain weight (TGW) which ultimately cause noticeably low grain productivity. Relationship between plant height (PH), leaf area, fertile tillers per plant, and grains per spike in wheat has been noticed at booting and anthesis phase which cause improvement in grain yield under water deficit condition (Gupta *et al.* 2014). Blum & Pnuel (1990) investigation depicted that under drought conditions grain filling period and spikelets per spike were associated with high grain production. Therefore, it is suggested that these morphological attributes should be selected for screening diverse wheat genotypes under drought in successful breeding schemes.

Plant height is affected by the association of genetic constitution and environmental conditions of the plant. Several experiments were made to relate plant height and yield under receding soil moisture conditions. Though plant height is genetically controlled, water stress also influences its regulation. Drought stress affects both elongation and expansion of growth due to the low turgor pressure (Bhatt and Srinivasa Rao, 2005). Among the crops, wheat is probably more susceptible to drought stress than most other plant species. Reduction in plant height and stem length reported in wheat crop under drought condition (Wu *et al*., 2008). The decrease in plant height might be due to decrease in relative turgidity and dehydration of protoplasm which is associated with a loss of turgor and reduced expansion of cell and cell division. Manivannan *et al.* (2007) also mentioned that the reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress. The plant height in wheat reduced from 77 cm to 61.66 cm when irrigation was skipped at tillering stage of crop growth (Gupta *et al.* 2014). Abdul Raziq *et al.* (2014) reported that tallness in wheat plants is mostly associated with the genetic makeup of the variety. Therefore it is suggested that these morphological attributes should be selected for screening diverse wheat genotypes under drought in successful breeding schemes. The adverse and unfavorable outcomes of water deficit stress on wheat sensitive stages of crop growth such as reproductive, booting and grain filling stage can be minimized by preventing stress at these stages to develop genotypes showing drought tolerance (Abdul Raziq *et al.*,2014).

2.3. Physiological traits of wheat influenced under water stress

Different types of plant physiological responses have been reported by various Plant physiologists in their findings under drought stress situation. Zaharieva *et al.* (2001) reported that in globally drought affected area; physiological mechanism is very handy approach in evaluating and screening the extraordinary genotypes having drought resistant mechanism. Comprehensive information of physiological mechanisms permits plant researcher to develop promising genotypes that would be utilized efficiently, continue its growth and production under water deficit stage (Ashraf *et al*., 1994).

Plants may survive drought by minimizing its adverse effects that is combating the stress by developing the capacity to tolerate the stress conditions (Puijalon *et al*., 2011). Plants improve their survival by enhancing their root density, and reducing their transpiration rate, low stomatal conductance, leaf rolling, slow wilting, and delayed senescence. The main physiological processes affected by water stress are transpiration, photosynthesis, and stomatal conductance (Anjum *et al*., 2011). Cell water relationship is also affected by water deficiency, as insufficient water may reduce water potential and relative water content in leaf and stem.

2.3.1. Chlorophyll contents

Chlorophyll is one of the major chloroplast components for photosynthesis and chlorophyll content has a positive association with photosynthetic rate. Under drought stress, the decrease in chlorophyll content has been considered as a typical side effect of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are responsible for soil dehydration (Farooq *et al.,* 2009). Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought (Zhang *et al.,* 2002).

Higher leaves chlorophyll contents is significantly correlated with photosynthesis and regarded as encouraging selection trait in crop productivity (Teng *et al.,* 2004). It has been reported in many studies that under water stress photosynthesis exhibit direct relationship with wheat grain production because less stomata opening frequency and low amount of CO_2 fixation lead to reduction in photosynthetic amount (Mafakheri *et al.*, 2010). Severe water stress restricts the photosynthesis by damaging the chlorophyll contents (CC) and changing the photosynthetic machinery (Iturbe-Ormaetxe *et al.*, 1998). Decreased photosynthetic amount under water stress condition is an outcome of inhibition of RuBisCO (ribulose-1, 5-bisphosphate carboxylase/oxygenase) enzyme activity and development of ATP (Dulai *et al.,* 2006).

There are reports about decrease of chlorophyll in the water stress conditions. Almeselmani *et al*. (2011) reported that chlorophyll content of resistant and sensitive cultivars reduced in response to drought and thermal stress. Chlorophyll maintenance is essential for photosynthesis under water stress and higher chlorophyll content and lower percent of reduction under stress in tolerant varieties of wheat has also been reported (Farooq *et al.*, 2009). According to Manivannan *et al*. (2008), chlorophyll is one of the major chloroplast components for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate and flag leaf chlorophyll content is an indicator of the photosynthetic activity and its stability for the conjugation of assimilate biosynthesis.

Many studies have been reported that under water stress, photosynthesis shows a direct relationship with wheat grain production because less stomata opening frequency and low amount of CO_2 fixation lead to the reduction of amount in photosynthesis (Mafakheri *et al.*, 2010). A severe water deficit stress restricts the photosynthesis by damaging the chlorophyll components (CC) and changing the photosynthetic machinery. Inhibition of RuBisCO (ribulose-1, 5-bisphosphate carboxylase/oxygenase) enzyme activity and development of ATP decreased photosynthetic amount under water stress condition (Dulai *et al.*, 2006).

2.3.2. Relative water content

Leaf relative water content (RWC) is very frequently taken as substitute to the measure of plant water status, thereby serving as measure of the metabolic activity level within the tissues (Taiz & Zeiger, 2006). Relative water content of the plants growing under water stress was significantly low in comparison to the well watered plants. Momentous pattern of divergence can be observed in relative water content (RWC) among diverse genotypes during various plant growth stages. Under water stressed condition, decrease in water status and osmotic potential in plants is the ultimate outcome of lower relative water content. Osmoregulation mechanism plays a phenomenal role in preserving turgor pressure which helps in soil water absorption and continue plant metabolic activities for its survival.

During drought stress, the relative water content (RWC) and water potential of the plants decrease (Bajji *et al*. 2001), leading to a significant decline in photosynthetic CO_2 fixation. The reduction in the net CO_2 assimilation rate is mainly due to stomatal closure, which is the most efficient way to reduce water loss (Cornic, 2000). When the stomata are closed, so that the conductance of water vapors through the stomata is low, the diffusion of CO_2 into the leaves is limited, resulting in a decrease in the leaf intercellular CO_2 concentration (Cornic 2000). However, during drought stress, the diffusion of CO2 from the leaf intercellular spaces to the chloroplast of the mesophyll cells may also be reduced depending on the structure of the mesophyll cell and the rearrangement of the intercellular spaces (Loreto *et al*., 2003).

2.4. Biochemical response of plants to water stress

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 (Oliveira *et al*., 2012). Osmotic adjustment accomplished by accumulation of various solutes such as sugar, organic acids, amino acids (proline) and also inorganic ions (Oliveira *et al.*, 2012). Zivcak *et al.* (2009) stated that osmotic adjustment play 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 supply of water. 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. Genotypes that have higher osmotic adjustment ability were less affected than those with lower ability of osmotic adjustment. Also yield of wheat genotypes with higher osmotic adjustment showed lower yield reduction as a result of drought induced than those with lower level of osmotic accumulation (Zivcak *et al.*, 2009).

2.4.1. Proline

Plants accumulate various solutes like proline, sucrose, soluble carbohydrates, glycine, betaine, and others to maintain turgidity in the cell wall under drought stress (Rhodes and Samaras, 1994). Proline, being an essential amino acid, plays a key role in maintaining the osmotic potential of cells and protecting them from severe dehydration (Anjum *et al*., 2011). Higher levels of proline help in overcoming the adverse effects of drought and it also minimizes the damage caused by ROS to cells. Proline level is associated with the ability of plants to tolerate drought; hence, the higher the level of proline, the more tolerant plant will be to drought. Compared to any other amino acid present in plants, proline is the one that is accumulated at far higher levels under water stress (Ghaderi and Siosemardeh, 2011). Proline works as a signaling molecule to regulate mitochondria, cell production and cell death.

Proline production is triggered by various types of stresses and it plays a great role in drought tolerance, but the accumulation depends on the type and intensity of the water stress. Oraki *et al*. (2012) reported that proline content increase with drought stress especially with severe water stressed condition. Results from Moayedi *et al*. (2009) showed that accumulation of proline depends on the developmental stage of the plant. The amount of proline produced increased by 22 %, 47 % and 114 % by water stress which occurs from one leaf stage to floral initiation; from floral initiation to anthesis and after anthesis, respectively. The accumulation of proline in plant tissues is a clear indication of plants inherent ability of tolerance against environmental stress, particularly in plants under drought stress. Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al*., 2002). Therefore, the more the proline, better the ability of plants to withstand drought, which is one of the most common compatible osmolytes in drought stressed plants.

2.4.2. Total soluble sugar

Accumulations of soluble sugars in plants give drought tolerance ability and protect cells from damage in two ways. Sugars create hydrophilic interaction with membrane protein because of their OH group; these prevent protein denaturation during desiccation. Also sugars form biological glass in the cytoplasm of dehydrated cell and this can prevent the movement of reactive compounds in the cell and extend the life of dehydrated tissue (Oraki *et al*., 2012). Under severe water stressed condition, total soluble sugars play a great role in osmotic adjustment (Oliveira *et al*., 2012). Mohammadkhani and Heidari (2008) reported that the seedling of maize variety showed an increase in total soluble sugar content as the water potential becomes lower. Keyser (2010) reported that soluble carbohydrate of flag leaf obtained from drought stress at grain filling stage is higher in cultivars of wheat than the amount on other stages.

3. MATERIALS AND METHODS

3.1. Description of the study area

The pot experiment was carried out at Kulumsa Agricultural Research Center (KARC) from March to July, 2019 under lath house conditions. The site is located at 8°08' N and 39°08' E at an elevation of 2200 masl in Arsi Administrative Zone of Oromia Regional State, 167 km southeast of Addis Ababa. The agro ecology of the study area is characterized as cool highland to semi-arid. The area has a uni-modial kind of annual rainfall, which extends from March to September; however, the peak season is from July to August. The data obtained from the weather station located at KARC revealed that the area receives a mean annual precipitation of 809 mm whereas the mean maximum and minimum temperature are 23.08°C and 9.9°C, respectively. The dominant soil type of the study area is vertic luvisol (IUSS Working Group WRB, 2014).

3.2. Experimental design and treatments

The pot experiment was designed to characterize drought resistant bread and durum wheat varieties morphologically, physiologically and biochemically. The experiment had two factors; twelve varieties and two water management combined factorially, which made up a total of 24 treatments, and was arranged in RCBD with three replications. The six bread wheat (*Triticum aestivum* L.) varieties were (Wane, Danka, Kakaba, Ogolcho, Paven-76 and Kingbird,) whereas the six durum wheat (*Triticum durum* desf.) varieties were Donmatia, Mangudo, Utuba, Alemtena, Ude and Mukuye. The bread and durum wheat varieties were collected from KARC and Debrezeit Agricultural Research Center (DARC), respectively.

The 12 wheat varieties were planted in pots with capacity of 5 liters each. The soil was collected from the nearby site where the experiment was conducted in the lath house. The soil was air-dried, sieved to pass through a 2-mm mesh, and its residual moisture content (17%) and bulk density (1.05 g cm^{-3}) were determined prior to repacking. Each pot was repacked with 4.5 kg air-dried soil. The repacked soil was saturated with water and kept overnight. The next day, 16 March 2019, a total of 15 and 10 seeds of bread and durum wheat respectively were sown in each pot. The germinated wheat were thinned to 10 and 8 plants of bread and durum wheat, respectively after emergence to meet the recommended seed rate of wheat for sowing (125 kg ha^{-1}). The numbers of seedlings per pot were attained considering the area of the pot (334 cm^2) and the mean TKW of six bread wheat (41.08 g) and six durum wheat (54.25 g) varieties. Based on the recommended package for wheat, 0.53 g nitrogen (N) and 0.50 g phosphorous (P) fertilizers from urea and triple super phosphate per pot were applied at sowing, which were equivalent to 73 and 30 kg N and P ha^{-1}, respectively.

The water management under well-watered treatment was equivalent to 100% FC and was measured according to Khakwani *et al*.(2011). The 100% FC was equivalent to replenishing the same amount of water lost in the form of evapotranspiration per a certain period. The water stress was induced by refilling the soil to only 30% FC. The application of water treatment was started after germination of the crop continued until maturity.

Table 1 : Weather variables and reference evapotranspiration of wheat in the study area during March – July 2019.

Month	Max Temp (°C)	Min Temp (°C)	Relative humidity (%)	wind speed (m s^{-1})	Sunshine (hrs)	ETo (mm day^{-1})
March	28.4	12.8	34	1.0	8.2	4.64
April	26.4	13.2	51	0.7	6.2	3.83
May	26.0	12.8	48	1.1	8.0	4.46
June	24.2	13.1	69	0.8	5.9	3.45
July	22.9	12.3	65	0.5	5.0	3.13

Note: Max Temp is average maximum temperature; Min Temp is average minimum temperature and ETo is average total reference evapotranspiration.

The irrigation water requirement for the 100% FC, that is the amount of water that had to be applied to replenish the crop's evapotranspiration without significant reduction in yield was, therefore, computed based on the CWR of the test crop per its stages of growth and weather conditions. The 30% FC was calculated once the 100% FC value was obtained. Irrigation water was applied every four days since germination to physiological maturity.

3.3. Data collection

The morphological and physiological data were collected (computed) from the pot experiment at KARC whereas the biochemical test in the laboratory experiment was carried out at Melkassa Agricultural Research Center (MARC).

3.3.1. Evaluation of morphological traits

The morphological traits measured for this experiment included plant height (PH), spike length (SPL) and number of spikelet per spike (SPS). The measurement of each of the morphological traits was recorded from the randomly selected three sample plants from each pot at physiological maturity and the values were expressed as a mean per each pot. The PH was measured using a meter scale from the surface of the pot (soil) to apex of the plant excluding awns. The SPL was measured using a meter scale from the base of spike to its tip excluding awns. The SPS was counted from three plants randomly selected and the mean value was taken.

3.3.2. Evaluation of physiological traits

3.3.2.1. Determination of chlorophyll content

The relative amount of chlorophyll present in wheat leaves (chlorophyll index), is an indicator parameter to predict nitrogen fertilization in wheat, was measured with a SPAD 502 meter (Konica Minolta, Inc.) just after anthesis stage from the randomly selected three sample flag leaves of the plant from each pot. The SPAD-502 meter is a small portable device employed for quick, accurate and non-destructive measurements of relative chlorophyll concentrations in plant leaves (Richardson *et al*., 2002). The technique instantly measures the CC of the leaves by simply clamping the meter over the leaf and obtaining the reading on a scale. The measurement values were expressed as a mean of three individual SPAD meter readings per pot.

3.3.2.2. Determination of relative water content

The RWC was determined according to procedure of Schonfeld *et al.* (1988) on one fully expanded leaf per pot. The leaf from each pot was cut at the base of lamina, preserved within a polyethylene plastic bag and immediately brought to laboratory. The fresh weight of leaf was weighed immediately after reaching at the laboratory using a sensitive balance (precision = 0.01 g) and the turgid weight was measured after saturating the leaf within distilled water for 24 h at room temperature. After saturating, the leaf was carefully transferred to a tissue paper and its dry weight was recorded after drying it in an oven at 70°C for 24 h. RWC was calculated by using the following formula.

$$\text{RWC (\%)} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} * 100$$

Where, FW is the weight of freshly collected leaf, TW is the weight after rehydration (saturation) for 24 hours at room temperature and DW is the weight after drying the leaf at 70°C for 24 hours.

3.3.3. Evaluation of Biochemical traits

3.3.2.1. Proline determination

Analysis of proline in the leaves was measured following method of Bates et al. (1973). The reagents used for this analysis were (1) Acid-ninhydrin, which was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid, with agitation, until dissolved and kept cool (stored at 4°C), (2) extraction buffer 3% (w/v) aqueous sulfosalicylic acid, (3) glacial acetic acid, (4) toluene and (4) L-Proline reagent was prepared.

In order to examine the amount of proline content in response to water stress, analysis of leaf samples was done at flowering stage after water stress was induced. Fresh plant sample (0.5 g) was homogenized with 10 ml of 3% aqueous sulfo salicylic acid and the homogenate was filtered using Watman No. 2 filter paper. The filtrate was then used for proline concentration determination. Two milliliter ml of the filtrate was mixed 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°C. Then, it was allowed to be cooled, producing reddish 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. Chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction was separated from aqueous phase to other test tube and was warmed at room temperature and absorbance was read at 520 nm by spectrophotometer. The proline concentration was determined from the standard curve constructed from the known concentration of proline, and was expressed in µmol of proline per g fresh weight of the leaf and calculated on a fresh weight basis as follows.

$$Proline\ content = \frac{\left[(\mu g\ proline^{-ml}) * \frac{ml\ toluene}{115.5\ \mu g^{-\mu mole}}\right]}{\left[\frac{g\ sample}{5}\right]}$$

Where the content of proline was expressed in units of µmol/g fresh weight of leaves (µmol/g FW).

3.3.2.2. Total soluble sugar

Total soluble sugar in the leaf samples was determined according to DuBois *et al*. (1956) with some modifications (Tiwari *et al*., 2016). Reagents used in this analysis was 5% phenol solution, which was prepared by mixing 5 g of phenol in 95 ml of double distilled water (DDW), and 90% ethanol, which was prepared by mixing 90 ml of ethanol and 10 ml of DDW. A 0.25 g of fresh leaf material was kept in 10 ml of 90% ethanol for 1 h at 60°C in a water bath. The extract was then filtrated using filter paper into a volumetric flask and the residue was re-extracted. Final volume was made up to 25 ml by adding 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 95% sulfuric acid was added to it and mixed thoroughly by vertical agitation. For exothermic reaction, the test tube was cooled in the air (Appendix 5). Absorbance was recorded at 490 nm on spectrophotometer and soluble sugar was calculated from standard glucose graph.

$$\text{Total soluble sugar}\left(\frac{\text{mg}}{\text{g}}\right)=\frac{\text{Sugar value from graph }(\mu\text{g})}{\text{Aliquot sample used}} * \frac{\text{Total volume of extract}}{\text{Weight of sample}}\text{x } 100$$

3.4. Data analysis

The wheat physiological, morphological and biochemical data were subjected to two-way analysis of variance (ANOVA) using Statistical Analysis System (SAS) version of 9.0 (SAS Institute Inc., Cary NC, USA). Means comparison for treatments with significant effects was performed using the least significant difference test at 5%. Simple correlation analysis between traits was also carried out using the same statistical tool. Graphs were made using Microsoft Excel 2010 (Microsoft® Corp., Redmond, Washington, USA).

4. RESULTS AND DISCUSSIONS

Analysis of variance (ANOVA) indicated that there were significant differences ($p<0.05$) among the bread and durum wheat varieties in terms of the studied morphological, physiological and biochemical characters (Table 2). The ANOVA also indicated that water stress negatively affected all of the studied morphological, physiological and biochemical traits of the wheat varieties (Table 2). Since there were no significant interaction effects between variety and water management in all traits the main effects was only variety and water management.

Table 2: ANOVA table for the influence of varieties and water management on the morphological, physiological and biochemical characteristics of wheat.

Sources of variation	**Plant height (cm)**	**Spike length (cm)**	**Spikelet No spike^{-1}**	**Relative water content (%)**	**Chlorophyll content (SPAD unit)**	**Proline (µmol g^{-1})**	**Total Soluble Sugar (mg/g)**
Rep	*	Ns	Ns	***	ns	ns	ns
Variety (V)	Ns	***	***	Ns	***	***	***
Water management (WM)	***	***	***	***	***	***	***
V*WM	Ns	Ns	Ns	Ns	ns	ns	Ns
Mean	**56.23**	**5.25**	**10.69**	**69.52**	**44.22**	**3.43**	**48.34**
CV	8.07	10.8	9.4	15.54	9.64	17.99	5.28
LSD_V	5.27	0.66	1.16	12.56	4.95	0.71	2.96
LSD_{WM}	2.15	0.27	0.47	5.12	2.02	0.29	1.21

Note: ns, * and *** indicated not significant at $p < 0.05$, significant at $p < 0.01$ and significant at $p < 0.001$, respectively. CV= coefficient of variation; LSD_V = list significant difference of varieties; LSD_{WM} = list significant difference of water management

4.1. Morphological, physiological and biochemical characteristics of wheat as influenced by water stress

4.1.1. Morphological traits of wheat as affected by water stress

The influence of water stress on the morphological, physiological and biochemical characteristics of wheat varieties has been shown in Table 3. Water deficit adversely affected the plant height (PH), spike length (SPL) and number of spikelet per spike (SPS). Overall, the mean PH of wheat was very stunted under water stress (48 cm) compared to the well-watered treatment (65 cm). Water stress was highly significantly ($p < 0.001$) and affected the mean SPS and SPL of wheat varieties. Compared to the well-watered treatment (12.0), the mean number of spikelet per spike was lower by 2.7, which was 23% reduction (Table 3). Similarly, the SPL of wheat varieties was found shorter under water stressed (5 cm) in contrast to the well-watered treatment (5.5 cm).

The stunted growth in the wheat varieties could ascribe to the decrease in relative turgidity and dehydration of the protoplasm, which in turn associated to the loss of turgor as well as the reduced division and expansion of cell. Similar findings have been reported by Manivannan *et al*. (2008) that the stunted growth attributed to the decline in the cell enlargement and more leaf senescence in the plant under water stressed condition.

4.1.2. Physiological characteristics of wheat as affected by water stress

The relative water content (RWC) estimated for the wheat varieties under controlled and water stressed condition was shown in Table 3. In this study, the RWC was determined to give an indication on the plant water status under drought condition. As expected, water stress resulted in significantly lower ($p < 0.001$) RWC under water stressed condition (63.54 % RWC) compared to the control treatment (75.51 % RWC), which was found inferior by 11.97 %. During water stress, the water balance of plants is disrupted, as a result of which the RWC and water potential of leaves decrease (Bajjii *et al*., 2001). Decreased RWC with water stress in all the tested varieties were also reported in common bean (Korir *et al*., 2006).

Water stressed was also very highly significantly ($p < 0.001$) influenced the chlorophyll content (CC) of wheat varieties. Deficiency in water availability (41.62 SPAD units) brought significant reduction in CC in contrast to the well-watered treatment (46.83 SPAD units) (Table 3), which was found inferior by 11%. This finding is in similar with the work of Araus *et al*. (1998) who reported that drought treatment caused a 20% reduction in leaf chlorophyll content. The reduction in CC under the water stressed treatment could attribute to destruction of the chlorophyll and deterrence of its synthesis. Several researchers including Lessani and Mojtahedi (2002) reported the damage of leaf pigments because of water deficit. A further reason for the diminution in chlorophyll content in response to water stress was that drought or heat stress as a consequence of the production of reactive oxygen species (ROS) such as O^{2-} and H_2O_2 can lead to lipid peroxidation and thus, chlorophyll destruction (Foyer *et al*., 1994). Also, with decreasing chlorophyll content due to the changing green color of the leaf into yellow, the reflectance of the incident radiation is increased (Schelmmer *et al*., 2005). It is also supported by the findings of Sikuku *et al*.(2010) noting the inhibition of chlorophyll synthesis and inability of wheat to withstand water deficit.

High chlorophyll content is a desirable characteristic because it indicates a low degree of photo inhibition of photosynthetic apparatus; therefore, reducing carbohydrate losses for grain growth (Farquhar *et al*., 2009). Chlorophyll is one of the major components of chloroplast, which helps for photosynthesis (Manivannan *et al*., 2007). CC has a positive relationship with photosynthetic rate, and the flag leaf chlorophyll content is an indicator of the photosynthetic activity and its stability for the conjugation of assimilate biosynthesis. Several researchers have successfully employed this trait for screening and selection of drought tolerance in wheat cultivars (Almeselmani *et al*., 2011). Finally, the assessment of photosynthetic pigments and consequently their relationships is an important indicator of senescence (Brown *et al*., 1991).

4.1.3. Biochemical characteristics of wheat as affected by water stress

Proline was significantly ($p < 0.001$) affected by water stress (Table 3). ANOVA revealed significantly higher amount of PRO under water stress (3.85 µmol g^{-1}) than well-watered condition (3.01 µmol g^{-1}). This implies that the tested varieties produced significantly higher amount of PRO under water stress than well-water condition as a mitigation strategy to cope up with the drought condition. The accumulation of proline in plant tissues is a clear indication of plants inherent ability of tolerance against environmental stress, particularly in plants under drought stress. Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al*., 2002). Therefore, the more the proline, the better ability of plants to withstand drought, which is one of the most common compatible osmolytes in drought stressed plants.

Total soluble sugars was also significantly ($p < 0.001$) affected by water stress (Table 3). ANOVA revealed significantly higher amount of TSS under water stressed (49.84 mg/g) than well-watered condition (46.84 mg/g). This implies that the tested varieties produced significantly higher amount of TSS under water stress than well-water condition as a mitigation strategy to cope up with the droughty condition. Leaf osmotic adjustment via soluble sugars has been a well-known mechanism to resist water stress in many plants (Wu and Xia, 2006). This is because the synthesis of soluble sugars plays great role in osmotic adjustment for drought tolerance. According to Zhang *et al*. (2002), solutes are accumulated during stress serve as an aid to cope with the existing condition.

Table 3: Mean comparison of the Plant height, Spike length. Spikelet No spike-1 Relative water content, Chlorophyll content Proline and Total Soluble Sugar characteristics of wheat varieties under well water and water stress condition.

Sources of variation	Plant height (cm)	Spike length (cm)	Spikelet No spike^{-1}	Relative water content (%)	Chlorophyll content (SPAD unit)	Proline (µmol g^{-1})	Total Soluble Sugar (mg/g)
Well watered	64.6^{a}	5.5^{a}	12.0^{a}	75.51^{a}	46.83^{a}	3.01^{b}	46.84^{b}
Water stress	47.9^{b}	5.0^{b}	9.29^{b}	63.54^{b}	41.62^{b}	3.85^{a}	49.84^{a}
Mean	**56.23**	**5.25**	**10.69**	**69.52**	**44.22**	**3.43**	**48.34**
CV	8.07	10.8	9.4	15.54	9.64	17.99	5.28
LSD_{WM}	2.15	0.27	0.47	5.12	2.02	0.29	1.21

4.2. Morphological, physiological and biochemical characteristics of wheat as influenced by varieties

4.2.1. Morphological traits of wheat as affected by varieties

The ANOVA indicated that there were marginally significant ($p < 0.05$) differences among the tested varieties related to the plant height (PH). The tallest plant (61.1 cm) was recorded from the Ude variety; however, this result was statistically similar to Ogolcho (58.4cm), Danka (58.2 cm) Mangudo (49.69 cm), paven-76 (56.4 cm) and Utuba (56.3 cm) (Figure 1) varieties. The shortest wheat variety was noted from Donmatia variety (50.8 cm) (Figure 1). Nevertheless, this result found statistically similar to Wane (54.08 cm), Kakaba (54.18 cm) Mukuye (55.5 cm), Kingbird (55.6 cm) and Alemtena (55.8 cm) varieties. The difference in PH of the varieties should be attributed to the difference in their genetic makeup (Jemal *et al*., 2015). On top of the genetic makeup of a variety, environmental factors can also control the PH of plants (Shahzad *et al*., 2007). Manivannan *et al*., (2007) also mentioned that the reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress. The plant height in wheat reduced from 77 cm to 61.66 cm when irrigation was skipped at tillering stage of crop growth (Mushtaq *et al*., 2011). These results are in agreement with the findings of Bayoumi *et al*., (2008) who observed that drought caused reduction in plant height by 14.7 %.

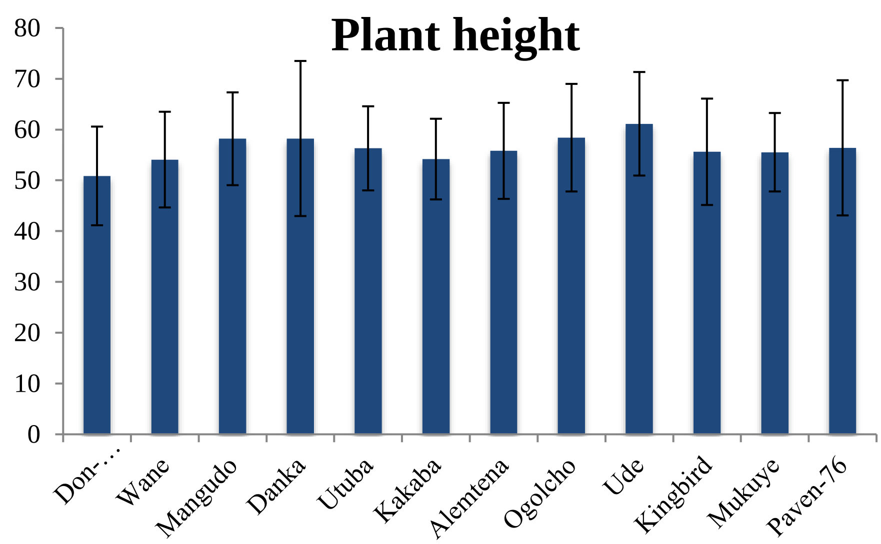


Figure 1: Mean result of plant height differences among bread wheat and durum wheat varieties under water stress.

The statistical analysis results revealed that spike length was significantly ($p<0.001$) affected by water stress and varieties (Table 2; Figure 2). The lengthiest and statistically superior SPL (6.9 cm) was recorded from Ogolcho variety (Figure 2). However statistically lesser compared to the Ogolcho variety, the next longest SPL was recorded from King bird (6.2 cm), Danka (6.1 cm), Paven-76 (5.9 cm) and Kakaba (5.6 cm) varieties, which were statistically similar to each other. The shortest SPL was recorded from Mukuye variety (4.2 cm), however, it was statistically similar to Ude (4.4 cm), Alemtena (4.5 cm), Utuba (4.5 cm), Mangudo (4.7 cm) and Wane (4.9 cm) varieties. Compared to Ogolcho, the SPL of Mukuye, Ude, Alemtena, Utuba, Mangudo and Wane varieties were found shorter by 39%, 37%, 36%, 35%, 32% and 29%, respectively. The varietal difference in spike length is governed by genetic makeup of the varieties and the environmental effect (Shahzad *et al.*, 2007). This result is in agreement with Jemal *et al*. (2015) who reported that with increasing the stress, the spike length was declined by 8.57%. Many researchers have reported similar effect of irrigation on spike length in wheat (Ahmad, 1994).

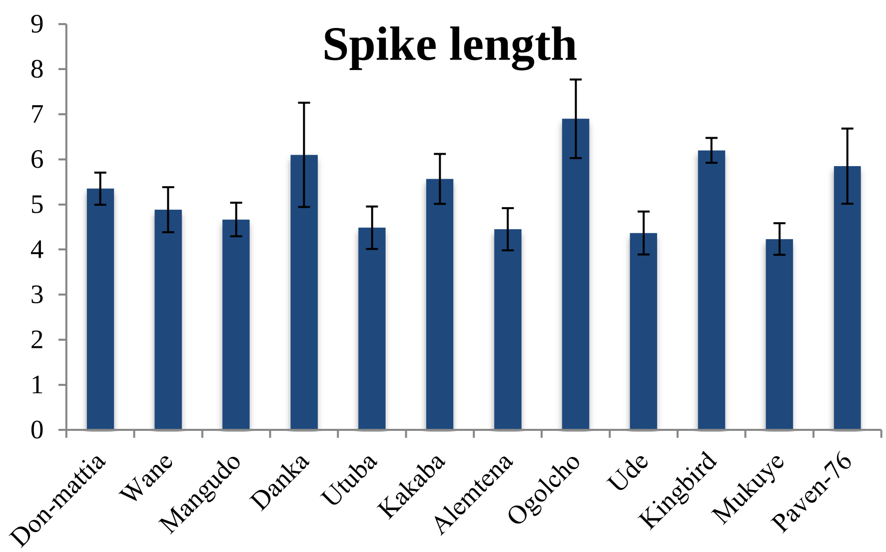


Figure 2: Mean of the spike length among bread wheat and durum wheat varieties.

Likewise, there were very highly significant ($p < 0.001$) differences among the tested varieties regarding the SPS (Table 2; Figure 3). The maximum number of SPS (12.16) was recorded from the Donmatia variety; however, this result was statistically similar to the Alemtena (11.95), Kingbird (11.38), Mangudo (11.16), and Danka (11.1) varieties (Figure 3). The lower most number of SPS was noted from the Wane (8.55) variety. Nevertheless, this result found statistically similar to Kakaba (8.81) variety (Figure 3). Wane and Kakaba varieties yielded 29.68 and 27.54 % lower SPS in contrast to the Donmatia variety. The potential of wheat spike is determined by the spikelet per spike, which is an important yield component of grain yield. The result of this study was consistent with the findings of Rahim *et al.* (2012) who reported that water stress produced significantly decreased number of grains (kernels) per spike.

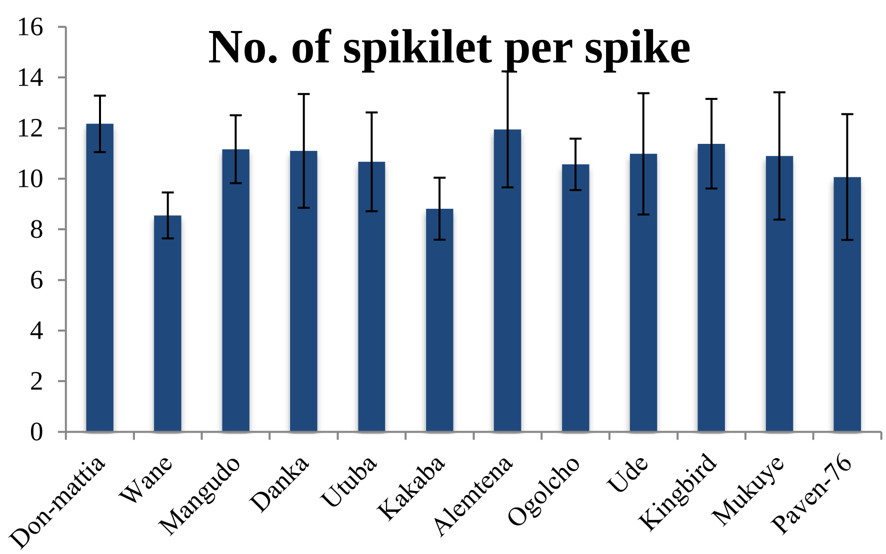


Figure 3: Mean of the number of spikelet per spike among bread wheat and durum wheat varieties.

4.2.2. Physiological characteristics of wheat as affected by varieties

Considering the relative water content (RWC), ANOVA did not present statistically significant difference ($p < 0.05$) in the tested varieties (Table 2 and Figure 4). The only difference regarding the RWC was in Ogolcho (60.41%) varieties. That variation in RWC might attribute to the differences in the abilities of the varieties to absorb more water from the soil and/or the ability to control the water loss through their stomata. The RWC values in control and water deficit treatments were comparable with those reported by Abdul Raziq *et al*. (2004). Water deficit caused a 36.7% reduction of the RWC of the leaves but genotypes were varied in maintaining their RWC under both water deficit and well-watered conditions. During drought stress, the water balance of a plant is disrupted and as a result of which the RWC and water potential of leaves decreased (Bajjii *et al*., 2001). Changes in the RWC of leaves are considered as a sensitive indicator of drought stress and more useful integrator of plant water balance than the leaf water potential (Strauss and Agenbag, 2000).

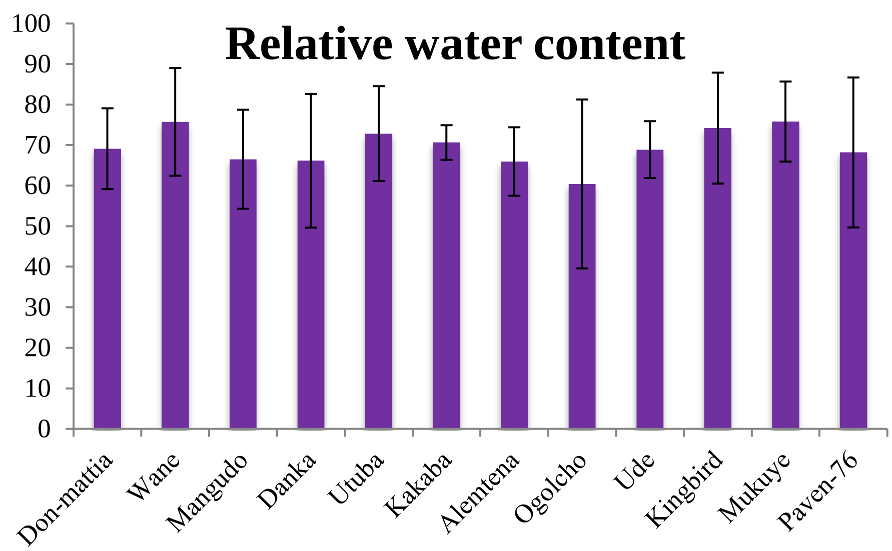


Figure 4: Mean result of relative water content in the leaves of bread wheat and durum wheat varieties.

The result indicated that chlorophyll content (CC) differed significantly ($p < 0.001$) among varieties (Table 3). The highest CC (49.55) was recrded from the Mangudo variety (Figure 5). Statistically similar CC was also noted from the Donmattia, Alemtena, Mukuye, Utaba and Ude varieties, which were (49.13), (47.58), (47.03), (46.48) and (44.67), respectively (Figure 5). Conversely, the lowest CC was recorded in Danka variety (39.38), which were also statistically similar to Paven-76 (39.38), Kakaba (40.88), Kingbird (41.15), Ogolcho (42.52) and Wane (42.92) varieties. Compared to the Mangudo variety, the RCC measured from the Danka, Paven-76, Kakaba, Kingbird, Ogolcho and Wane varieties were lesser by 20.5%, 20.5%, 17%, 17%, 14%, and 13%, respectively. These findings was in agreement with Araus *et al.* (1998) who reported that drought treatment caused a 20% reduction in leaf chlorophyll content. The result was supported by the findings of Sikuku *et al*. (2010) noting the inhibition of chlorophyll synthesis and inability of sensitive wheat to withstand water deficit. High chlorophyll content is a desirable characteristic because it indicates a low degree of photoinhibition of photosynthetic apparatus, therefore reducing carbohydrate losses for grain growth.

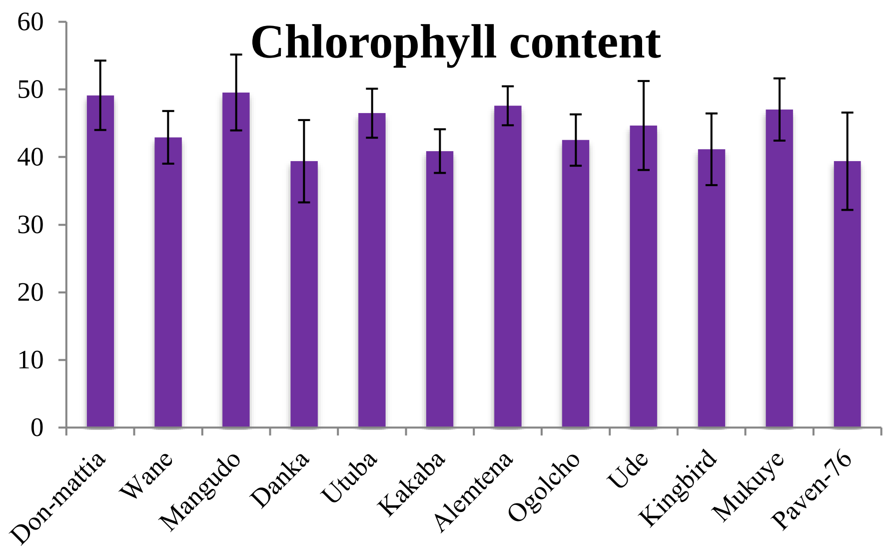


Figure 5: Mean result of chlorophyll content in the leaves of bread wheat and durum wheat varieties.

4.2.3. Biochemical characteristics in wheat as affected by in response to varieties

All varieties produced significantly ($p < 0.05$) higher amount of proline content (PRO) due to water stress at varying extents. ANOVA presented significant differences among the tested varieties concerning the production of PRO when exposed to stress. Utuba variety yielded the highest PRO (4.78 µmol g^{-1}) followed by Alemtena (4.65 µmol g^{-1}), Don-mattia (4.36 µmol g^{-1}) and Mukuye (4.28 µmol g^{-1}) varieties, respectively (Figure 6). The lowest PRO was produced by Danka variety (2.21 µmol g^{-1}). However, this result was found statistically similar to Ogolcho (2.25 µmol g^{-1}), Wane (2.75 µmol g^{-1}) and Paven -76(2.9 µmol g^{-1}) varieties (Figure 6). Overall, these varieties yielded 53.9%, 52.9 %, 42.4% and 39.3% reductions in the PRO production compared to Utuba under water stressed condition respectively. Varieties which had high PRO content might increase ability to synthesize osmotic regulators for protection from the damage of soil water deficits. Furthermore, PRO may play a role as an enzyme-stabilizing agent and has the ability to mediate osmotic adjustment, stabilized sub-cellular structure and scavenge free radicals (Hassanein, 2004). Proline is one of the osmoprotectant that accumulate in a lot of crops in response to different kinds of stresses including drought (Mafakher *et al*., 2010). According to Taize and Zeiger (2006), proline is a compatible solute which plays a great role in osmotic adjustment through osmotic balance in cytoplasm and vacuole and protection of membrane and proteins from oxidative damage during water stress (Schulze *et al*., 2005).

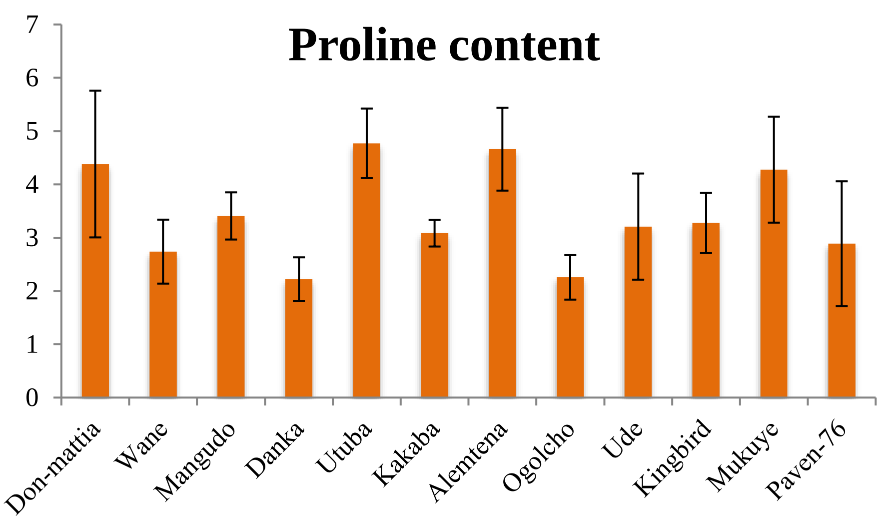


Figure 6: Mean result of proline content (µmol/g fresh weight) in the leaves of bread wheat and durum wheat varieties.

All varieties produced significantly ($p < 0.05$) higher amount of total soluble sugar (TSS) due to water stressed at. ANOVA presented significant differences among the tested varieties concerning the manufacture of TSS when exposed to stress. Ogolcho yielded the highest TSS (51.32mg/g) followed by Utuba (51.05mg/g), Alemtena (49.6mg/g), Kakaba (49.6mg/g) and Kingbird (49.03mg/g) varieties, respectively (Figure 7). The lowest TSS was manufactured by Mangudo variety (44.1). However, this result was found statistically similar to Mukuye (46.86) and Paven-76 (46.9) varieties, respectively (Figure 7). Overall, these varieties yielded 8.6% - 14% reductions in the TSS production compared to Ogolcho variety under stressed condition.

These results were in accordance with Irigoyen *et al*. (1992), who reported that the sugar content in leaves of the plant could increase under drought conditions. Moreover, one of the mechanisms plants use to withstand water stress is by regulating osmotic potential of the cell, especially if water stress increase gradually from mild stress to severe one (Lipiec *et al*., 2013). Osmotic cell potential can be adjusted by increasing the concentration of TSS, which can decrease water potential of cells without inhibiting the function of the enzyme and does not reduce turgidity of the cell. Sugar accumulation in water stressed conditions helps to maintain the stability of the membrane prevents and protects membrane fusion, and keeps protein so as to remain functional (Arabzadeh, 2012; Lipiec *et al*., 2013).

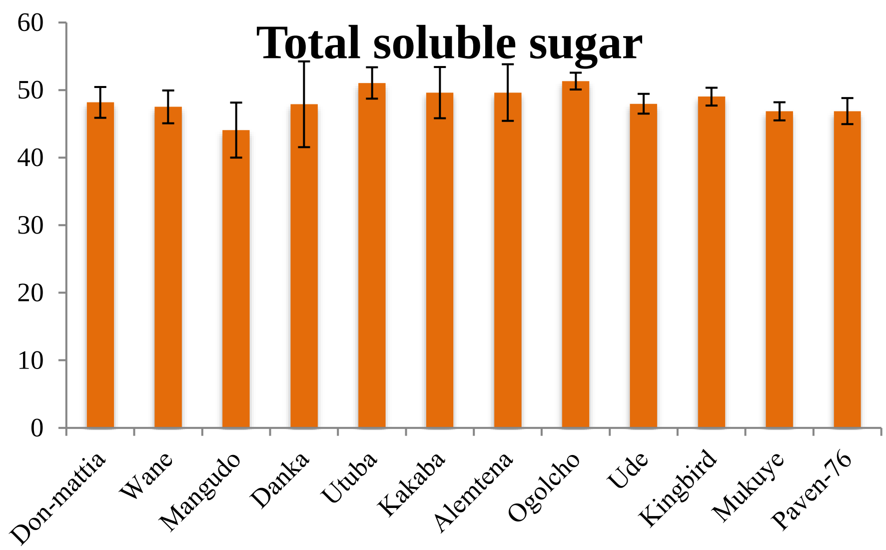


Figure 7: Mean result of soluble sugar content (mg/g fresh weight) in the leaves of bread wheat and durum wheat varieties.

5. CONCLUSION AND RECOMMENDATIONS

In the present study, plant height was not significantly affected by water stress, however it was significantly but negatively correlated with proline content and total soluble sugar. Solutes were accumulated during stress, and served as an aid to cope with the existing condition. In general, the result of proline and total soluble sugar content with the highest value was obtained in the drought stress treatment and the lowest in the well water treatment. The highest proline content in drought stress treatment (30% FC) was obtained in the variety of Utuba (4.78 μmol g^{-1} FW), and the lowest in variety of Danka (2.21 μmol g^{-1} FW). Whereas, the total soluble sugar content was the highest in Ogolcho (51.31 μmol g^{-1} FW) and the lowest in Mangudo (44.1 μmol g^{-1} FW). The differences in the synthesis of proline and total soluble sugar with an increase in drought stress among the selected wheat varieties showed that each variety has different ability to cope up with stresses. This difference in the responses is also likely to be due to the genetic differences among the varieties.

Based on the study the following recommendations were made:

- Further work is required on the molecular identification the genes controlling the morphological, physiological and biochemical traits

- Since the current research was done under controlled condition, the reliability of observed traits should be studied under field and different environmental condition to solve the problem in water stress. Therefore, it is of paramount importance to select and recommend varieties for the area.

- Among the parameters, the present findings revealed that plant height and relative water content was not significantly affected ($p > 0.05$) by water stress among the varieties that it will recommended to replace with other traits to see their effects towards water stress.

- Soluble sugar content and proline content can be used as potential biochemical traits to select drought tolerant wheat varieties.

6. REFERENCES

Abd-El-Haleem, S., Reham, M.A. and Mohamed, S.M. (2009). Genetic Analysis and RAPD polymorphism in some Durum Wheat Genotypes. *Global J of BiotechnoL and Biochem.* **4**: 1-9.

Abdul Raziq, S., Sana U., Shahbaz K., Baber M., Waseem B., Hafeez N., Rameez A, Abdul H, Salih A., Kamran R., Ayaz A. and Ashraf M. (2014). Influence of Seed Size on Germinability and Grain Yield of Wheat (*Triticum aestivum* L.) Varieties. *J Natural Sci Resch.* **4** (23): 124-42.

Ahmad, J. (1994). Effect of varying irrigation frequencies on phenology, growth and yield of three wheat varieties. *J. Exp. Biol.* **208**:2819-2830.

Ali, Y., Manzoor, B., Akhter, J., Monneveux, P. and Lateef, Z. (2008). Genetic Variability, Trait Association and Diversity Studies in Wheat. *Pakistan J. Biot.*, **40**: 2087-2097.

Almeselmani, M., Abdullah, F., Hareri, F., Naaesan, M., Ammar, M., Kanbar, O. and Saud, A. (2011). Effect of drought on different physiological characters and yield component in different Syrian durum wheat varieties. *J. Agrl. Sci*. **3**:127-133.

Anjum, F., Yaseen, M., Rasul, E., Wahid, A. and Anjum, S. (2003). Water stress in barley (*Hordeum vulgare L.*). I. Effect on morphological characters. *Pakistan J. Agric. Sci.* **40**: 43-44.

Anjum, S., Xie, X., Wang, L., Saleem, M., Man, C., and Lei, W., (2011). Morphological, physiological and biochemical. *J Nanopart Res*., **15**:1-26.

Arabzadeh, N. (2012). The effect of drought stress on soluble carbohydrates (Sugars) in two species of *Haloxylon persicum* and *Haloxylon aphyllum. Asian J. Plant Sci*. **11**(1):44-51.

Araus, J.L., Amaro, T., Voltas, J., Nakkoul, H., and Nachit, M.M. (1998). Chlorophyll fluorescence as selection criterion for grain yield in durum wheat under Mediterranean conditions. *Field Crop Res.* **55**: 209-223.

Ashraf, M. and Fooled, M. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Env'l and Exp Bot*. **59**: 206-216.

Ashraf, M., Azmi, A., Khan, A. and Ala, S. (1994). Effect of water stress on total phenols, peroxidase activity and chlorophyll content in wheat (*Triticum aestivum* L.) genotypes under soil water deficits. *Acta Physiol. Plant.* **16**: 185-191.

Bajjii, M., Kinet, J.M. and Lutts, S. (2001). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. **In** *Plant Growth Reg.* pp.1-10.

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

Baser, I., Sehirali, H., Orta, A., Erdem, T., Erdem, Y. and Yorgancilar, O. (2004) Effect of different water stresses on the yield and yield components of winter wheat. *Cereal Res Comm.*, **32**: 217-23

Bayoumi, T., Eid, M. and Metwali, E. (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afri. J. Biotechnol., **7**:2341-2352.

Bechere, E., Belay, G., Mitiku, D. and Merker, A. (1996). Phenotypic diversity of tetraploid wheat landraces from the Northern and North- Central regions of Ethiopia. *Hered.* **24**: 165-172.

Bekele Hundie, Verkuijl, H., Mwangi, W. and Tanner, D. (2000). Adaptation of improved technologies in Adaba and Dodola Weredas of the Bale highland Ethiopia. International Maize and Wheat Improvement Centre (CIMMYT) and Ethiopian Agricultural Research Organization (EARO) Addis Ababa Ethiopia.

Beltrano, J. and Marta, G. (2008) Improved tolerance of wheat plants (Triticum aestivumL.) to drought stress and rewatering by the arbuscular mycorrhizal fungus Glomus claroideum: Effect on growth and cell membrane stability. *J of the Brazilian Soci of Plant Physiol.*, **67**: 569-572.

Bhatt, R. and Srinivasa, N. (2005). Influence of pod load response of okra to water stress. *Indian J. Plant Physiol*. **10**: 54-59.

Bilal, M., Rashid, R., Rehman, S., Iqbal, F., Ahmed, J., Abid, M., Ahmed, Z. and Hayat, A. (2015). Evaluation of wheat genotypes for drought tolerance. *J. green physiol, genetics and genomics*. **1**: 11-21.

Blum, A. (1996). Crop responses to drought and interpretation of adaptation. *Plant Growth Regulation.* **20**: 135-148.

Blum, A. (2011). Plant Breeding for Water-Limited Environments. Springer. New York, USA.

Braun, H., Pfeiffer, W.H. and Pollmer, W.G. (1992). Environments for selecting widely adapted spring wheat. *Crop Science*. **32**, 1420-1427.

Brown, S.B., Houghton, J.D., and Hendry, G.A.F. (1991). Chlorophyll breakdown. **In**: Scheer, H. (Ed.), Chlorophyllus. CRC Press, Boca Raton, pp. 465-489.

Buchanan, B., Gruissem, W. and Jones, R. (2000). Biochemistry and Molecular Biology of Plants. *Amer. Soc. Plant Physiol.* Rockville., **13**(3): 114-119.

Blum, A. and Pnuel, Y. (1990). Physiological attributes associated with drought resistance on wheat cultivars in a Mediterranean environment. Australian Journal of Agricultural Research., **41**: 799-810

CSA (Central Statistical Agency). (2015). Report on Area and Production of Crops (Private Peasant Holdings, Meher Season) The Federal Democratic Republic of Ethiopia Central Statistical Agency Agricultural Sample Survey Volume I, 2014 / 2015 (2007 E.C.) (March, 2015). Addis Ababa, Ethiopia.

CSA (Central Statistical Agency). (2018). Report on Area and production of major crops for 2014 / 2015 (private Peasant Holdings, Meher Season). Volume I. Statistical Bulletin No. 586. Addis Ababa, Ethiopia.

Dercon, Stefan. (2004). "Growth and Shocks Evidence from Rural Ethiopia." J of Dev't Economics., **74** (2): 309–29.

Demirevska, K, Simova, L., Vassileva, V., Vaseva, I., Grigorova, B. and Feller, U. (2008) Drought induced leaf protein alteration in sensitive and tolerant wheat varieties. Plant Physiol **20**: 29-37.

Dhanda, S., Sethi, G. and Behl, R. (2004). Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. and Crop Sci*. **19**: 6-8.

Dencic, S., Kastori, R., Kobiljski, B. and Duggan, B. (2000) Evaluation of grain yield and itscomponents in wheat cultivars and landraces under near optimal and drought conditions. *Euphytica* **113**: 43-52.

Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A. and Smith, F. (1956) Calorimetric methods for the determination of sugars and related substances. *Analyt Chem*., **28**: 350-56.

Dulai, S., Molnár, I., Prónay, J., Csernak, A., Tarnai, R. and Molnár-Láng, M. (2006). Effects of drought on photosynthetic parameters and heat stability of PSII in wheat and in Aegilops species originating from dry habitats. *Acta Biol. Szeged.* **50**: 11-17.

FAO (2015) Food and Agriculture Organisation of the United Nations Statistics Division..

FAO. (2018). FAOSTAT Statistical Database. Food and Agriculture Organization of the United Nations. Rome, Italy.

Farquhar, G. and Richards, R. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *J. Aust. Plant Physiol.* **11**: 539-552.

Farquhar, G., Wong, S., Evans, J. and Hubick, A. (1989). Photosynthesis and gas exchange. Plants under stress: biochemistry, physiology, and ecology and their application to plant improvement. **39**: 214-127.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S. (2009). Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev*., **29**: 185-212.

Foyer, C., Descourvieres, P. and Kunert, K. (1994). Photo oxidative stress in plants. *Plant. Physiol*., **92**: 696-717.

Gebre-Mariam, H. (1991) Wheat production and research in Ethiopia. **In**: H. Gebre-Mariam, D. G. Tanner and M. Hulluka (Eds.) Wheat Research in Ethiopia: A Historical Perspective, pp. 1-16. IAR/ CIMMYT, Addis Ababa, Ethiopia.

Ghaderi, N. and Siosemardeh, A. (2011). Response to drought stress of two strawberry cultivars (cv. Kurdistan and Selva). *Hort. Environ. Biotechnol*. **52**: 6-12.

Gupta, O., Meena, N., Sharma, I. and Sharma, P. (2014). Differential regulation of microRNAs in response to osmotic, salt and cold stresses in wheat. *Molec Biol Rep.* **41**: 4623-4629.

Gustafson, P., Raskina, O., Ma, X and Nevo, E. (2009). Wheat evolution, domestication, and improvement. **In** 'Wheat science and trade'.pp. 3–30. (Wiley-Blackwell: Ames, IA, USA).

Hassanein, A. (2004). Effect of relatively high concentrations of mannitol and sodium chloride on regeneration and gene expression of stress tolerant (*Alhagi graecrum*) and stress sensitive (*Lycopersicon esculentum* L) plant species. *Bulg. J. Plant Physiol.* **30**: 19-36.

Ibrahim, K. (1994). Association and path coefficient analysis of some traits in some bread wheat. *Ann Agril Sci*. **32**: 1189-1198.

Ingram, J. and Bartels, D. (1996). The Molecular basis of dehy- dration tolerance in plants. Annual Review of Plant Physiol and Plant Molar Biology. **47**: 337-403.

Irigoyen, J. Emerich, D. and Sanchez-Diaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant*. **84**:55-60.

Iturbe, O., Escuredo, I., Arrese-Igor, C. and Becana, M. (1998). Oxidative damage in pea.

IUSS Working Group WRB. (2014).International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome, Italy.

IWGSC (The International Wheat Genome Sequencing Consortium) (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. *J Science* **345**: 1251788.

Jaleel, A., Gopi, R., Manivannan, P., Gomathinayagam, M., Riadh, K., Ines, J., Chang-Xing, Z., Hong-Bo, S. and Panneerselvam, R. (2009). Antioxidant defense responses: Physiological plasticity in higher plants under abiotic constraints. *Acta Physiol Plant* **3**: 427-436.

Jatoi, W., Baloch, M., Kumbhar, M., Khan, N. and Kerio, M. (2011). Effect of water stress on physiological and yield parameters at anthesis stage in elite spring wheat cultivars. *Sarhad J. Agricul*. **27**: 59-65.

Jemal Abdulkerim, Tamado Tana and Firdissa Eticha. (2015). Response of Bread Wheat (*Triticum aestivum* L.)Varieties to Seeding Rates at Kulumsa, South Eastern Ethiopia. *Asian Journal of Plant Sciences,* **14**: 50-58.

Keyser, B. (2010). The wheat bZIP factor, TaABF1, mediates ABA-induced gene expression in bombarded barley aleurone layers. Honors Thesis. Pp. 593.

Khakwani, A., Dennett, M. and Munir, M. (2011). Drought tolerance screening of wheat varieties by inducing water stress conditions. *Songklanakarin J. Sci. Technol.* **33** (2): 135-142.

Khosh, K., and Ando, B. (1995) Effect of food environments, particularly sodium ion on the synthesis of chlorophyll and plant growth C4. Third Crop Science Congress of Iran. Tabriz University.

Korir, P., Nyabundi, J. and Kimurto, P. (2006). Genotypic response of common bean (*Phaseolus vulgaris* L). to moisture stress condition in Kenya. Asian Journal of

Kusaka, M., Ohta, M. and Fujimura, T. (2005). Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. Physiol Plant **5**: 474-489.

Lessani, H. and Mojtahedi, M. (2002). Introduction to Plant Physiology (Translation). 6th Edn., Tehran University press, Iran, ISBN: 964-03-3568-1, pp: 726.

Lipiec J., Doussan C., Nosalewicz, A. and Kondracka, K. (2013). Effect of drought and heat stresses on plant growth and yield: *A review. Int. Agrophys.* **27**:463-477.

Loreto, F., Centritto, M. and Chartzoulakis, K. (2003) Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. *Plant, Cell and Environment* **26**,595-601.

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.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P. and Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian J. crop Sci*. **4**: 580-585.

Maggio, A., Miyazaki, S., Veronese, P., Fujita, T., Ibeas J., Damsz, B., Narasimhan, M., Hasegawa P., Joly, R. and Bressan R. (2002). Does proline accumulation play an active role in stress-induced growth reduction. *Plant J* **31**: 699-712.

Manivannan, P., Jaleel, C., Somasundaram, R. and Panneerselvam, R. (2008). Osmoregulation and antioxidant metabolism in drought stressed Helianthus annuus under triadimefon drenching. *Comptes Rendus Biologies* **331**: 418-425.

Martin, S. (1996). CROPWAT: A computer program for irrigation planning and management. FAO Irrigation and drainage paper No. 46. Food and Agriculture Organization of the United Nations. Rome, Italy.

Martinez, J., Silva, H., Ledent, J. and Pinto, M. (2007). Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). *European J. Agron*. **26**: 30-38.

Mishra, V. and Cherkauer, K. (2010). Retrospective droughts in the crop growing season: Implications to corn and soybean yield in the Midwestern United States. Agrical and Forest Meteorology. **150**: 1030-1045.

MOA (Ethiopia Ministry of Agriculture). (2012). Crop Variety "Animal and Plant Health Regulatory Directorate."

Moayedi, A., Boyce, A. and Barakbah, S. (2009). Study on osmotic stress tolerance in promising durum wheat varieties using drought stress indices. *Resear. J. Agril and Biol Scien*. **5**: 603-607.

Mohammadkhani, N. and Heidari, R. (2008). Drought-induced accumulation of soluble sugars and proline in two maize varieties. *J World Appl. Sci*. **13**: 448-453.

Mushtaq, T., Hussain, M., Bukhsh, H., Iqbal, J. and Khaliq. T.(2011). Evaluation of two wheat genotypes performance under drought conditions at different growth stages. *J* *of Crop and Environment*. **2**(2): 20-27.

Naghavi, M., Aboughadareh, A. and Khalili, M. (2013). Evaluation of drought tolerance indices for screening some of corn (*Zea mays* L.) cultivars under environmental conditions. *Notulae Scientia Biologicae* **5**(3): 388-393.

Nevo, E. and Chen, G. (2010). Drought and salt tolerances in wild relatives for wheat and barley improvement. *Plant Cell and Env't*. **33**: 670-685.

Nezhadahmadi A, Prodhan ZH, Faruq G (2013) Drought tolerance in wheat. *Sci World Journal* **2013**, 1–12.

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

Oraki, H., Khajani, F. and Aghaalikhana, M. (2012). Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (Helianthus annuus L.) hybrids. *Afric. J. Biotech.* **11**:164-168.

Pan, X., Wang, Y., Wang, G., Cao, Q. and Wang, J. (2002). Relationship between growth redundancy and size inequality in spring wheat populations mulched with clearplastic film. *Acta PhytoecolSinica,* **26**: 177-184.

Rahim, N., Abas, S., Hamid, K., Amir, M. and Kamvan N. (2012). Effect of plant density on grain yield, yield components and associated traits of three durum wheat cultivar in western Iran. *Interl J of Agril and Crop Sci.* **4** (2):79-85.

Rhodes, D. and Samaras, Y. (1994). Genetic control of osmoregulation in plants. **In**: Stronge K (ed.). Cellular and Molecular Physiology of Cell Volume Regulation. Boca Raton: CRC Press, pp. 347-361.

Richardson, A.D., Duigan, S.P. and Berlyn, G.P. (2002). An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol*. **153**: 185–194.

Rizhsky, L., Liang, H. and Mittler, R. (2002). The combined effect of drought stress and heat shock on gene expression in tobacco, Plant Physiol. **130**(3): 1143-1151.

Sangtarash, M. (2010). Responses of different wheat genotypes to drought stress applied at different growth stages. *Pak. J. of Biological Sci*., **13**(3): 114-119.

SAS Institute Inc. (2002). SAS/STAT Software Version 9.0. SAS Institute, Cary, NC, USA.

Save, R., Biel, C., Domingo, R., Ruiz-Sanchez, M. and Torrecillas, A. (1995). Some physiological andmorphological characteristics of citrus plants for drought resistance. *J Plant Sci*, **110:** 167-172.

Schlemmer M., Francis, D., Shanahan, J. and Schepers, J. (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agron. J*., **97**:106-112.

Schonfeld, M., Johnson, R., Carver, B. and Mornhinweg, D. (1988). Water relations in winter wheat as drought resistance indicators. *Crop Sci*. **28**: 536-541.

Shahzad, M., Wasi, D., Sahi, S., Khan, M.., Ehsanullah and Ahmad, M. (2007). Effect of sowing dates and seed treatment on grain yield and quality of wheat. *Pakistan J of Agril Sci*, **44**: 581-583.

Shao, H.B., Chu, L.Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R., and Shao, M.A. (2009). Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. *Crit. Rev. Biotech.* **29**: 131-151.

Shulze, E.D., Beck, E. and Muller-Hohenstain, K. (2005). *Plant Ecology*. Springer, Berlin, Heidelberg, pp. 132, 134.

Sikuku, P.A., Onyango, J.C. and Musyimi, D.M. (2010). Chlorophyll fluorescence, protein and chlorophyll content of three nerica rainfed rice varieties under varying irrigation regimes. *J. Agric. Biol. Sci.,* **5**: 19-25.

Strauss, J., and Agenbag, G. (2000). The use of physiological parameters to identify drought tolerance in spring wheat cultivars. *South Africa J of Plant and Soil.,* **17**(1): 20-29.

Tang, A., Kawamitsa, Y., Kanechi, M. and Boyer, J.(2002). Photosynthesis at low water potentials in leaf discs lacking epidermis. *Annals of Botany* **89**(7): 861-870.

Taiz, L. and Zeiger, E. (2006). Plant physiology. (4th ed). Sinauer Associates Inc., Sunderland, Massachusets, USA. pp. 474-96.

Tanno, K. and Willcox, G. (2006). How fast was wild wheat domesticated? *Sci*., **311**: 1886.

Teng, S, Qian, Q., Zeng, D., Kunihiro, Y., Fujimoto, K. and Zhu, L. (2004). QTL analysis of leaf photosynthetic rate and related physiological traits in rice (*Oryza sativa* L.). *Euphytica*. **135**: 211-217.

Tesemma, T., Belay, G. and Worede, M. (1991). Morphological diversity in tetraploid wheat landrace populations from the central highlands of Ethiopia. *Hereditas.* **114**: 171-176.

Tiwari, S., Lata, C., Chauhan, P. and Nautiyal, C. (2016). Pseudomonas putida attunes morphophysiological, biochemical and molecular responses in Cicer arietinum L.

during drought stress and recovery. *Plant Physiol and Biochem.*, **99**: 108-117.

Turner, N. and Meyer, R. (2011). Synthesis of regional impacts and global agricultural adjustments. **In** '*Crop adaptation to climate change'*. Pp. 156-165.

Vajrabhaya, M., Kumpun, W. and Chadchawan, S. (2001). The Solute Accumulation: The Mechanism for Drought Tolerance in RD23 Rice (*Oryza sativa L.*) Lines. *J Sci. Asia* **27**: 93-97.

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. Agril. Sci.* **5**: 10-25.

Wu, Q., Xia, X. and Zou, N. (2008). Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European J. Soil Biol.* **44**: 122-128.

Wu, Q.-S., and Xia, R.-X. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under wellwatered and water stress conditions. *J. Plant Physiol*. **163**: 417-425.

Xonostle, C., Ramirez-O.,Flores, E. and Ruiz, M. (2011). Drought tolerance in crop plants. *Am. J. Plant Physiol*. 1-16.

Yancey, P. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* **208**:2819-2830.

Zaharieva, M., Gaulin, E., Havaux, M., Acevedo, E. and Monneveux, P. (2001). Drought and Heat Responses in the Wild Wheat Relative Roth. *J Crop Science* **41**: 1321-1329.

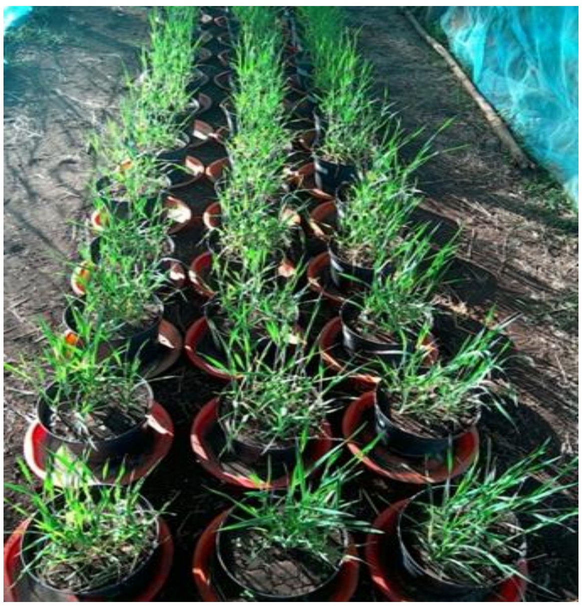
Zhang, S., Shan, L., Deng, X. (2002). Change of water use efficiency and its relation with root system growth in wheat evolution. *Chinese Science Bulletin* **47**:1879-1883.

Zhu**,** Y., Chen, J., Fan, Y., Wang, Y., Fan, S., Yang, L. (2000): Genetic diversity and disease control in rice. *Nature.,* **406,** 718-722.

Zivcak, M., Repkova, J., Olsovska, K. and Brestic, M. (2009). Osmotic adjustment in winter wheat varieties and its importance as a mechanism of drought tolerance. *Cereal research communications.* **37**: 569-572.

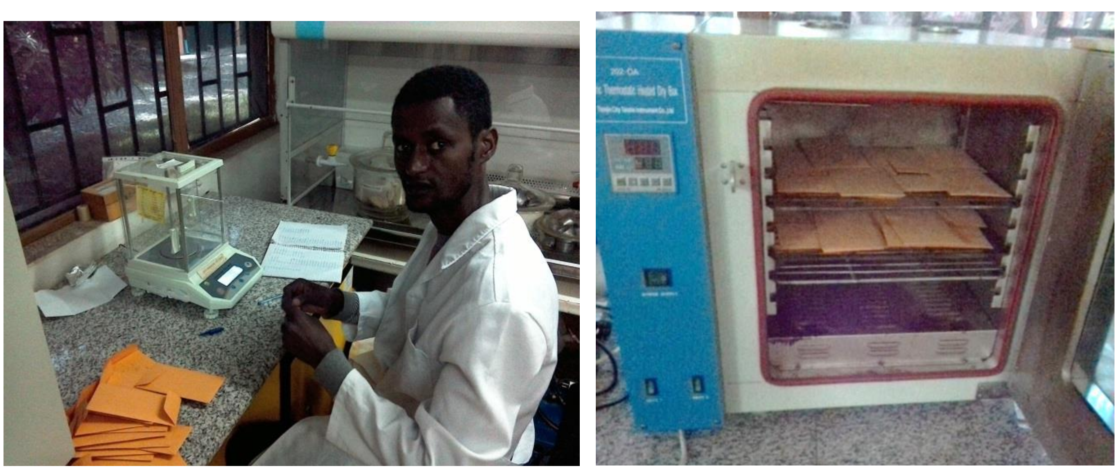
7. APPENDICES

Appendix 1: Partial view of the pot experiment at KARC



Appendix 2: Leaf sampling for the relative water content, proline and total soluble sugar determination

Appendix 3: Partial view of the samples measurements conducted for the laboratory experiment.



Appendix 4: Partial view of the preparation of samples for the determination of proline concentration

Appendix 5: Partial view of the preparation of samples for the determination of soluble sugar.

Appendix 6: Partial view of field and laboratory equipment