MOLECULAR AND MORPHOPHYSIOLOGICAL APPROACHES FOR A BETTER UNDERSTANDING OF DROUGHT RESISTANCE MECHANISMS IN SOME WHEAT GENOTYPES

by

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ABSTRACT

Drought resistance is the main challenge of wheat genetics and breeding programs. Resistance is a complex mechanism involving physiological, biochemical, and molecular processes. The effects of drought on these processes were studied in four bread wheat (*Triticum aestivum*) genotypes (Sahal-1, Giza-163, Ozcan and BVD-22) that were selected from a screening study. The selected genotypes were grown in the greenhouse and subjected to water deficit induced by withholding water supply for one week, at three different growth stages:-40, 60 and 80 days after sowing.

The results revealed that 1) drought adversely effected the plant height, biomass, number of leaves per plant, leaf and soil water content, macro and micro nutrients concentration whereas proline accumulation, soluble carbohydrate, lipid peroxidation, and antioxidant enzymes activities except catalase were positively affected; 2) Drought resistance was almost seen in Sahal-1 and BVD-22 genotypes but its extent varied from one genotype to another and even within genotype from growth stage to other stages. Differential display technique was used to study the expression profile of Sahal-1 and BVD-22 which was exposed to drought at 40 DAS. We observed ten differentially expressed genes. These fragments were isolated, cloned, sequenced, and compared with nucleotide and protein sequence databases using BLASTN and BLASTX algorithms.

Under field condition, the response of forty-nine wheat genotypes to drought significantly reduced the plant height, biomass, harvest index, NDVI, SPAD, and yield components as well as delayed the heading date, and increased canopy temperature of most genotypes.

Key words: drought, proline, lipid peroxidation, soluble carbohydrate, antioxidant enzymes, mRNA DD, SPAD, NDVI, canopy temp., harvest index, biomass, yield.

ÖZET

Kuraklık stresi buğday genetik ve ıslah programlarının ana sorundur. Direnç; fizyolojik, biyomedikal ve medikal süreçler içeren karmaşık bir mekanizmadır. Susuzluğun bu süreçler üzerindeki etkisi daha önce yapılan bir tarama çalışmasından seçilen dört tip (*Triticum aestivum*) ekmeklik buğday genotipi kullanılarak incelenmiştir (Sahal-1, Giza163, Ozcan and BVD-22). Seçilen genotipler serada yetiştirilmiş ve ekildikten 40, 60 ve 80 gün sonra üç farklı büyüme safhasında, bir hafta boyunca susuzluğa maruz bırakılmıştır.

Ortaya çıkan sonuçlara göre: 1) kuraklık bitki boyu, biyokütlesi, bitki başına düşen yaprak sayısı, yaprak ve toprak su içeriği ile makro ve mikro besin konsantrasyonlarını olumsuz yönde etkilerken; prolin birikimi, çözünebilir karbonhidrat, lipit peroksidasyonu ve katalaz dışındaki antioksidan enzim aktivitelerini olumlu olarak etkilemektedir; 2) BVD-22 ve Sahal-1 susuzluğa karşı direnç gözleminde daha iyi bir performans göstermiştir; 3) Kuraklık direnci Sahal-1 ve BVD-22 genotiplerinde az da olsa gözlenmiş; fakat kapsamı bir genotipten diğerine değiştiği gibi farklı büyüme safhalarında da farklılıklar ortaya çıkmıştır. 40. günde susuzluğa maruz bırakılan Sahal-1 ve BDV-22'nin ifade grafiği çalışılırken mRNA diferansiyel görüntü tekniği kullanılmıştır. Farklı seviyelerde ifade edilen 10 gen saptanmıştır. Bu genler izole edilmiş, klonlanmış, dizilenmiş, ardışık sıralanmış ve BLASTN ile BLASTX algoritmaları kullanılarak nükleotid ve protein dizi veritabanları ile karşılaştırılmıştır.

Tarla koşulları altında incelenen kırk dokuz buğday genotipinin kuraklığa tepkisi belirgin bir şekilde bitki boyunu, biyokütlesini, hasat endeksini, NDVI, SPAD ve verim birleşenlerini azaltmış, aynı zamanda çiçeklenme zamanını da geciktirmiş ve çoğu genotipin kanopi sıcaklık derecesini arttırmıştır.

Anahtar Kelimeler: Kuraklık, prolin, lipit peroksidasyonu, çözünebilir karbonhidrat, antioksidan enzimler, mRNA DD, SPAD, NDVI, kanopi derecesi, hasat endeksi, biokütle, verim.

أهديها لأبي، أمي، أخواتي، زوجتي وأبنائي أميرة وسيف الله

To my father, mother and sisters

To my wife, and my children’s Amera and Seyfullah

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AM	Ante Meridiem (before noon)
APO	Ascorbate peroxidase
ABRE's	ABA responsive elements
ABA	Abscisic acid
ABS	Absorbance
ATP	Adenosine triphosphate
APO	Ascorbate peroxidase
bp	Base pair
B	Boron
BHT	Butylated hydroxytoluene
Ca	Calcium
CT	Canopy temperature
CO_2	Carbon dioxide
CAT	Catalase
cm	Centimeter
cDNA	Complementary DNA
CRD	Completely Randomized Design
Cu	Copper
DAS	Days after sowing
da	Decare 1 da = 1000m2 = 0.1 hectare
^oC	Degree Celsius
DRE	Dehydration responsive element
DREB	Dehydration-responsive element binding protein
DNA	Deoxyribonucleic acid
DAP	Di-ammonium phosphate
DEPC	Diethylpyrocarbonate
dw	Distilled water
DSI	Drought susceptibility index
DW	Dry weight
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
ET	Evapo-transpiration
Fig.	Figure
FAO	Food and Agriculture Organization
FW	Fresh weight
G	Genotype
GAA	glacial acetic acid
GR	Glutathione reductase
g	Gram
h.	Hour

RH	Humidity
H_2O_2	Hydrogen peroxide
OH	Hydroxyl
ICP-OES	Inductively coupled plasma optical emission spectroscopy
Fe	Iron
kg	Kilogram
LEA	Late embryogenic abundant proteins
l.s.d.	Least significant differences
LPO	Lipid peroxidation
L	Litter
LB	Luria Bertani
Mg	Magnesium
MDA	Malondialdehyde
Mn	Manganese
m	Meter
µg	Microgram
µl	Microliter
meq	Milliequivalent
mg	Milligram
ml	Milliliter
mm	Millimeter
mM	Millimolar
MT	Million metric ton
min.	Minute
Moi.	Moisture
M	Molar
MW	Molecular weight
ng	Nanogram
nm	Nanometers
nmol	Nanomole
NADPH	Nicotinamide adenine dinucleotide
N	Nitrogen
NDVI	Normalized difference vegetation index
No.	Number
NGM	Number of grains per m^2
NGS	Number of grains per spike
NLP	Number of leaves per plant
NSM	Number of spikes per m^2
O.C	Organic carbon
O.M	Organic matter
O_2	Oxygen
ppm	Part per million
%	Percent

POD	Peroxidase
P	Phosphorus
Ph	Plant height
PEG	Polyethylene glycol
PCR	polymerase chain reaction
K	potassium
Pro	Proline
Put	putrescine
ROS	Reactive oxygen species
RY	Relative grain yield
RY_S	Relative grain yield under water stress
RY_W	Relative grain yield under well water
RWC	Relative water content
RY	Relative yield
rpm	Revolution per minuet
RNA	Ribonucleic acid
s.	Second
SDM	Shoot dry mass
SFM	Shoot fresh mass
SOM	Soil organic matter
SPAD	Soil Plant Analysis Development
SWC	Soil water content
SC	Soluble carbohydrates content
Spd	Spermidine
Spm	Spermine
S.D.	Standard deviation
SSA	Sulfosalicylic acid
S	Sulphur
O_2	Superoxide
SOD	Superoxide dismutase
Temp.	Temperature
TBA	Thiobarbituric acid
TGW	Thousand grain weight
T	Treatment
TCA	Trichloroacetic acid
TBE	Tris Borate EDTA
TW	Turgid weight
UNEP	United nations environment programme
V	Volume
H_2O	Water
Zn	Zinc

1 INTRODUCTION

Agriculture is highly dependent on climatic conditions; therefore, any changes in these conditions may negatively affect agricultural crops and lead to a shortage in the world food supply (**Maqsood** and **Ali**, **2007**). Drought dramatically affects plant functions, metabolism, limiting normal growth and causes a sharp decrease in crop productivity (**Yamaguchi**, ***et al***., **2002**). **Wang**, ***et al***., (**2003**) reported that drought stress reduced average yields of most crops by more than 50%. Drought occurs when the available water in the soil decreased and atmospheric conditions causes a continuous loss of water from the plant by transpiration process (**Jaleel**, ***et al***., **2009**).

Wheat is one of the most important cereal crops all over the world (**Amjad**, ***et al***., **2009**). It is the second important crop on the globe (**Johari-Pireivatlou**, ***et al***., **2010**). Furthermore, wheat is essential component for human food and animals feed in many countries, especially in developing countries. Wheat growth and productivity are adversely affected by drought stress. Nearly half of the cultivated areas of wheat are found in developing countries and up to 70% of these areas suffer from drought (**Bhutta**, ***et al***., **2006**). Moreover, freshwater resources are limited especially those used in agricultural sector. Meanwhile, the world population is increase. It is projected to reach 9.2 billion by 2050 (**World population prospects**, **2007**). Thus, to achieve a high output of agricultural crops under drought stress, it is necessary to develop new wheat genotypes, which are characterized by drought resistance, at the same time, high yield to meet the food demands of the growing population.

Studying the influence of drought stress on growth and the physiological characteristics of different wheat genotypes is a helpful tool for development and improved wheat resistance toward stressful conditions. The resistance to drought has not been defined very well and it is still not clear which aspects of the plant are important for such kind of resistance (**Abdelhady** and **Elnaggar**, **2007**). On the other hand, wheat is an attractive study system because of it is wide natural genetic variation in traits related to drought tolerance (**Loggini**, ***et al***., **1999**).

For a successful development of drought resistant genotypes, it is necessary to study all changes that occur in genotypes of differing susceptibility caused by the drought stress (**Ramiz** and **Mehraj**, **2004**), and compare between tolerant and susceptible genotypes under stress and non-stress conditions. The genetic improvement for drought resistance requires a search for possible physiological and morphological components of drought resistance and exploration of their genetic variation.

To understand the components of drought resistance, two experiments were designed. The first one was a greenhouse experiment that was conducted at the Biological Sciences and Bioengineering Department, the Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey, during the 2009 season. The aim was to study the influence of water deficit during three growth stages of four bread wheat genotypes, two genotypes from Egypt and two from Turkey. The sowing date was done on 6 January 2009, in pots using three replicates. The treatments were two water regimes (stress and non-stress), the stress treatment was induced by withholding irrigation for one week at 40, 60, and 80 DAS (days after sowing) and non-stress (well watered).

The second experiment was an open field experiment. It was designed to examine and evaluate the differences in some morphological, physiological characters among 49 bread wheat genotypes in response to drought stress. The evaluation was done under supplementary irrigation and rain-fed conditions. This experiment was conducted at Anatolian agricultural research institute, Eskisehir, Turkey, during 2008 and 2009 seasons. The sowing was done on 20 October 2008, in rows 20cm apart using three replicates.

The objectives of this study were: 1) Assess the growth and yield of some bread wheat genotypes under drought stress conditions; 2) Characterize the changes that occur at different levels, in response to drought; 3) Understand and identify some drought resistance mechanisms; and 4) Identify, clone, and characterize the differentially expressed drought- responsive genes in some bread wheat genotypes. These genotypes were used in screening for genes that alter their expression levels by using a genomic tool called mRNA differential display.

2 OVERVIEW

2.1 Wheat production and importance

Wheat is one of the most important cereal crops all over the world; it is the second important crop on the globe (**Johari-Pireivatlou**, ***et al***., **2010**). In 2007, the world production of wheat was nearly 606 million metric tons (**FAO**, **2007**). On the other hand, Turkey ranked eighth among the world's wheat producers (**Table 2.1**) with 17.2 million tons (**FAO**, **2007**).

Table 2.1: World wheat production

Rank		Production (MT)
1	China	109.3
2	India	75.8
3	USA	55.8
4	Russia	49.4
5	France	32.8
6	Pakistan	23.3
7	Canada	20.1
8	Turkey	17.2
9	Argentina	16.5

Source: - http://faostat.fao.org/site/339/default.aspx

Wheat plays a significant role in human food and animal feed; moreover, it provides one-third of the world population with nearly half of their calorie and protein intakes (**Sibel** and **Birol**, **2007**). Furthermore, it is an important source for many minerals such as iron and zinc (**USDA**, **National Nutrient Database**, **2006**). Wheat could be divided into three types according to planting time: winter, spring, and facultative wheat. In addition, it could be divided into 1) Diploid, with two sets of chromosomes, and 2) Polyploid: - (a) Tetraploid, with four sets of chromosomes (*Triticum durum*), represents nearly 4% of cultivars and is used for making macaroni and pizza. (**Debasis** and **Paramjit**, **2001**), (b) Hexaploid, with six sets of chromosomes (*Triticum aestivum*), represents about 95% of the wheat grown worldwide (**Shewry**, **2009**), and used for making bread and baked products.

2.2 Climatic changes and its effects on crops productivity

Greenhouse gases result from human activities. The accumulation of these gases in the atmosphere leads to an increase in the planet temperature and causes changes in global climate (**Nguyen**, **2004**). In the past century, the global temperature was increased by more than 0.6°C. It is expected by 2100, it will increase by between 1.4 and 5.8°C (**IPCC**, **2001**). As a result of that, the global precipitation could increase; meanwhile, the global evapotranspiration could also increase, but it will be greater than the precipitation, so there will a potential for drought in many parts of the world. On the other hand, agriculture is highly dependent on climatic conditions; therefore, any changes in these conditions negatively affect crops yield and causing a shortage in the world food supply (**Maqsood** and **Ali**, **2007**). Many reports on crop productivity, suggest that the productivity of crops, especially tropical crops, will decrease because of increasing global temperature (**Nguyen**, **2004**). **Peng**, ***et al***., (**2004**) reported that rice yield decreased by as much as 15% for each 1°C increase in the growing season. Similarly, **Chipanshi**, ***et al***., (**2003**) concluded that climate changes might decrease the maize yield by between 10 -36 %.

2.3 World population growth and global water resources situation

In 2007, the world population was nearly 6.7 billion, and it is expected to reach 9.2 billion by 2050 (**World population prospects**, **2007**). With continuous increasing of the population, the need for food and water will increase. However, the water resources are limited (**Farooq**, ***et al***., **2009**), especially freshwater resources. Less than 3% of the world's water is freshwater, while the rest is seawater and undrinkable. 2.5% of these freshwater resources are in a frozen form and not available for human use. Therefore, humanity must rely on only 0.5% for all needs. On the other hand, agriculture accounts for more than 70% of the total global consumption of water (**Molden**, **2007**). Furthermore, about one third of the current world population lives in water-stressed locations and it is expected to increase to two thirds within the next 25 years (**Ortiz**, ***et al***., **2007**). Therefore water saving and a development of new genotypes with drought resistance and highly yield to meet food demand of the growing world

population will be logical targets in the next future and the main challenge of wheat researchers.

2.4 Drought definition

Drought is meteorological term; commonly defined as a period without a significant rainfall (**Jaleel**, ***et al***., **2009**), and includes all problems due to water shortage in the soil. However, agricultural drought could be defined as a climatic excursion involving deficiency of sufficient precipitation, which adversely affects crops productivity (**Royo**, ***et al***., **2000**). It occurs when the available water in the soil is decreased and at the same time, the atmospheric conditions (high temp. and low precipitation) cause continuous loss of water from plant by transpiration (**Jaleel**, ***et al***., **2009**).

2.5 Effects of drought

2.5.1 Effects of drought on soil and microbial activity levels

Drought has many negative effects on the soil, especially the surface layer (topsoil), which is the most fertile layer**.** One of these effects is soil erosion, which enhanced during drought stress period. The potential for global climate changes to increase the risk of soil erosion is clear (**Zhang** and **Nearing**, **2005**). Because of lack of water in the soil, topsoil becomes drier and soil aggregates decrease, which can be easily removed by wind. There are many microorganisms in plant rhizosphere. Some of them are useful for plants such as nitrogen fixation, micorhiza, and some of them are harmful and cause diseases to plants. That lack of moisture in the soil may limit or inhibit microbial activity levels. **Borken**, ***et al***., (**2006**) reported that the low soil moisture inhibited microbial decay of soil organic matter (SOM). **Streeter**, (**2003**) found that drought stress conditions reduced the N_2-fixing activity of legumes crops. On the other hand, there are some soil organisms, which can survive during this kind of dry conditions by the formation of cysts, capsules and spores (**Borken**, ***et al***., **2006**).

2.5.2 Effects of drought on nutrient availability

Plant resistance to drought stress depends on plants nutrient status (**Marschner**, **1995**). Drought has negative effects on the nutrient accumulation level in plant (**Baligar**, ***et al***., **2001**); it reduces nutrient uptake (**Marschner**, **1995**), decreases nutrient diffusion rate in the soil to the root surfaces (**Alam**, **1999**), and decreases the transport from roots to shoots (**Hu** and **Schmidhalter**, **2005**). **Brown**, ***et al***., (**2006**) found that soil drying significantly decreases nutrient uptake (Ca, Fe, Mg, N, P, and K). On the other hand, plant species may vary in their response to mineral uptake under water stress (**Farooq**, ***et al***., **2009**). The negative effects of drought could be due to stomatal closure, which reduces transpiration rates from leaves and impaired active transport from root to shoot (**Alam**, **1999**). In addition, it may be due to effects on root growth (**Fageria**, ***et al***., **2002**) and root distribution in the soil. The mineral nutrients are divided into two groups: macronutrients and micronutrients.

2.5.2.1 Effect of drought stress on macronutrients

Macronutrients are divided into two groups: primary and secondary nutrients. The primary nutrients are - nitrogen (N), phosphorus (P), and potassium (K), while the secondary nutrients are - calcium (Ca), magnesium (Mg), and sulfur (S). These macronutrients play multiple essential roles in plant metabolism and plant growth.

Phosphorus (P) which is the key component of nucleic acids, phospholipids and phosphor-proteins (**Hu** and **Schmidhalter**, **2005**); plays significant roles in 1) cellular energy transfer in form of adenosine triphosphate (ATP), 2) respiration and photosynthesis (**Alam**, **1999**). Furthermore, it is important and required for root growth (**Hopkins**, **1999**). Several reports have suggested that phosphorus has positive effects on plant growth under stress conditions. **Garg**, ***et al***., **(2004**) found that phosphorus fertilization enhanced plant growth under stress. The positive effects of phosphorus could be due to it is role in increasing water-use efficiency, as well as the stomatal conductance (**Bruck**, ***et al***., **2000**), also it could be due to it is role in increasing cell-membrane stability (**Sawwan**, ***et al***., **2000**).

Like phosphorus, nitrogen (N) is also an essential nutrient for plant growth; it is an important constituent of plant cells components such as proteins, amino and nucleic acids (**Hu** and **Schmidhalter**, **2005**). Nitrogen uptake and it is transport from roots to shoots is negatively affected by drought stress. **Bloem**, ***et al***., (**1992**) found that drought stress reduced soil-N mineralization and reduced nitrogen availability. Thus, there was a nitrogen deficiency symptom, which significantly affects and inhibits plant growth.

Among all nutrients, potassium (K) helps in osmotic adjustment (**Farooq**, ***et al***., **2009**). Drought also affects on K availability to plants, due to decreasing mobility under such conditions (**Hu** and **Schmidhalter**, **2005**). **McWilliams**, (**2003**) found that drought stress reduced K uptake in cotton plants. The application of potassium fertilizers reduced the adverse effects of drought on mung bean growth (**Sangakkara**, ***et al***., **2001**). The roles of potassium in improving plant resistance to drought may be due 1) stomatal regulation under stress conditions (**Kant** and **Kafkafi**, **2002**), 2) increasing the retention of water in plants (**Umar** and **Moinuddin**, **2002**), 3) osmoregulation and osmotic adjustment (**Bajji**, ***et al***., **2000**), 4) charge balance (**Marschner**, **1995**), and 5) maintaining turgor pressure and reducing transpiration rate under stress conditions (**Andersen**, ***et al***., **1992**). **Morgan**, (**1992**) found that the wheat lines that accumulated more potassium in their shoot tissues, showed highly osmotic adjustments. Furthermore, the accumulation of potassium in *Brassica napus* leaves accounted for about 25% of drought-induced changes in osmotic adjustment (**Ma**, **2004**). The application of potassium fertilizers enhanced the photosynthetic rate, plant growth and yield under stress conditions (**Egila**, ***et al***., **2001**; **Umar** and **Moinuddin**, **2002**).

Calcium (Ca) is an essential nutrient for regulating many physiological processes within plant cells through it effects cell membrane structure, stomatal function, cell division and cell-wall synthesis (**Mclaughlin** and **Wimmer**, **1999**). Similar to other macronutrients, also water stress conditions affect calcium uptake (**Hu** and **Schmidhalter**, **2005**). Calcium plays significant roles under drought stress conditions through 1) osmoregulation **(Bartels** and **Sunkar**, **2005**), 2) signaling in plant defense and repair of damage; it is a key signal messenger for regulating a plant's resistance to drought (**Hu** and **Schmidhalter**, **2005**), **Sadiqov**, ***et al***., (**2002**) reported that calcium participates in the signaling mechanisms of drought-induced proline accumulation, 3) also it has an important role in ensuring membrane integrity (**Hirschi**, **2004**).

2.5.2.2 Effect of drought stress on micronutrients

Micronutrients are those elements required for plant growth, which are needed in small amounts. These elements are sometimes called minor elements. The micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo), and zinc (Zn).

Zinc (Zn), plays an important role in plant growth under stress conditions. It is protect plant cells from the damage effects that caused by reactive oxygen species (**Cakmak**, **2000**), reduces free radicals production by superoxide radical producing enzymes. Zn also has a role in protection of chloroplasts from photo-oxidative damage that occur by ROS (**Wang** and **Jin**, **2005**). Zinc has in functional, structural and regulatory roles in several enzymes (**McCall**, ***et al***., **2000**). Zn also, is involved in carbohydrate metabolism through its effects on photosynthesis and sugar transformations (**Coruh**, **2007**). There are many negative effects of zinc deficiency, one of which is susceptibility to stress and decreased synthesis of carbohydrates (**Singh**, **2005**). Zn may probably play a crucial role in the metabolism of starch (**Alloway**, **2004**). Such as zinc, copper (Cu) is also a necessary element for plant growth, it acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (**Marschner**, **1995**; **Raven**, ***et al***., **1999**). Cu ions act as cofactors in many enzymes such as Cu/Zn superoxide dismutase (**Yruela**, **2005**).

Iron (Fe) is an important component, functions as a cofactor and catalytic site of important enzymes. Some of these enzymes are utilized in chlorophyll metabolism (**Davenport**, **1983**), transfer of electrons (redox reactions such as cytochromes and iron-sulfur proteins) (**Salazar-Garcia**, **1999)**; also it is involved in N_2 fixation, and respiration (**Taiz** and **Zeiger**, **1991**). Manganese (Mn) also is an essential nutrient to all plants. It is involved in disease resistance (**Graham** and **Webb**, **1991**), via production of lignin. Mn also is involved in photosynthesis, respiration, and amino acid biosynthesis (**Todorovic**, ***et al***., **2009**). It plays an essential role in activation of several enzymes, such as isoenzymes of superoxide dismutase (**Campanella**, ***et al***., **2005**). Mn also involved in scavenging of superoxide and hydrogen peroxide (**Ducic** and **Polle**, **2005**).

The effects of drought stress on micronutrients availability are not great as for macronutrients because the plant requires only small quantities of these nutrients (**Hu** and **Schmidhalter**, **2005**). **Oktem**, (**2008**) reported that water deficiency decreased micronutrients concentrations (Fe, Zn, and Cu) in *Zea mays* plants. The drought stress induces deficiencies in all micronutrients, but boron deficiency is the common one. The availability of Mn and Fe increased under well-watered conditions because of its presence in more soluble forms (**Havlin**, ***et al***., **1999**). Some reports referred that micronutrients application increased plant drought resistance (**Rahimizadeh**, ***et al***., **2008**).

2.5.3 Effects of drought on plant

Plants are made up of tissues and cells, which are filled with water in order to maintain their turgor. However, if the turgor not maintained, the plant begins to wilt (**Unruh** and **Elliott**, **1999**). The plants absorb water from soil through the roots system; then water moved throughout the plant and eventually released via stomata through a process known as transpiration (**Salisbury** and **Ross**, **1992**). Under drought stress plant reduces evaporation through stomata closing (**Turner**, **1986**), which negatively affects plant growth, and all functions. In addition, the gas exchange and CO_2 supply will be very limited (**Jaleel**, ***et al***., **2009**). As well as drought inhibiting seed germination (**Kaya**, ***et al***., **2006**). **Okcu**, ***et al***., (**2005**) reported that drought stress impaired the germination of *Pisum sativum*. Furthermore, drought reduces development and distribution of roots in the soil (**Pace**, ***et al***., **1999**), decreases cell elongation and enlargement (**Nonami**, **1998**). Moreover, drought reduces leaf size, and stem extension (**Farooq**, ***et al***., **2009**).

2.5.3.1 Effect on photosynthesis process and photosynthetic rate

Plant growth requires energy, which comes from sun light through the photosynthesis process, where the chlorophyll absorbs this energy and uses it with water (H_2O), and carbon dioxide (CO_2) to produce oxygen (O_2) and sugars. Under drought stress conditions, the plant reduces water loss through leaf transpiration via stomatal

closure (via ABA signaling) which in turn leads to reduce CO_2 supply and its assimilation by leaves (**Farooq**, ***et al***., **2009**). Reduction in CO_2 influx reduces carboxlation and indirectly affects photosynthesis process; moreover, drought also decreases photosynthetic rate (**Fernandez**, ***et al***., **1999**), reduces chlorophyll content through chlorophyll degradation (**Anjum**, ***et al***., **2003**; **Nayyar** and **Gupta**, **2006**; **Farooq**, ***et al***., **2009**), inhibits the photochemical activities, decreases activities levels of enzymes that are related to CO_2 fixation and Calvin Cycle such as Rubisco (**Monakhova** and **Chernyadev**, **2002**) , and accelerates leaf senescence (**Rivero**, ***et al***., **2007**).

2.5.3.2 Organic solutes accumulation

As a response to drought stress, the water potential in the soil and plant root zone decreases, at the same time the osmotic potential increases, so the plants synthesizes several organic solutes (sugars, proline, mannitol, and glycine betaine) to maintain cell volume and turgor against dehydration. These solutes classified into two categories: one is nitrogen-containing compounds such as proline and amino acids, and the other group is hydroxyl-containing compounds, such as oligosaccharides and sucrose (**Mccue** and **Hanson**, **1990**).

2.5.3.2.1 Proline accumulation (Pro)

Among all amino acids, the accumulation of proline under drought stress has been recognized by many researchers (**Vendruscolo**, ***et al***., **2007**; **Tatar** and **Gevrek**, **2008**). Proline is a basic amino acid and one of 20 amino acids. It has highly hydrophilic characteristics, which accumulate at high amounts in plant cells without interfering with macromolecules or metabolism (**Samaras**, ***et al***., **1995**). This accumulation was recognized as beneficial drought tolerance indicator and plays a significant role in minimizing the damages that caused by drought within plant cells (**Mohammadkhani** and **Heidari**, **2008**). Proline acts as a compatible solute in regulating and reducing water loss from cells prevents cell membrane damage and protein denaturation. Some stressed plants used proline as a source of storage for carbon and nitrogen (**Samaras**, ***et al***., **1995**). It has been reported that proline accumulation could be only useful as a possible

drought injury sensor instead of its role in stress tolerance mechanism (**Zlatev** and **Stoyanov**, **2005**). However, other reports suggest that proline is involved in tolerance mechanisms against oxidative stress, and it was the main strategy of plants to avoid the damage impacts of drought stress (**Vendruscolo**, ***et al***., **2007**). **Yamada**, ***et al***., (**2005**) reported that the exogenous application of proline enhanced the endogenous accumulation of free proline and improved drought tolerance of petunia plants. The accumulation of proline in plant cells is a result of two pathways: first, increase expression of proline synthesis enzymes and thus increase the proline biosynthesis and the second, is inhibition of proline oxidation and proline degradation (**Delauney** and **Verma**, **1990**; **Peng**, ***et al***., **1996**).

2.5.3.2.2 Soluble carbohydrate accumulation (SC)

Soluble carbohydrate accumulation in the shoot and root parts of plant is enhanced by exposure to stresses (**Prado**, ***et al***., **2000)**. It has a key role in drought tolerance (**Johari-Pireivatlou**, ***et al***., **2010**). High carbohydrate concentration, beside its role in maintaining protein structure and cell membrane stabilization (**Hoekstra**, ***et al***., **2001**), plays a significant role in osmotic adjustment (**Mohammadkhani** and **Heidari**, **2008**). It also serves as signal molecule (**Smeekens**, **2000**) for sugar-responsive genes which enhancing the defense responses, as well as it acts as regulators for gene expression (**Koch**, **1996**).

2.5.3.2.3 Polyamine accumulation

Polyamines, mainly diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm), are polycationic compounds of low molecular weight that are present in cells of all living organisms (**Liu**, ***et al***., **2007**). The positively charged polyamines plays a key role in responding to the drought stress, through the interaction with negatively charged macromolecules such as DNA, RNA, and proteins, which in turn leads to change the physical and chemical properties of the membranes (**Galston** and **Kaur**, **1990**; **Bouchereau**, ***et al***., **1999**; **Alcazar**, ***et al***., **2006**). Polyamines help to detoxify the ROS accumulation during a biotic stress (**Groppa** and **Benavides**, **2008**; **Rider**, ***et al***., **2007**). Moreover, polyamines considered secondary messengers and are

important compounds for regulating stress response (**Liu**, ***et al***., **2007**). It is closely associated with the resistance of plants to drought stress (**Aziz**, ***et al***., **1997**). The exogenous application of polyamines enhanced stress tolerance of wheat seedlings (**Liu**, ***et al***., **2004**). The exogenous application to stressed plants could lead to injury alleviation and growth promotion (**Liu**, ***et al***., **2007**).

2.5.3.2.4 Glycinebetaine

Glycinebetaine has important roles under drought stress via improving the growth and production of plants. The plants produce and accumulate glycinebetaine but in a small quantity, and not enough to address the damage caused by the environmental stresses (**Subbarao**, ***et al***., **2000**), and thus the exogenous application of glycinebetaine perhaps improve drought tolerance. **Hussain**, ***et al***., (**2008)** reported that the exogenous application of glycinebetaine improved drought tolerance of sunflower. **Sakamoto** and **Murata**, (**2002**) found that the foliar-application of glycinebetaine had a significant role in the protection of plants from stress by maintenance in leaf water status through osmotic adjustment and enhanced photosynthesis. Also the glycinebetaine application alleviates the negative effects of drought stress in tobacco plants via increasing anti-oxidative enzyme activities (**Ma**, ***et al***., **2007**).

2.5.3.3 Phytohormones accumulation

There are many hormones that play important roles in responding to drought stress; abscisic acid is one of these phytohormones. The drought stress induces ABA accumulation (**Jiang** and **Zhang**, **2002**). ABA plays central roles under drought stress: 1) it regulates plant response to drought (**Davies** and **Zhang**, **1991**; **Shinozaki** and **Yamaguchi**, **1997**), 2) it stimulates stomatal closure, also 3) it induces expression of some stress-related genes (**Shinozaki** and **Yamaguchi**, **2007**). There are many genes that are induced as a result of exogenous treatments of ABA (**Yamaguchi** and **Shinozaki**, **2005**). ABA is a stress signal (**Jiang** and **Zhang**, **2002**), has a role in increasing antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) in plant cells (**Bellaire**, ***et al***., **2000**; **Jiang** and **Zhang**, **2001**). Therefore, ABA referred to as a stress hormone (**Taylor**, ***et al***., **2000**).

2.5.3.4 Relative water content (RWC)

RWC is an appropriate measure and useful indicator for plant water status in terms of physiological consequence of cellular water stress. It is strongly affected by exposure to drought stress. The decrease in RWC content is an indication to decrease in turgor pressure in plant cells and plant growth; this decrease may be because of plant vigor reduction (**Liu**, ***et al***., **2002**). **Blokhina**, ***et al***., **(2003**) reported that drought stress affect on cell membrane caused an increase in penetrability and decrease in sustainability. The maintenance of favorable plant water relations is vital for the development of drought resistance in crop plants (**Passioura**, **2002**). The water-stressed wheat had lower relative water content than non-stressed (**Farooq**, ***et al***., **2009**). **Schonfeld**, ***et al***., **(1988**) showed that the wheat cultivars that had high RWC were more resistant to drought stress.

2.5.3.5 Reactive oxygen species (ROS)

ROS are reactive molecules that contain the oxygen atom. Under drought stress, the plant leaves receive sunlight much more than they can utilize in photosynthesis process, which causes the excessive accumulation of absorbed light, and activate molecular oxygen to ROS (superoxide O_2, hydrogen peroxide H_2O_2 and hydroxyl OH). The ROS may react with proteins, membrane lipids and nucleic acids (DNA, RNA), causing oxidative damage and impairing the normal functions of cells, which in turn leads to cell death (**Mittler**, **2002**; **Mittler**, ***et al***., **2004**). Furthermore, ROS inhibit plant growth (**Kong**, ***et al***., **2005**; **Yao** and **Liu**, **2007**). It serves as a second messenger, which involves in stress signal transduction pathways (signaling molecules) and activates stress response (antioxidant enzymes and defense pathways) (**Torres**, ***et al***., **2002**).

Under non-stress conditions, ROS produced as byproducts of aerobic metabolic processes such as respiration and photosynthesis, but in low concentrations. However, under stress conditions, the level increases too much, as well as during senescence (**Woo**, ***et al***., **2004**). The ability to reduce the damaging effects of ROS in plants may be associated with drought tolerance. Plants use antioxidant defense mechanisms includes enzymatic and non-enzymatic systems, to prevent these damages (**Agarwal** and **Pandey**, **2003**). The non-enzymatic systems include 1) β-carotenes, 2) ascorbic acid and

3) α-tocopherol (**Tayebeh** and **Hassan**, **2010**), while the enzymatic systems include 1) superoxide dismutase, 2) ascorbate peroxidase, 3) catalase, and 4) glutathione reductase. The tolerant cells activate their enzymatic antioxidant system, which then starts to detoxify the ROS radicals and protecting the cell. **Selote** and **Khanna-Chopra**, **(2004**) found that the plant-water relations play role in activation of these defense mechanisms. **Khanna-Chopra** and **Selote**, **(2007**) reported that the activities of antioxidant enzymes generally, increases under a biotic stress.

2.5.3.6 Effect of drought stress on yield and yield components

Among of all abiotic stresses, drought is the most damaging one, which affects all plant functions and leads to a sharp decrease in crop productivity. **Yao**, ***et al***., (**2009**) reported that the growth of wheat has been seriously influenced by drought in many regions. The selection for high yield under drought stress is effective and very important in breeding for drought-tolerance. The high yield potential under drought conditions is the main target of crop breeders (**Jaleel**, ***et al***., **2009**). The wheat grain yield can be assessed in terms of three yield components, namely: 1) number of spikes per unit area, 2) number of kernels per spike and 3) kernel weight (**Moayedi**, ***et al***., **2010**). A complex of different morphological, physiological and phenological traits of that genotype, which are in turn influenced by the drought stress (**Nouri-Ganbalani**, ***et al***., **2009**), influences the grain yield of any wheat genotype.

In arid and semi-arid regions, drought is one of the major a biotic environmental factors that caused a significant reduction in grain production of rained wheat (**Bhutta**, ***et al***., **2006**). In barley, drought stress reduced grain yield by 49–57% (**Samarah**, **2005**), while in maize drought stress at grain filling reduced yield by 79–81% (**Monneveux**, ***et al***., **2006**). The world wide losses in yield caused by drought and salinity are greater than losses caused by all other environmental factors (**Kramer**,**1980**).The reduction in crop yield could be due to: 1) reducing harvest index, 2) decreasing radiation-use efficiency, and 3) reducing canopy absorption of photo-synthetically active radiation (**Earl** and **Davis**, **2003**). **Nouri-Ganbalani**, ***et al***., **(2009)** referred that the drought caused low harvest index, decreased 1000-grain weight and reduced grain yield. **Edward** and **Wright**, **(2008**) pointed to a decrease in yield components of wheat under stress conditions.

2.6 Plant strategies under drought stress conditions

To maintain growth and productivity under drought stress conditions, plants must adapt to these conditions and exercise specific tolerance mechanism (**Wang**, ***et al***., **2003**). Plants adapt to drought at different levels: 1) molecular, 2) cellular, and 3) whole plant level, by using different morphological, physiological, biochemical and molecular mechanisms. These mechanisms are controlled by assortment and network of genes, which are activated or repressed as a response to drought (**Bartels** and **Sunkar**, **2005**; **Yamaguchi** and **Shinozaki**, **2005**).The drought resistance mechanisms could be divided into three categories, 1) drought escape, 2) drought avoidance and 3) drought tolerance (**Mitra**, **2001**).

2.6.1 Drought escape

It is defined as the ability of plants to complete their life cycles before serious soil water deficits develop with short life cycle and rapid growth during wet season. This mechanism involves a) rapid phonological development (early flowering and early maturity), and b) developmental plasticity (variation in duration of growth period depending on the extent of water-shortage).

2.6.2 Drought avoidance

It is defined as the ability of plant to maintain relatively high tissue water potential despite shortage in soil water content (**Mitra**, **2001**).

Drought avoidance mechanisms

The major avoidance mechanisms include reduces water loss and increase water uptake:-

A- Mechanisms of reducing water loss from the plant leaves

1) decreasing canopy size, 2) producing thick cuticles and fleshy leaves, 3) stomata closing during the day or during the drought stress period, 4) reducing evaporation surface area (**Turner**, **1986**) by producing smaller leaves **(Farooq**, ***et al***., **2009**), and 5) decreasing the amount of absorbed radiation via leaves rolling and folding (**Begg**, **1980**).

B-Mechanisms of maintaining and enhancing water uptake

The ability to extract water from soil under water deficit conditions is a major attribute of drought adaptation (**Olivares-Villegas**, ***et al***., **2007**). Root depth plays a key role in drought resistance **(Farooq**, ***et al***., **2009**) and high biomass production. It is associated with high water and nutrients uptake. The genotypes that have a well-developed root system have the ability to reach residual moisture depth in the soil, as well as improving nutrient uptake by increasing the surface area. **Manske**, ***et al***., (**2000**) reported that the wheat genotypes that had higher root length density were able to take up more nutrients from soil especially phosphorus. The wheat genotypes that had grown under low moisture conditions used deeper root systems to reach soil moisture from the depths of soil (**Mian**, ***et al***., **1993**). In perennial plants, the drought avoidance mechanisms contribute to the survival of the plants and complete their life cycle. However, in annual crops such as wheat, these mechanisms reduce crops yield and productivity (**Rivero**, ***et al***., **2007**).

2.6.3 Drought tolerance

Drought tolerance means the ability of plant to withstand water-deficit with low tissue water potential (**Mitra**, **2001**). Tolerance to drought is a complex mechanism, because of the different interactions between drought stress and various physiological, biochemical and molecular processes, which affect plant growth (**Razmjoo**, ***et al***., **2008**).

Drought tolerance mechanisms

2.6.3.1 Osmotic adjustment

Among all adaptive mechanisms, the accumulation of compatible solutes (osmotic adjustment) has drawn much attention (**Mohammadkhani** and **Heidari**, **2008**). Osmotic adjustment is one of the most effective physiology mechanisms, which helps plant to resist drought (**Bhutta**, ***et al***., **2006**). As a response to drought stress, the water potential in plant root zone decreases and the osmotic potential increases. In order to maintain cell volume and turgor against dehydration stress, the plant cells synthesizes organic solutes as osmoprotectants. The plant adaptation to drought is associated with metabolic adjustments, which lead to accumulate kind of solutes, such as carbohydrate, betaines and proline (**Unyayar**, ***et al***., **2004**). The osmoprotectants are involved in signaling and regulate the plant responses to drought stress **(Farooq**, ***et al***., **2009**).

2.6.3.2 Molecular control mechanisms of drought stress tolerance

The drought tolerance mechanisms are based on expression of specific stress-related genes, which activate or deactivate as a response to drought. These genes could be divided into three major categories:-

A) Genes play role in signaling pathways and in transcriptional control, such as MAP kinases (**Zhu**, **2001**), and phospholipases (**Frank**, ***et al***., **2000**). Transcription factors (TFs), which considered gene activators; binds to specific sequence of promoter region of the target genes which will be activated as a response to drought (**Shinozaki** and **Yamaguchi**, **2000**). These promoter regions include dehydration responsive elements (DRE) and ABA responsive elements (ABRE's) which are involved as a response to drought. Dehydration-responsive element binding (DREB) proteins constitute a large family of transcription factors that are involved in abiotic stress tolerance. DREBs regulate many functional genes related to drought stress (**Ito**, ***et al***., **2006**). DREB genes consist of two subclasses, 1) DREB gene1, which induced by cold stress, and 2) DREB gene2, which induced by dehydration stress (**Choi**, ***et al***., **2002**). It is possible to engineer stress tolerance in transgenic plants by manipulating the expression of these

genes (**Agarwal**, ***et al***., **2006**). **Ito**, ***et al***., (**2006**) concluded that DREB1-type genes are useful for the improvement of stress tolerance to environmental stresses.

B) Genes involved in cell membrane stabilization and protein protection. Drought causes the activation of many genes that leading to accumulation of stress-induced proteins. Most of these proteins are soluble in water, and therefore contribute to stress tolerance by hydration of cellular structures (**Wahid**, ***et al***., **2007**). These proteins play a major role in protection of other proteins from degradation **(Farooq**, ***et al***., **2009**); also, they prevent protein denaturation during environmental stresses (**Gorantla**, ***et al***., **2006**). The stress-induced proteins could be divided into two groups (**Wang**, ***et al***., **2003**): 1) late embryogenesis abundant (LEA) proteins, and 2) heat shock proteins (Hsps). Both types play a significant role in protection of plant cell from the harmful effects of stress (**Wang**, ***et al***., **2003**). **Farooq**, ***et al***., (**2009**) reported that Hsps had significant roles in stabilizing structures of other proteins. The synthesis of these stress proteins associated with drought tolerance (**Taiz** and **Zeiger**, **2006**). **Mahajan** and **Tuteja**, (**2005**) reported that drought stress changed the expression levels of LEA /dehydrin- genes. The Hsps induced by different stresses such as drought, as well as high temperature (**Wahid** and **Close**, **2007**). **Close**, (**1997**) found that dehydrins, accumulated in response to dehydration stress.

C) Genes function in water uptake and transport such as aquaporins and ion transporters (**Blumwald**, **2000**). It is possible to improve the plant stress tolerance, through transformation of genes, which plays role in protection and maintenance the function of cellular components (**Wang**, ***et al***., **2003**).

2.6.3.3 Canopy temperature (CT)

As a result of decreasing soil water content, there is a significant rise in the temperature of plant leaves (**Shakya** and **Yamaguchi**, **2007**). There is a negative correlation between transpiration rate and leaf temperature. Leaf cooling is one of the important functions of transpiration process. Under drought conditions, the transpiration rate decrease due to stomatal closing and thus the leaf temperature increase. The change in leaf temperature can be important factor in controlling leaf water status under stress

conditions (**Farooq**, ***et al***., **2009**). Canopy temperature can be sensed remotely by using infrared thermometry. It has been associated well with the yield of wheat cultivars (**Fischer**, ***et al***., **1998**). The genotypes that maintain a lower canopy temperature as compared to other genotypes under drought stress conditions are probably able to resist drought. **Reynolds**, ***et al***., **(2001**) reported that the drought-susceptible genotypes showed warmer canopies than did the drought-tolerant genotypes. Furthermore, the CT showed a strong and reliable association with yield under drought stress conditions (**Saint Pierre**, ***et al***., **2010**). On the other hand, the canopy temperature utilized as a screening tool for predicting high -yielding wheat genotypes or as an important predictor of yield performance under drought (**Olivares-Villegas**, ***et al***., **2007**). The potential of CT as screening tool for wheat genotypes under drought-stress (**Rashid**, ***et al***., **1999**) based on its significant association with grain yield **(Reynolds**, ***et al***., **2001**).

2.6.3.4 Green leaf retention and photosynthetic pigments

Drought stress inhibits photosynthesis and accelerates leaf senescence (**Hafsi**, ***et al***., **2000**). Senescence is a type of cell death program (**Rivero**, ***et al***., **2007**). The ability to maintain the functionality of the photosynthetic machinery under drought stress is an important mechanism in drought tolerance. It could be possible to enhance drought tolerance of plant by delaying leaves senescence (**Rivero**, ***et al***., **2007**). On the other hand, the carotenoids play also a vital role in drought tolerance via, light harvesting and protection from oxidative damage. Thus, increased pigment contents in plants specifically carotenoids is very important for stress tolerance (**Jaleel**, ***et al***., **2009**).

2.6.3.5 Normalized differential vegetation index (NDVI)

NDVI basically based on the properties of the green leaf to absorb solar radiation in red (RED) spectrum through chlorophyll a, b and cell wall scatter (reflect and transmit) in near-infrared (NIR) spectrum through the spongy mesophyll. The NDVI is express as: $$\mathrm{NDVI} = \frac{R_{\mathrm{NIR}} - R_{\mathrm{RED}}}{R_{\mathrm{NIR}} + R_{\mathrm{RED}}}$$

Where, R_{RED} is the reflectance of red light at a wavelength of 670 nm, R_{NIR} is the reflectance of infrared light at 800 nm wavelength. NDVI is used widely as 1) standard vegetation index for estimating biomass (**Barbosa**, ***et al***., **1999**), 2) to estimate the plants under stress (**Johnsen**, ***et al***., **2009**), and 3) a stress indicator (**Fitz-Rodriguez** and **Choi**, **2002**). **Bahrun**, ***et al***., (**2003**) used NDVI to assess turfgrass plants under stress conditions.

Because of the close relationship between vegetation vigor and soil moisture, NDVI used in assessment of vegetation drought stress in arid and semi-arid regions (**Ji** and **Peters**, **2003**), also there is a positive correlation between NDVI and plant moisture content (**Fenstemaker-Shaulis**, ***et al***., **1997**). The NDVI was a better estimator of chlorophyll content in turfgrass (**Bell**, ***et al***., **2004**) but it was not a strong predictor of biomass (**Fenstemaker-Shaulis**, ***et al***., **1997**). **Kruse**, ***et al***., (**2005**) found that there is no correlation between biomass and NDVI. On the other hand, **Fenstemaker-Shaulis**, ***et al***., (**1997**) reported a negative correlation between NDVI and canopy temperature. In wheat, the measurements of NDVI at the milky-grain stage closely correlated to yield more than earlier measurements (**Royo**, ***et al***., **2003**). **Babar**, ***et al***., (**2006)** found that there was a positive correlation between NDVI and yield, especially if measured during the last part of the crop cycle. The NDVI measurements that taken between anthesis and milky stage were the best estimates of yield and final biomass, compared with other developmental stages (**Marti**, ***et al***., **2007**). The healthy vegetation gives high NDVI values, while the unhealthy plant gives low values (**Shakya** and **Yamaguchi**, **2007**).

2.7 mRNA differential display

The differential display technique is an important tool used to identify the differentially expressed genes between two cells or within a single cell under different conditions (**Liang** and **Pardee**, **1992**). In addition, it used to obtain gene expression profiles (**Canli**, **2007**). This technology includes several steps. The first step is to isolate mRNAs from plant cells and convert it to first strand cDNAs by using oligo-dT primers (reverse transcription). Then the cDNAs amplified by using a set of primers that are short and arbitrary in sequence and recognize 50-100 mRNAs (**Liang** and **Pardee**, **1992**). Next, the PCR products are visualized using gel electrophoresis; the comparisons

of such cDNA patterns between relevant mRNA samples reveal the differences in gene expression profiles. The differentially expressed fragment bands excised from the gel, and cloned and sequenced. The obtained sequences compared with the known sequences from databanks. **Cebeci**, (**2006**) used this method to obtain and identify cadmium responsive genes in durum wheat. Also **Isik**, (**2007**) used same technique to study gene expression in a Zn deficiency tolerant wheat genotype grown in varying conditions of Zn.

3 MATERIALS AND METHODS

In this research, we studied effects of drought stress on morphological, physiological and molecular characters of some bread wheat genotypes at different levels.

3.1 Materials

3.1.1 Plant materials

The plant materials used in these studies kindly provided by Egyptian agricultural research centre and by Anatolian agricultural research institute, Eskisehir, Turkey (**Tables 3.1**, **3.2**, and **3.3**).

Table 3.1: The bread wheat genotypes that were used in laboratory experiment.

Egyptian genotypes	Turkish genotypes
Gimeza-7	BVD-22
Giza-163	Bezosta
Sahal- 1	Bolal
Sakha-93	Ozcan
Sids-1	Seval

Table 3.2: The bread wheat genotypes that were used in greenhouse experiment.

Egyptian genotypes	Turkish genotypes
Sahal- 1	BVD-22
Giza-163	Ozcan

3.1.2 Growth media, stock solutions and buffers

All growth media, stock solutions and buffers that used during this study were prepared according to **Sambrook**, ***et al***., **(2001)**.

3.2 Methods

Laboratory and greenhouse experiments were conducted at Sabanci University, Istanbul, Turkey, during two successive seasons 2008 and 2009, to evaluate the effect of drought stress on some bread wheat genotypes.

3.2.1 Laboratory experiment

Ten bread wheat genotypes (**Table 3.1)** were used to study the effects of water stress, simulated by using polyethylene glycol (PEG) of molecular weight (MW) 6000 **(Fluka biochemika**). For surface sterilization, hand selected seeds were initially treated with 70% ethanol for 5min. followed by 5% sodium hypochlorite (bleach) for 10min., the residual chlorine was eliminated by washing the seeds 3 times with sterilized distilled water. Ten seeds were transferred to Petri dishes, containing 20ml of MS medium (**Murashige** and **Skoog**, **1962**). All Petri dishes were placed in a germination chamber at random to germinate in the dark condition for 72h., at $20^{o}C \pm 1^{o}C$. The seedlings were transferred to square Petri dishes, containing 20ml MS medium and 30ml of PEG 15% (w/v), for one week. All treatments were replicated two times.

3.2.1.1 Parameters

- **Root length** (mm)
- **Shoot length** (mm)
- **Fresh and dry weight of shoot** (mg)
- **Fresh and dry weight of root** (mg):- Dry weights were measured after drying samples at $70^{o}C$ in oven until a constant weight was achieved.

3.2.2 Greenhouse experiment

From the screening study, we selected four bread wheat genotypes (**Table 3.2**), for a greenhouse study.

Table 3.3: The bread wheat genotypes that were used in the open field experiment

G1	Altay2000	G26	Kirac66
G2	Aytin98	G27	Kirgiz95
G3	Bayraktar	G28	Kirkpinar79
G4	Bezostaya1	G29	Krc/bez/3/1150-18/vgdwf/4/ye2453
G5	Bolal2973	G30	Ks82w422/swm754308//ks831182/ks83w422
G6	Ca8055/krc66	G31	Ktk/ye2453
G7	Century	G32	Kutluk94
G8	Dagdas	G33	Lov/bll//mir264/5/pnc/cm//nb61977
G9	Ekg15//tast/sprw/3/2*id800994.w/vee	G34	Mnch/5/br12*¾/ias55*4/ci14123/3/ias55*4/...
G10	Es00-ke3	G35	Momtchill
G11	Es84-24//ks82w409/spn	G36	Momtchill/gun//gun
G12	Es84-24/seri//seri	G37	Mufitbey
G13	F12.71/coc//kauz	G38	Pastor
G14	F12.71/coc//prl"s"/vee#6/4/c126-15/cofn"s"/3/n10b/p14//p101	G39	Pyn/bau
G15	Flamura85	G40	Seval
G16	Gerek gm	G41	Sonmez01
G17	Gerek79	G42	Soyer
G18	Gun91	G43	Stk52/trumbull
G19	Harmankaya99	G44	Suzen97
G20	Hawk	G45	Tosunbey
G21	Ikizce96	G46	Vona//no57//probex/3/car/torım
G22	Izgi01	G47	Vorona/kauz
G23	Jagger	G48	Weston
G24	Karahan	G49	Zitnica
G25	Katia		

3.2.2.1 Greenhouse conditions

The temperature was maintained at 23/15°C ± 2°C during day/night regimes respectively.

3.2.2.2 Pot filling and soil fertilization

First, the plastic pots were washed with diluted HCl and rinsed in ddH_2O for 5min. After drying, 3000g of air-dried soil (pH: 8.04, $CaCO_3$ 14.9%, organic matter 0.69%, salt 0.08%, and clay 60.6%) was placed the pots. The wheat plants received fertilizers, the basal treatment was 200mg N/kg soil in the form of calcium nitrate Ca $(NO_3)_2$, 100mg P/kg soil as potassium diphosphate KH_2PO_4, 125mg K/kg soil in the form of potassium sulphate (K_2 SO_4), 2.5mg Fe/kg soil in the form of Fe-EDTA and 5 mg Zn/kg soil as $ZnSO_4.7H_2O$. The fertilizers mixed well with the soil.

3.2.2.3 Seeds

About 10 seeds were sown in each pot, spaced about 3cm apart, and slightly pressed into the topsoil. Then the seeds were covered with 2cm soil layer leaving a 3cm space for water application. The sowing date was 6 January 2009, after 15 days of emergence; the seedlings were thinned to five plants per pot.

3.2.2.4 Watering

In order to avoid the mineral content of tap water from influencing the results the plants were watered with distilled water throughout the growing period. The pots were shifted around randomly every 4 to 5 days in order to prevent exposure to different conditions that could have applied to different areas in the greenhouse.

3.2.2.5 Treatments

The bread wheat genotypes were subjected to water deficit induced by withholding the water supply in the soil for 7 days, during plant growth stages:-

- 40 Days after sowing (DAS)
- 60 Days after sowing (DAS)
- 80 Days after sowing (DAS)

3.2.2.6 Data measurement

Various parameters were used to measure the response of wheat grown under drought stress in greenhouse conditions.

3.2.2.6.1 RWC

Fully expanded leaves were excised from control and stressed plants and the fresh weights (FW) were immediately recorded; the leaves were soaked for 4h., in distilled water at RT under a constant light, and the turgid weight (TW) was recorded. After drying for 24h., at 80°C total dry weights (DW) were recorded. RWC was calculated according to **Barrs** and **Weatherley**, **(1962)** using the formula given below.

RWC = [(fresh weight – dry weight)/ (turgid weight- dry weight)] x 100.

3.2.2.6.2 Soil water content (SWC)

SWC was measured according to (**International Atomic Energy Agency**, **2008**). At each growth stage, three soil samples (10g) were taken from each treatment, left in a previously dried and weighed metal cans with lid. Soil and cans were weighed (wet soil), then dried in oven at 105°C overnight. Soil and cans were weighed again (dry soil), after cooling them down for 30 minutes at room temperature. SWC was calculated as follows:-

$$SWC\ \% = \frac{\text{Wet soil (g)} - \text{Dry soil (g)}}{\text{Dry soil (g)}} \times 100$$

3.2.2.6.3 Vegetative growth

-Plant height (cm)

Plant height was recorded from the base of the stem to the tip of the longest leaf during the vegetative growth phase and to the tip of the spike on the main shoot, after anthesis, using a measuring tape.

- Shoot fresh mass (g)

At each growth stage the shoots (leaves and stems) were harvested using a sharp scissor, the fresh mass was determined by using a digital scale.

- Shoot dry mass (g)

The shoots were placed in a paper bag and dried in an oven at 70ºC (±2ºC) for 72 hours, and then the dry mass was determined by using a digital scale.

- Number of leaves/plant

Three plants were taken from each plot, at each growth stage, the numbers of leaves were counted, and the average was taken.

3.2.2.6.4 Macro and micro element concentration

At each growth stage, the shoots were harvested and dried at 70°C and the dried samples were grounded. Approximately 0.4g of the grounded samples were ashed in a microwave by using 2ml Hydrogen peroxide (H_2O_2) and 5ml Nitric acid (HNO_3) for 1h.; then the ashed samples were analyzed for macro and micro elements concentration (Ca, K, P, S, Mg, Fe, Mn, Cu, and Zn), by using inductively coupled plasma optical emission spectroscopy (**ICP-OES**, **Varian**, **Australia**) at 214.44nm emission wavelength.

3.2.2.6.5 Proline determination

Approximately 0.5g from the second fully expanded leaves from of apices (to reduce sample variation) were harvested and immediately frozen in liquid nitrogen (N_2) and kept at -80^{o}C until they were analyzed. Extraction and determination of free proline were carried out according to **Bates**, ***et al***., (**1973**).The leaves of the control and stressed plants were homogenized in 10ml of 3% aqueous sulfosalicylic acid (SSA) and filtered through Whatman's no.2 filter paper. 2ml from the filtrate was mixed with 2ml of acid–ninhydrin and 2ml of glacial acetic acid (GAA) in falcon tubes. The mixture was placed in a water bath for 1h., at 100°C. After cooling, 4ml of toluene was added and strongly

shaken. The absorbance of the toluene phase was measured at 520nm. The appropriate proline standards (**Sigma Chemical Co**., **USA**) were included for calculation of proline in the sample. The content of proline was expressed as μg/g fresh weight.

3.2.2.6.6 Soluble carbohydrates content (SC) determination

A sample of 0.1g of dried leaves was shaken in 10ml 80% (v/v) ethanol. The insoluble fraction was washed with 5ml of 80% ethanol. All soluble fractions were centrifuged at 5000*g* for 10min. The supernatants were collected and stored at +4°C. Glucose was analyzed by reacting 0.5ml extract with 2.5ml freshly prepared anthrone and placed in a boiling water bath for 5min. (**Halhoul** and **Kleinberg**, **1972**). After cooling, the absorbance was determined at 625nm.

3.2.2.6.7 Lipid peroxidation

Lipid peroxidation was measured according to **Hodges**, (**1999**), by determining the level of malondialdehyde as indictor of lipid peroxidation. Approximately 0.5g of fresh leaf from control and stressed plants were grounded, using autoclaved mortars and pestles, under liquid nitrogen and homogenized with 10ml of 80% (v/v) ethanol. The homogenates were transferred to falcon tubes. The samples were centrifuged at 3000*g* at 4^{o}C for 15min., approximately 4ml from the aliquot, was transferred to two 10ml centrifuge tubes. 4ml of 20% TCA (w/v) + 0.01% BHT (w/v) solution (TCA: trichloroacetic acid, BHT: butylated hydroxytoluene) was added to the first tube, and 4ml of 20% TCA (w/v) + 0.01% BHT (w/v) + 0.65% TBA (w/v) solution (TBA: 2-thiobarbituric acid) was added to the second tube, all tubes were vortexed and incubated at 95^{o}C in water bath for 25min., the samples were immediately cool-down by using ice and centrifuged at 3000*g* at 4^{o}C for 15min. The absorbance was measured at 532nm and 600nm for the first tube and at 532, 600 and 440nm for the second tube. The results were calculated as follows:

1. $[(ABS\ 532_{+TBA})-(ABS\ 600_{+TBA})]-[(ABS\ 532_{-TBA})-(ABS\ 600_{-TBA})]=A$
2. $[(ABS\ 440_{+TBA}-ABS\ 600_{+TBA}) \times 0.0571] = B$
3. MDA equivalents (nmol ml^{-1}) = $(A-B / 157\ 000) \times 10^{6}$

ABS: absorbance, MDA: malondialdehyde.

3.2.2.6.8 Antioxidant enzymes

Approximately, 1g of fresh leaf samples were homogenized using mortar and pestle, in 12ml of ice-cold 50mM phosphate extraction buffer (pH 7.6) containing 0.1mM Titriplex (Na-EDTA). The homogenized samples were transfered to falcon tubes 15ml and centrifuged at 4600g for 15min, the pellet were discarded and the supernatants were transfrered to anthoer falcon tubes and centrifuged again at 15000g for 15min. Resultant supernatant was used for enzyme analysis. All operations until analysis were carried out at +4°C except SOD enzyme, all enzyme activities were measured in a final volume of 1 ml using various aliquots of the supernatants. The Ascorbate peroxidase (AP) activity was determined as outlined by **Cakmak**, (**1994**), while the activity of other enzymes glutathione reductase, superoxide dismutase, and catalase were measured according to **Cakmak** and **Marschner**, (**1992**).

3.2.2.6.9 mRNA differential display (mRNA DD)

Total RNA Isolation

Trizol reagent (**Invitrogen**) has been used to isolate total RNA from wheat samples, approximately 0.3g of leaves samples were grounded with 1.7ml Trizol, using autoclaved mortars and pestles in liquid nitrogen. The homogenates were incubated for 5min., at RT., and then 1ml of homogenate was transferred to eppendorf tube, which kept, on ice while the other samples were being homogenized with Trizol. 0.2ml of chloroform was added to each tube and the tubes were shaken and incubated at RT for 5min. then, the samples were centrifuged at 12,000g for 15min. at 4°C. Approximately 500µl of the upper aqueous phase containing RNA was transferred to a 1.5ml micro-centrifuge tube. Then, 0.5ml of isopropanol was added to precipitate RNA. The tubes were mixed gently and then incubated at RT for 10min., and centrifuged at 12,000g for 15min. at 4°C. The supernatants were discarded and the pellets were washed with 1ml ethanol (75%). Then the samples were vortexed and centrifuged at 7,500g for 5min., at 4°C. The supernatants were discarded and the RNA pellets were air-dried for 15min. Then the pellets were dissolved in 50µl diethyl pyrocarbonate (DEPC)-treated H_2O and incubated in water bath at 55°C for 1h.

The qualities of the isolated RNAs were verified on a 2% agarose gel. The concentrations of RNAs were calculated by using Nanodrop spectrophotometer, at 260nm wavelength.

The first strand of cDNA was synthesized from total RNA samples by using M-MuLV Reverse Transcriptase (**Fermentas**). The reaction mix contained 1.5mg of total RNA, 1µl oligo$(dT)_{18}$ primer (0.5µg/µl), 4µl reaction buffer (5X), 0.5µl RNase inhibitor, 2µl dNTP Mix (10mM), 1µl M-MuLV reverse transcriptase and completed the volume to 12.5µl by DEPC-treated water. The tubes were incubated at 37°C for 1h., followed by 10 min. at 70°C (to terminate the reaction). The cDNAs samples were kept and stored at -20°C until use. The ss-cDNAs samples were subjected to mRNA DD method by using 72 possible combinations of forward (P) and reverse (T) primer. The primers were purchased from Biogen. The sequences of these primers are listed in **Table 3.4**.

Table 3.4: Primers that were used in mRNA differential display

Primer	Sequence (5' - 3')
P1	ATT AAC CCT CAC TAA ATG CTG GGG A
P2	ATT AAC CCT CAC TAA ATC GGT CAT AG
P3	ATT AAC CCT CAC TAA ATG CTG GTG G
P4	ATT AAC CCT CAC TAA ATG CTG GTA G
P5	ATT AAC CCT CAC TAA AGA TCT GAC TG
P6	ATT AAC CCT CAC TAA ATG CTG GGT G
P7	ATT AAC CCT CAC TAA ATG CTG TAT G
P9	ATT AAC CCT CAC TAA ATG TGG CAG G
T1	CAT TAT GCT GAG TGA TAT CTT TTT TTT TAA
T2	CAT TAT GCT GAG TGA TAT CTT TTT TTT TAC
T3	CAT TAT GCT GAG TGA TAT CTT TTT TTT TAG
T4	CAT TAT GCT GAG TGA TAT CTT TTT TTT TCA
T5	CAT TAT GCT GAG TGA TAT CTT TTT TTT TCC
T6	CAT TAT GCT GAG TGA TAT CTT TTT TTT TCG
T7	CAT TAT GCT GAG TGA TAT CTT TTT TTT TGA
T8	CAT TAT GCT GAG TGA TAT CTT TTT TTT TGC
T9	CAT TAT GCT GAG TGA TAT CTT TTT TTT TGG

Amplification of cDNA fragments were performed in 50µl PCR reactions by using the ss-cDNAs as template and different combinations of P and T primers (72 combinations). Each reaction contained; 37.8µl ddH_2O, 1µl cDNA template, 5µl Taq buffer 10X (with $(NH_4)_2SO_4$), 3µl $MgCl_2$ (25mM), 1µl dNTP mix (10mM), 1µl of P primer (10µM),1µl of T primer (10µM) and 0.2µl Taq DNA polymerase (**Fermentas**). All mRNA differential display PCR reactions were carried out in a DNA thermocycler GeneAmp PCR System 9700 (**PE Applied Biosystems**) with the conditions written in **Table 3.5**.

Table 3.5: mRNA differential display PCR conditions

	Temperature	Time
1. Heating Lid:	105ºC	
2. Denaturation :	94ºC	4 min.
3.Non-specific annealing:	40ºC	5 min.
4. Extension:	72ºC	5 min.
5. Denaturation:	94ºC	1 min.
6.Non-specific annealing:	40ºC	1 min.
7. Extension:	72ºC	5 min.
8. Go to 5 Repeat cycle 1 time		
9. Denaturation:	94ºC	40 s.
10. Annealing:	58ºC	45 s.
11. Extension:	72ºC	2 min.
12.Go to 9 Repeat cycle 35 times		
13. Final elongation	72ºC	7min.

DNA extraction from agarose gels

50µl from the PCR products of mRNA DD were separated on 2% agarose gel using 0.5X TEB buffer. The differentially expressed fragment bands were excised from the gel and extracted by using QIAquick Gel Extraction kit (**Qiagen**) according to manufacturer instructions. The absorbance of fragments was determined by using the Nanodrop.

Re-amplification of the differentially expressed fragments

The eluted fragments were reamplified by using the same sets of primers and the same PCR conditions. The PCR products were run on 1% agarose gel for 40min. at 100 mV.

Ligation and Transformation

Ligation

The extracted fragments were ligated into pGEM-T easy vector (**Promega**), according to the manufacturer's protocol. The ligation reaction was contained 1µl 50ng diluted vector (1:5), 5µl ligation buffer (2X), 1µl T4 ligase enzyme (3unit), and 3µl cDNA insert. Then the reactions were incubated for 1 hr. at RT.

Transformation

E.coli competent cells (DH5α strains), were used for transformation. 5µl from ligation reaction were mixed gently with 50µl of competent cells in 1.5ml eppendorf tubes and incubated for 20min. on ice, then the tubes were incubated for 50 second at 42 ºC (for heat shocking), after that the tubes were incubated for 2 min. on ice. Then 950µl of Luria Bertani (LB) medium were added to the tubes and incubated with shaking for 1h. at 37ºC. After that the tubes centrifugated at 5,000rpm for 3min., 900µl from the supernatant was eliminated, and the pellet was re-suspended again and spread on LB plates using sterilized glass beads. Since the vector contains ampicillin resistance and LacZ genes, so the plates were contained ampicillin (100µg/ml), IPTG (100µl to each plate), and X-Gal (20µl to each plate). The plates were incubated at 37ºC for 16h.

Colony selection and Colony PCR

Depending on blue-white selection property of the pGEM-T easy vector, the positive white clones from transformation plates were chosen in three replicates (A, B and C). To confirm whether these positive clones have the correct fragments, colony PCR reaction has been done with the same combination of primers that were used in mRNA differential display.

Plasmid isolation

One colony of the positive white clones was transferred to sterile culture tube containing 5ml of LB broth medium containing 100µg/ml ampicillin. The culture tubes were incubated with shaking at 270rpm and 37°C for 16h. Then the bacterial cells were transferred to eppendorf tubes 2ml (3 tubes to each culture tube) and centrifuged at 8,500xg for 3min. The plasmids were isolated by using QIQprep spin minipreps kits and according to manufacturer instructions (**Qiagen**).The isolated plasmids concentrations were checked by using Nanodrop spectrophotometer at 260nm and 1% agarose gel for 40min. at 100V.

Restriction enzyme digestion

The pGEM-T easy vector has two EcoRI recognition sites, so to check if the plasmids containing the required fragments, the plasmids were digested with EcoRI restriction enzyme (**Promega**), and run on 1% agarose gel. The samples were kept at -20°C until send for sequencing.

Sequencing analysis

The differential expressed cDNA fragments were sequenced by using M13 forward primer. The sequence analyses were commercially provided by RefGen Company (**Ankara**). To eliminate vector contamination; the sequences were exposed to **VecScreen** algorithm (www.ncbi.nlmn.nih.gov). Then the sequences were compared with nucleotide and protein sequence databases using BLASTN (EST database), and BLASTX algorithms (NCBI).The open reading frames of the sequences were detected by using ORF finder tool of NCBI, and the amino acid sequences of the corresponding proteins were obtained. The protein sequences were searched for known protein motifs using Motif Scan algorithm of SIB-Swiss Institute of Bioinformatics by searching against PROSITE patterns, PROSITE patterns (frequent match producers), PROSITE profiles, Prefile (more profiles), Pfam HMMs (local models), Pfam HMMs (global models) databases (http://myhits.isb-sib.ch/cgi-bin/motif_scan). For pair wise and multiple alignments, ClustalW and ClustalX (EBI) algorithms were used.

Dreb

Dehydration-responsive element binding (DREB) proteins constitute a large family of transcription factors that are involved in a biotic stress tolerance. DREBs regulate many functional genes related to drought stress (**Ito**, ***et al***., **2006**). The cDNAs samples were performed in 50µl PCR reactions by using the ss-cDNAs as template and different dreb primers. Each reaction contained; 37.8µl ddH_2O, 1µl cDNA template, 5µl Taq buffer 10X (with $(NH_4)_2SO_4$), 3µl $MgCl_2$ (25mM), 1µl dNTP mix (10mM), 1µl of F dreb primer (10µM),1µl of R dreb primer (10µM) and 0.2µl Taq DNA polymerase (**Fermentas**). All reactions were carried out in a DNA thermocycler GeneAmp PCR System 9700 (**PE Applied Biosystems**).The sequences of dreb primers are listed in **Table 3.6**.

Table 3.6: Dreb primers sequences that were used in this study

DREB primers		Seq.
DERB 1A	R	5-ACG AAG GGC TAA AGC GGC AA-3
	F	5-TAT ACA GAG GAG TTC GTC GG-3
DERB 2A	R	5- CGG AGA AGG GTT TAG ATT CA-3
	F	5- ACC AAG AAG AGG AAA GTA CC-3
DERB 3A	R	5- CCA CGT ACA ACA CCT TCA AT-3
	F	5- GAA GTG AAC CAA CAA CTG GA-3
DREB 2B	R	5- GCT TCA CAC AAA CAT CAC CA-3
	F	5- GGC TGT TAA AGA AGG AGA GA-3
DREB 1	R	CTC GAG CTA ATA TGA GAA AAG ACT AAA CCC ATC ATC A
	F	GTC GAC ATG GAG ACC GGG GGT AG
DERB 3a	R	ACA ACC CTT CGA AGA ACT
	F	ATG ACG GTA GAT CGG AAG

3.2.2.6.10 Experimental design and Statistical analysis

The pots were arranged in completely randomized design (CRD) with three replicates. The data were statistically analyzed as completely randomized design; the results were expressed as means with standard deviation (±S.D.). The treatment means were compared using the least significant difference of the means; the significant difference (at $P < 0.05$) was evaluated by analysis of variance (ANOVA) by using GenStat Discovery Edition 3.

3.2.3 Open field experiment

This study was carried out at Anatolian agricultural research institute, Eskisehir, Turkey, during 2008/2009 season to evaluate the response of 49 bread wheat genotypes to drought stress under open field conditions. The experiment was conducted in a clay soil. The soil was analyzed in soil and water resources management institute, Eskisehir, Ministry of Agriculture and Rural, Turkey. Its chemical and physical characteristics presented in **Table 3.7**.

Table 3.7: Physical and chemical properties of the experimental soil

A. Mechanical analysis

Depth	Sand%	Silt%	Clay%	Texture
0 – 30cm	23.48	29.89	46.63	C
30 - 60	18.63	29.93	51.44	C
60 - 90	15.72	30.13	54.15	C

B- Chemical properties

Depth	pH (1: 2.5)*	Total Salt	O.M %	Phosphorus P_2O_5 kg/da**	Potassium K_2O kg/da	$CaCO_3$ %
0 – 30cm	8.39	0.131	0.17	1.71	78.8	13.0
30 - 60	8.67	0.134	0.15	0.52	43.6	13.4
60 - 90	8.64	0.142	0.18	0.45	52.4	10.5

*Soil suspension 1: 2.5 Soil: water

**1 da =1000 m2 = 0.1 hectare

The field moisture capacity and wilting pointing were of 36.4% and 9.6% volume, respectively.

3.2.3.1 Meteorological data

The average temperature and average rainfall during the growing season of the wheat crop are displayed in **Tables 3.8** and **3.9**.

Table 3.8: The rainfall measurement (mm/month)

Years	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Total Annual
2007-08	0.0	19.2	92.4	49.9	15.7	1.0	42.4	38.5	11.7	9.3	0.0	5.5	285.6
2008-09	30.7	6.4	49.6	34.5	66.3	82.0	40.9	28.0	15.4	10.2	19.4	2.0	385.4

3.2.3.2 Cultivars

To evaluate the response of bread wheat cultivars to drought stress conditions, seeds of forty-nine bread wheat (*Triticum aestivum*) were used (**Table 3.3**). The seeds sown in 20 October 2008, the sown rate was 450-500 seeds/m^2.

Table 3.9: Meteorological data

Month	Air temp. (C°)	Humidity RH%	Soil temp. (C°)	Soil Moi.	Radiation	Wind speed Km/h
Sep. 2008	17.3	75.0	16.0	120.3	41.0	2.4
Oct. 2008	11.2	86.6	11.4	193.4	27.6	1.9
Nov. 2008	6.6	90.0	5.9	74.3	17.8	1.8
Dec. 2008	2.9	96.2	2.5	32.6	13.4	2.0
Jan. 2009	2.6	97.3	2.3	22.3	14.5	1.7
Feb. 2009	2.8	94.0	2.4	22.5	16.5	2.3
Mar. 2009	3.2	90.3	3.5	21.8	25.5	2.4
Apr. 2009	8.9	82.6	9.0	117.4	37.2	2.0
May.2009	13.4	79.3	14.7	58.8	39.3	1.9
Jun. 2009	18.6	71.3	19.4	109.3	45.7	1.6
Jul. 2009	20.9	72.0	21.9	195.3	56.2	2.1
Aug. 2009	19.9	68.6	20.8	199.5	55.5	2.6

*Meteorological Laboratory, Anatolian Agricultural Research Institute, Eskisehir

3.2.3.3 Type of fertilizers and their application

The fertilizer management affects crop productivity under drought stress conditions and thus, the addition of nutrients can either enhance or decrease plants resistance to drought or have no effect, depending on the level of water availability (**Hu** and **Schmidhalter**, **2005**).Wheat plants (*Triticum aestivum)* received different levels of N and P as ammonium nitrate (33% N) and Di-ammonium phosphate (DAP) 18-46-0. The nitrogen fertilizers were added in two equal doses. The first application was added

at planting time, whereas the second one was applied in the early spring at 31 March 2009 (**Table 3.10**).

Table 3.10: Application of fertilizers

	Rainfall	Supplement irrigation
Ammonium nitrate	7 kg/da	9 kg/da
DAP	6 kg/da	6 kg/da

3.2.3.4 Herbicides control

2.4-D being the principal chemical used on wheat, it was add in 9 April 2009.

3.2.3.5 Treatments

1- Supplement irrigation	1st irrigation was at 1st November 2008 for 3.5h. 2nd irrigation was at 20 May 2009 for 4h.
2- Rainfall	Without irrigation

3.2.3.6 Harvesting

The wheat plants were harvested in 17 July 2009.

3.2.3.7 Experimental design

The experiment was in balanced lattice design with three replicates. The plot size was 1.2m width, 5m long (6m² plot areas), each plot contained six rows at 20cm apart, and each row contained 10 plants at 5cm apart.

3.2.3.8 Parameters measured

None of the parameters and observations was taken on plants in the border rows.

a- Plant height (cm)

Plant height was measured on ten randomly selected from the base of plant to the tip of the spike, by using a measuring tape.

b- Biomass and yield components

Total biomass and yield components were measured according to **Hobbs** and **Sayre**, **2001**). All aboveground biomass in a specific area (50cm) were cut, the border effects were avoided by sampling away from edges of plot. The sub-sample of tillers was selected randomly from the plot sample (50 tillers) and the fresh weight was measured (sub-sample fresh weight). Then the fresh weight of remaining plot sample (plot fresh weight) was measured.

The sub-sample of tillers were put in closed bags to avoid loss of grains, and then dried at 70ºC. The dry weight was measured (sub-sample dry weight). The plot sample was threshed for fresh grain weight (plot grain weight). One hundred grain was randomly selected; the fresh and dry weights were recorded (100-grain fresh and dry weights, or 100fw/dw). The Harvest index (%), Yield, Biomass, 1000- GW (g), Number of spikes m^{-2}, Number of grains m^{-2}, and Number of grains $spike^{-1}$, were measured according to **Table 3.11**.

Table 3.11: Harvest index, Biomass and Yield components

Harvest index (HI)	PGW*(100dw/100fw)/Pfw*(SSdw/SSfw)
Yield (g m^{-2})	[(PGW*100dw/100fw) + (SSdw*HI)]/A
Biomass (g m^{-2})	[(Pfw+SSfw)*(SSdw/SSfw)]/A
1000-GW (TGW) (g)	100dw*10
Tillers dw (g)	SSdw/ 50 (no. of tillers)
Number of spikes m^{-2}	Biomass/ $tillers^{-dw}$
Number of grains m^{-2}	Yield/TGW*1000
Number of grains $spike^{-1}$	Grains m^{-2}/spike m^{-2}

PGW= plot grain weight, Pfw= plot fresh weight, SSdw= sub-sample dry weight, SSfw= sub-sample fresh weight, A = plot area harvested (50 cm).

c- Heading date: The date when 50% of shoots had reached this stage recorded for each replicate.

d- Normalized difference vegetation index: NDVI was measured with a hand-held Green Seeker (NTech Industries Inc. 888-728-2436 Stillwater, Oklahoma, USA) on 8 and 17 June. The Measurements were taken around midday on sunny days by passing the sensor over the plots at a height of approximately 40–50 cm above the canopy.

e- Flag leaf stay green duration and chlorophyll content measurements: Chlorophyll contents (mean of six readings per leaf -10 plants per plot) measured using a Minolta SPAD-502 meter (**Spectrum Technologies Inc., Plainfield, IL.**) during 9 and 16 June.

f- Canopy temperature

Canopy temperature was red using handheld infrared thermometer (**Model no. 39650-20, Cole-Parmer Instruments, Co., Chicago, Ill., USA**) at sunny days. At least two readings were collected for each replicates, the thermometer was held so that the sensor viewed only the canopy at an oblique angle above the horizontal, and this position gave an elliptical canopy target (**O' Toole** and **Real, 1984**) and prevented the thermometer from sensing the soil surface when the leaves were rolled. Cloudy or windy conditions were avoided because it had effects on leaf temperature. Canopy temperature measurements were taken at 2 and 8 June between noon and 2:00 p.m.

g- Drought susceptibility index for each genotype (DSI)

It provides a measure of stress resistance based on minimization of yield loss under stress as compared to non-stress conditions (**Fischer** and **Maurer, 1978**) from the following formula:

$$DSI = \frac{1- (Y_d / Y_w)}{D}$$

Where $\mathbf{Y_d}$ (mean grain yield under drought stress conditions), $\mathbf{Y_w}$ (mean grain yield under non-stress conditions), and **D** (stress intensity) = 1 - (mean $\mathbf{Y_d}$ of all genotypes / mean $\mathbf{Y_w}$ of all genotypes).

h- Relative yield (RY)

The relative yield was calculated as the yield of a specific genotype under drought divided by that of the highest yielding genotype in the population.

3.2.3.9 Statistical analyses

The results were expressed as means with standard deviation (±S.D.). The treatment means were compared using the least significant difference of the means; the significant difference (at $P < 0.05$) was evaluated by analysis of variance (ANOVA by using GenStat Discovery Edition 3. Phenotypic relationships among traits were investigated using regression /correlation analysis.

4 RESULTS

In this study, the response and growth of wheat genotypes to drought stress conditions has been evaluated, in laboratory, greenhouse and open field environments. The study covered morphological, physiological and molecular aspects of the drought response.

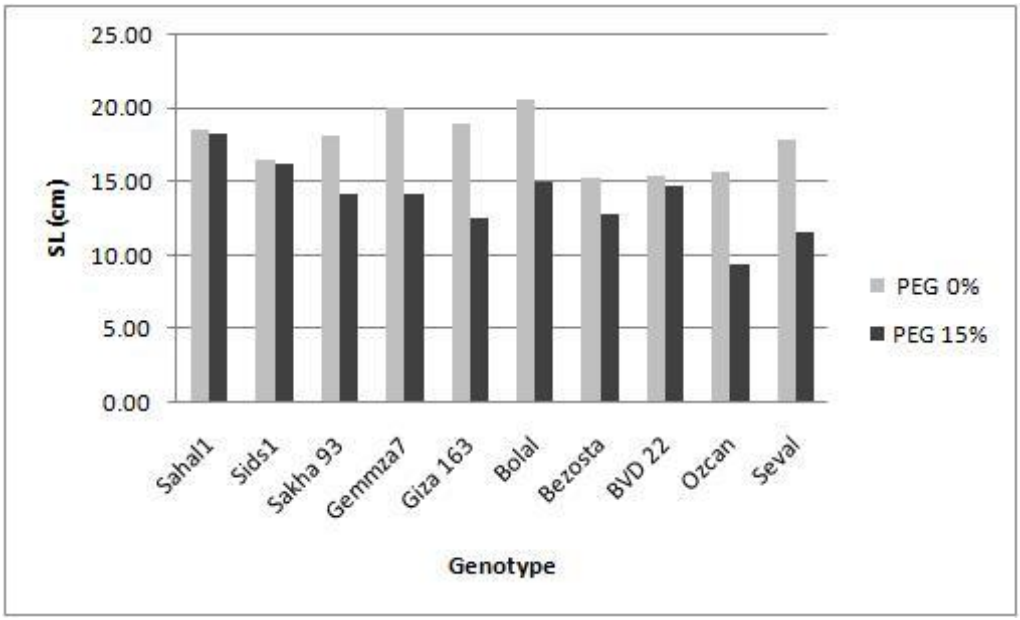
4.1 Laboratory experiment

In laboratory experiment, water deficiency was simulated by polyethylene glycol (PEG) of MW 6000 in the following concentrations viz., 0, and 15 % (w/v).

4.1.1 Effects of drought stress induced by polyethylene glycol on Egyptian and Turkish wheat genotypes

The response of ten wheat genotypes to chemical desiccation induced by PEG 6000 during the seedling stage is shown in **Table 4.1**. Generally, the genotypes differed in their response to stress. The PEG treatment caused inhibition to all measured traits. The PEG treatment decreased, shoot and root length (**Fig.4.1**), shoot and root fresh weight (**Fig.4.2**), shoot and root dry weight, of all genotypes.

Among the Egyptian genotypes, Sahal-1 showed a good performance in all traits under stress conditions (**Fig.4.3**), while Giza-163 recorded the worst performance in all traits except shoot fresh weight and root dry weight. On the other hand, Ozcan recorded the greatest reduction in all traits among all Turkish genotypes, while the lowest reduction was observed in BVD-22 genotype. **Bayoumi**, ***et al***., (**2008**) demonstrated similar results.



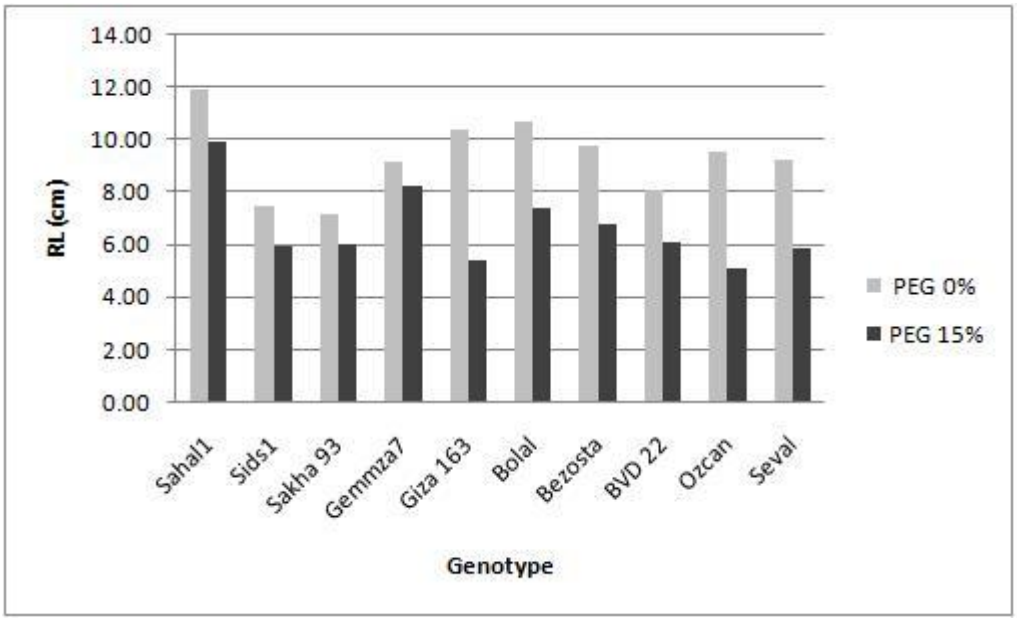


Fig. 4.1: Effect of drought stress induced by PEG 6000 on shoot and root length (cm) of ten *Triticum aestivum* genotypes.

4

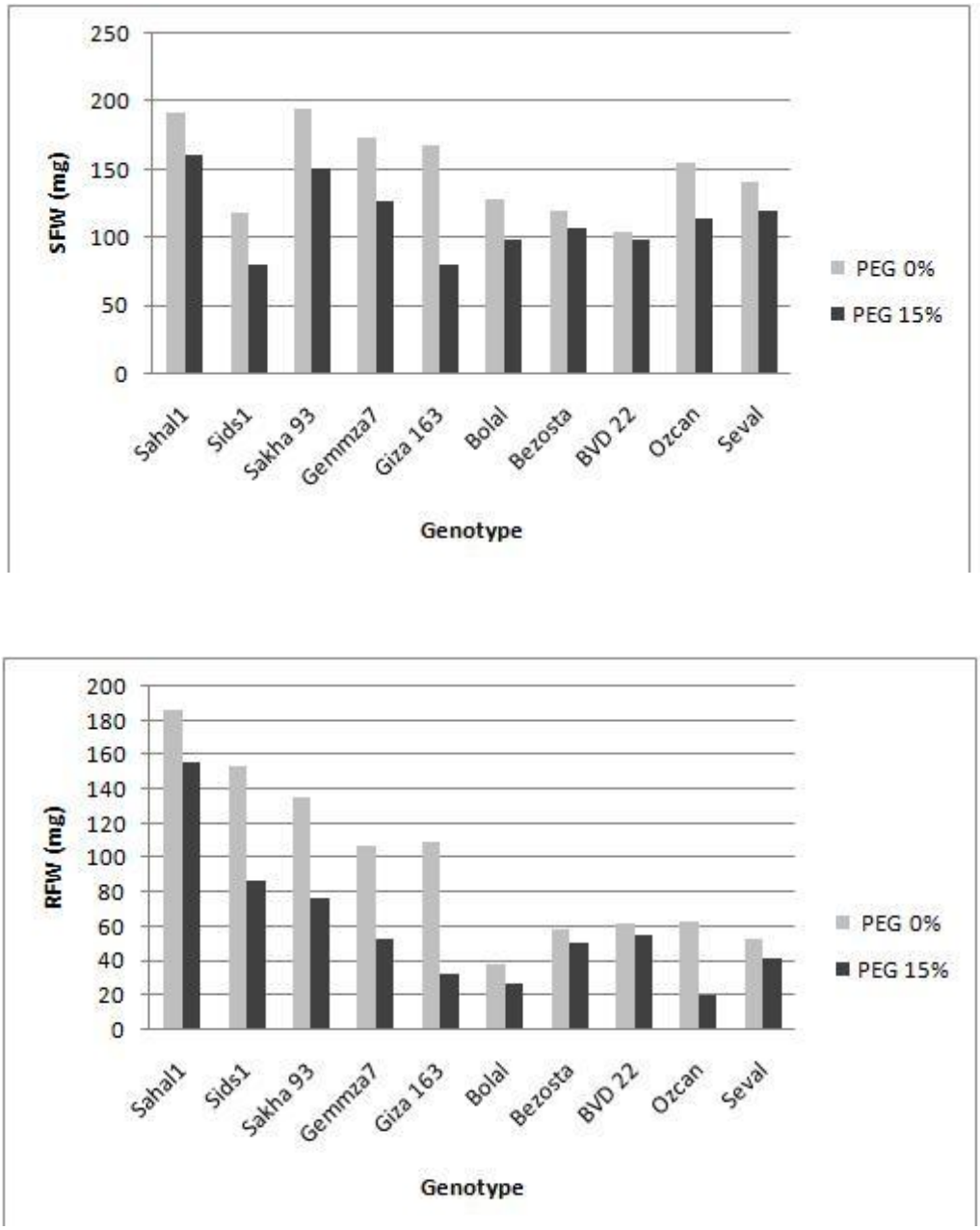


Fig. 4.2: Effect of drought stress induced by PEG 6000 on shoot and root fresh weight (mg) of ten *Triticum aestivum* genotypes

Fig. 4.3: Effect of drought stress induced by PEG 6000 on shoot and root length of four *Triticum aestivum* genotypes.

Table 4.1: Effect of drought stress induced by PEG on some Egyptian and Turkish wheat seedling traits.

Genotype		Control PEG 0%						PEG 15%						Differ. %					
		SL (cm)	RL (cm)	SFW (mg)	RFW (mg)	SDW (mg)	RDW (mg)	SL (cm)	RL (cm)	SFW (mg)	RFW (mg)	SDW (mg)	RDW (mg)	SL	RL	SFW	RFW	SDW	RDW
Sahal-1		18.50	11.93	192.0	186.0	34.1	44.9	18.30	9.90	160.0	155.5	32.8	39.7	0.01	0.17	0.17	0.16	0.04	0.12
Sids1		16.43	7.43	118.5	153.3	32.5	23.9	16.15	5.90	80.0	86.3	27.9	16.2	0.02	0.21	0.32	0.44	0.14	0.32
Sakha-93		18.20	7.13	194.3	135.0	38.7	42.8	14.20	6.00	150.7	75.9	32.5	29.5	0.22	0.16	0.22	0.44	0.16	0.31
Gemmza7		20.10	9.15	173.3	107.0	34.0	40.0	14.15	8.20	126.3	52.0	28.1	28.7	0.30	0.10	0.27	0.51	0.17	0.28
Giza-163		18.90	10.35	167.3	109.0	30.2	30.8	12.50	5.35	80.2	32.3	23.9	19.1	0.34	0.48	0.52	0.70	0.21	0.38
Bolal		20.60	10.70	127.3	38.0	28.2	18.2	14.95	7.40	97.3	26.0	25.0	14.7	0.27	0.31	0.24	0.32	0.11	0.20
Bezosta		15.27	9.80	119.3	58.5	26.2	29.3	12.73	6.77	106.7	50.0	20.9	23.5	0.17	0.31	0.11	0.15	0.20	0.20
BVD -22		15.45	8.10	103.7	61.0	31.7	25.2	14.65	6.10	97.5	54.5	31.3	24.7	0.05	0.25	0.06	0.11	0.01	0.02
Ozcan		15.70	9.57	155.3	63.0	47.5	30.2	9.30	5.10	113.7	19.0	17.2	11.4	0.41	0.47	0.27	0.70	0.64	0.62
Seval		17.90	9.20	140.0	52.3	36.0	26.7	11.57	5.83	120.0	40.6	23.0	16.6	0.35	0.37	0.14	0.22	0.36	0.38
L.S.D.	**Genotype**	0.95	1.44	37.34	59.33	8.89	9.07												
	Treatment	0.42	0.64	16.70	26.53	3.98	4.05												
	G X T	1.34	2.04	52.80	83.91	12.57	12.82												

* The data represent mean of two replicates, l.s.d.: least significant differences of means (5% level), SL = Shoot length, RL = Root length, SFW = Shoot fresh weight, RFW = Root fresh weight, SDW = Shoot dry weight, RDW = Root dry weight, Differ. = values represent decrease percent as compared to normal conditions.

4.2 Greenhouse experiment

From the laboratory experiment data, Sahal-1 and BVD-22 genotypes showed better performance under drought stress compared with other genotypes; in contrast, Giza-163 and Ozcan genotypes recorded the worst performance. These genotypes have been selected and grown in greenhouse under controlled environmental conditions.

4.2.1 Effect of drought stress on wheat growth and development

4.2.1.1 Effect of drought stress on wheat height (cm)

As described in **Table 4.2**, plant height increased with age under all conditions except BVD-22 genotype at 80 DAS under control conditions. Generally, drought stress significantly influenced the plant height of all wheat genotypes. Among all genotypes, Giza-163 was the tallest under both well watered and drought stress conditions (71.23 and 64.78cm respectively); in contrast Ozcan was the shortest one (**Fig.4.4**). The results were in accordance with those reported by **Mirbahar**, ***et al***., (**2009**).

4.2.1.2 Effect of drought stress on relative water content (RWC)

The wheat genotypes differed in their response to drought stress for RWC; the RWC decreased with age in all genotypes (**Table 4.2**). **Sairam** and **Saxena**, (**2000**) reported that the relative water content under irrigated and stress conditions showed a decreasing trend with age in all wheat genotypes. Generally, exposure to drought resulted in decline in RWC in all wheat genotypes when compared to irrigated plants. Under drought stress conditions, Sahal-1 had the highest RWC value of 25.8%. In contrast, Ozcan recorded the lowest RWC value (19%). Similar results were obtained by **Sibel** and **Birol**, (**2007**).

Table 4.2: Effect of drought stress on plant height (cm) and relative water content (%) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		Ph (cm)			RWC (%)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal-1	**Irrigated**	61.55 ± 2.8	71.49 ± 0.5	71.58 ± 1.4	68.89 ± 3.1	47.15 ± 2.4	31.57 ± 4.9
	Stress	52.18 ± 1.5	67.96 ± 1.9	69.39 ± 0.5	44.03 ± 2.2	25.91 ± 9.2	7.53 ± 0.0
	Average	56.87	69.73	70.49	56.46	36.53	19.55
Giza-163	**Irrigated**	67.83 ± 2.1	71.91 ± 2.0	73.95 ± 3.3	67.33 ± 4.3	47.72 ± 4.2	37.47 ± 1.9
	Stress	55.84 ± 0.9	68.79 ± 2.2	69.71 ± 1.6	38.08 ± 2.1	21.47 ± 6.7	6.67 ± 0.3
	Average	61.84	70.35	71.83	52.71	34.60	22.07
Ozcan	**Irrigated**	53.08 ± 1.4	56.69 ± 2.4	56.98 ± 4.3	65.38 ± 0.4	59.03 ± 3.1	47.37 ± 1.4
	Stress	50.88 ± 1.1	52.31 ± 2.9	52.48 ± 2.1	31.94 ± 1.4	19.56 ± 2.3	5.62 ± 0.4
	Average	51.98	54.50	54.73	48.66	39.30	26.5
BVD-22	**Irrigated**	59.91 ± 0.8	70.75 ± 0.6	66.78 ± 3.2	71.19 ± 3.1	68.26 ± 2.6	57.37 ± 2.5
	Stress	51.15 ± 2.4	63.33 ± 1.2	64.55 ± 1.6	42.72 ± 2.6	22.10 ± 1.2	7.23 ± 0.1
	Average	55.53	67.04	65.67	56.96	45.18	32.30
Irrigated	**Average**	60.59	67.71	67.32	68.20	55.54	43.44
Stress	**Average**	52.51	63.10	64.03	39.19	22.26	6.76
Grand mean		56.55	65.41	65.68	53.70	38.90	25.10
l.s.d.	**Genotype**	1.21			2.76		
	Stage	1.04			2.39		
	Treatment	0.85			1.95		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

Sahal- 1

Giza-163

Ozcan

BVD-22

Fig. 4.4: Effect of drought stress on plant height of four *Triticum aestivum* genotypes, the genotypes were exposed to drought stress at 40 days after sowing (DAS).

4.2.1.3 Effect of drought stress on number of leaves per plant (NLP)

Drought stress significantly influenced the leaves number per plant of all wheat genotypes. Among all genotypes, Sahal-1 had the lowest number of leaves under drought stress conditions, with seven leaves, while Ozcan recorded the highest number with 17 leaves (**Table 4.3**). Similar results were obtained by **Fuzhong**, ***et al***., (**2008**).

Table 4.3: Effect of drought stress on number of leaves per plant and soil water content (%) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		NLP			SWC (%)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal-1	**Irrigated**	8.3 ± 1.0	8.3 ± 0.3	8.9 ± 1.5	19.2 ± 0.6	20.5 ± 0.4	21.1 ± 2.0
	Stress	6.8 ± 0.6	6.8 ± 0.5	7.6 ± 0.5	10.0 ± 0.3	9.4 ± 0.2	7.9 ± 0.7
	Average	7.5	7.5	8.3	14.6	15.0	14.5
Giza-163	**Irrigated**	9.4 ± 0.5	10.5 ± 0.4	12.5 ± 1.8	18.3 ± 0.2	21.3 ± 1.5	21.7 ± 0.3
	Stress	7.5 ± 0.4	8.3 ± 0.5	9.4 ± 0.9	11.2 ± 0.3	9.3 ± 0.3	10.5 ± 1.3
	Average	8.4	9.4	10.9	14.7	15.3	16.1
Ozcan	**Irrigated**	25.5 ± 0.4	30.9 ± 1.3	37.0 ± 0.8	18.1 ± 3.0	18.7 ± 2.6	19.0 ± 0.6
	Stress	14.3 ± 1.0	18.7 ± 1.2	20.0 ± 2.2	10.8 ± 0.3	8.2 ± 1.1	10.2 ± 0.4
	Average	19.9	24.8	28.5	14.5	13.4	14.6
BVD-22	**Irrigated**	19.5 ± 0.7	19.9 ± 1.4	20.5 ± 2.5	20.5 ± 0.5	22.7 ± 0.7	21.6 ± 0.8
	Stress	12.4 ± 0.2	13.0 ± 0.8	13.6 ± 0.5	10.5 ± 0.3	10.5 ± 0.3	15.5 ± 3.2
	Average	15.9	16.4	17.1	15.5	16.6	18.6
Irrigated	**Average**	15.7	17.4	19.7	19.0	20.8	20.9
Stress	**Average**	10.2	11.7	12.7	10.6	9.4	11.1
Grand mean		13.0	14.6	16.2	14.8	15.1	16.0
l.s.d.	**Genotype**	0.6			0.7		
	Stage	0.6			0.6		
	Treatment	0.45			0.52		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.1.4 Effect of drought stress on soil water content (SWC)

The soil water content was more or less similar in all wheat genotypes under well-watered conditions for each growth stage. On the other hand, the SWC was decreased with drought stress in all cases. Under drought stress, Sahal-1 genotype recorded the lowest SWC (9.1%) compared with the other genotypes (**Table 4.3**).

4.2.1.5 Effect of drought stress on shoot fresh mass (g)

The reduction in fresh and dry biomass production is one of the most common adverse effects of drought stress on plant (**Farooq**, ***et al***., **2009**). In general, the shoot fresh mass (SFM) increased with age under all conditions except Ozcan under drought stress conditions, which showed the opposite trend. As shown in **Table 4.4**, increase of the shoot fresh mass of all four genotypes was inhibited by drought stress. The reduction in SFM of Giza-163, Sahal-1, BVD-22 and Ozcan compared to controls was 41.93, 45.30, 51.50 and 72.84 % respectively. Under drought stress conditions, BVD-22 had the greatest SFM (7.3g) when compared with other genotypes. In contrast, the lowest SFM of Ozcan (3.8g) showed its susceptibility to drought stress. The results were in accordance with those reported by **Fuzhong**, ***et al***., (**2008**).

4.2.1.6 Effect of drought stress on shoot dry mass (g)

As described in **Table 4.4**, imposition of drought stress had a significant reductive effect on shoot dry mass of the four wheat genotypes. Under controlled conditions, BVD-22 maintained the highest SDM (4.83g), while Ozcan had the lowest SDM (3.53g). Similarly, under drought stress, BVD-22 had the highest SDM (3.90g) and Ozcan had the lowest (3.17g). The results were in agreement with those found by **Tatar** and **Gevrek**, (**2008**).

Table 4.4: Effect of drought stress on shoot fresh mass (g) and shoot dry mass (g) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

Genotype	Variant	SFM (g)			SDM (g)		
		40 DAS	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS
Sahal- 1	**Irrigated**	9.3 ± 0.5	11.4 ± 0.5	11.5 ± 0.5	3.2 ± 0.1	4.2 ± 0.3	5.3 ± 0.5
	Stress	4.3 ± 0.2	6.2 ± 0.9	7.2 ± 2.1	2.7 ± 0.2	3.6 ± 0.4	4.5 ± 0.6
	Average	6.8	8.8	9.4	2.9	3.9	4.9
Giza-163	**Irrigated**	8.5 ± 0.5	13.3 ± 2.5	13.4 ± 0.8	3.1 ± 0.1	4.1 ± 0.1	5.3 ± 0.5
	Stress	4.5 ± 0.2	6.6 ± 0.8	9.3 ± 1.3	2.7 ± 0.1	3.9 ± 0.3	5.0 ± 2.2
	Average	6.5	9.9	11.3	2.9	4.0	5.1
Ozcan	**Irrigated**	11.0 ± 1.1	14.9 ± 2.7	16.6 ± 4.3	3.1 ± 0.2	3.7 ± 0.2	3.8 ± 0.3
	Stress	5.2 ± 0.5	4.8 ± 0.1	1.5 ± 1.3	2.8 ± 0.1	3.3 ± 0.1	3.4 ± 0.1
	Average	8.1	9.9	9.1	2.9	3.5	3.6
BVD -22	**Irrigated**	11.1 ± 0.4	16.7 ± 3.8	17.3 ± 3.1	3.2 ± 0.2	4.5 ± 0.6	6.8 ± 1.6
	Stress	4.6 ± 0.7	6.6 ± 0.3	10.6 ± 1.0	2.8 ± 0.2	3.8 ± 0.1	5.1 ± 1.1
	Average	7.9	11.7	14.0	3.0	4.2	5.9
Irrigated	**Average**	10.0	14.0	14.7	3.1	4.1	5.3
Stress	**Average**	4.7	6.1	7.2	2.7	3.7	4.5
Grand mean		7.3	10.1	10.9	2.9	3.9	4.9
l.s.d.	**Genotype**	1.0			0.4		
	Stage	0.9			0.3		
	Treatment	0.7			0.3		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.2 Effect of drought stress on macro and micronutrients

4.2.2.1 Effect of drought stress on macronutrients

The inorganic nutrients such as N, P, K^+, Ca^{2+}, and Mg^{2+} plays essential roles in metabolism and plant growth. **Marschner**, (**1995**) reported that the plant resistance to drought stress conditions depends on plants nutrient status.

4.2.2.1.1 Effect of drought stress on calcium (Ca) concentration (%)

Calcium **(**Ca^{2+}) ions play an essential role in osmoregulation under drought stress **(Bartels** and **Sunkar**, **2005**). Furthermore, they are a key signal messenger in regulating plant resistance to drought (**Hu** and **Schmidhalter**, **2005**). As described in **Table 4.5**, Ca^{2+} concentration decreased with age in all wheat genotypes under both stress and non-stress conditions (except Giza-163 genotype at 60 DAS). In addition, the Ca^{2+} concentration was decreased under drought stress in comparison with control in all wheat genotypes. Under drought stress conditions, Sahal-1 had the highest Ca^{2+} concentration at 40 DAS, while BVD-22 recorded the highest at 60 DAS. However, at 80 DAS the highest concentration was observed in Giza-163 genotype. The results were in accordance with those reported by **Brown**, ***et al***., (**2006**) and **Gunes**, ***et al***., (**2006**).

4.2.2.1.2 Effect of drought stress on potassium (K) concentration (%)

Potassium (K) plays a significant role in increasing the plant's drought resistance through stomatal regulation (**Kant** and **Kafkafi**, **2002**), osmoregulation and osmotic adjustment under stress conditions (**Bajji**, ***et al***., **2000**). Similar to calcium results, potassium concentrations were also decreased with age in all wheat genotypes under stress and non-stress conditions (**Table 4.5**). In addition, the availability of potassium was significantly affected by drought stress. Under drought and irrigated conditions, BVD-22 genotype had the highest K concentrations at 40 and 60 DAS growth stages; while at 80 DAS Ozcan recorded the highest concentration. **Morgan**, (**1992**) found that the water-stressed wheat plants that had accumulated more potassium in their tissues, showed large osmoregulation. The results presented here were in agreement with those obtained **Elkholy**, ***et al***., (**2005**) and **Gunes**, ***et al*., (2006**).

Table 4.5: Effect of drought stress on calcium and potassium concentrations (%) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		Ca (%)			K (%)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal- 1	**Irrigated**	0.69 ± 0.06	0.67 ± 0.06	0.47 ± 0.00	5.47 ± 0.0	4.15 ± 0.1	3.01 ± 0.1
	Stress	0.68 ± 0.02	0.56 ± 0.10	0.33 ± 0.00	5.16 ± 0.2	3.75 ± 0.0	2.15 ± 0.0
	Average	0.69	0.62	0.40	5.32	3.95	2.58
Giza-163	**Irrigated**	0.57 ± 0.01	0.61 ± 0.00	0.54 ± 0.01	4.90 ± 0.6	4.60 ± 0.1	3.00 ± 0.0
	Stress	0.52 ± 0.03	0.52 ± 0.07	0.42 ± 0.00	4.81 ± 0.1	4.15 ± 0.3	2.28 ± 0.0
	Average	0.55	0.57	0.48	4.86	4.38	2.64
Ozcan	**Irrigated**	0.67 ± 0.06	0.57 ± 0.01	0.48 ± 0.00	5.51 ± 0.1	4.49 ± 0.1	4.45 ± 0.1
	Stress	0.66 ± 0.02	0.56 ± 0.03	0.34 ± 0.01	5.07 ± 0.0	4.46 ± 0.1	3.13 ± 0.1
	Average	0.67	0.57	0.41	5.29	4.48	3.79
BVD -22	**Irrigated**	0.66 ± 0.02	0.60 ± 0.04	0.46 ± 0.00	7.53 ± 0.3	6.48 ± 0.4	2.70 ± 0.0
	Stress	0.63 ± 0.04	0.59 ± 0.03	0.32 ± 0.02	6.42 ± 0.2	4.92 ± 0.1	2.06 ± 0.0
	Average	0.65	0.60	0.39	6.98	5.70	2.38
Irrigated	**Average**	0.65	0.61	0.49	5.85	4.93	3.29
Stress	**Average**	0.62	0.56	0.35	5.37	4.32	2.41
Grand mean		0.64	0.59	0.42	5.61	4.63	2.85
l.s.d.	**Genotype**	0.03			0.19		
	Stage	0.03			0.17		
	Treatment	0.02			0.14		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.2.1.3 Effect of drought stress on magnesium (Mg) concentration (%)

Magnesium (Mg) involved in many biological processes e.g. photosynthesis (**Lesko**, ***et al***., **2002**), through increasing the activity of enzymes, such as ATPases, RNA polymerase and protein kinases (**Shaul**, **2002**). As shown in **Table 4.6**, the concentrations of Mg varied among wheat genotypes under different conditions, between 0.13% in Sahal-1 and BVD-22 genotypes at 80 DAS under stress conditions, and 0.30% in Sahal-1 at 40 and 60 DAS under irrigated conditions. Generally, Mg concentrations declined with age in all genotypes except Ozcan at 80 DAS under irrigated conditions, which showed the opposite trend. The imposition of water stress significantly reduced Mg concentrations in the shoots of all genotypes. The maximum increase of Mg concentrations were observed under stressed and non-stressed conditions in Sahal-1 genotype followed by BVD-22 at 40 and 60 DAS; while at 80 DAS Ozcan recorded the highest concentrations. The results were in harmony with those achieved by **Gunes**, ***et al*., (2006**).

4.2.2.1.4 Effect of drought stress on phosphorous (P) concentration (%)

Phosphorous (P) is a key component of nucleic acids, phospholipids and phosphoproteins (**Hu** and **Schmidhalter**, **2005**). Generally, P concentration in plant tissues decreased with age in all wheat genotypes (**Table 4.6**). Likewise, P content diminished with imposition of water stress. The highest values of phosphorous concentration were observed in the shoots of BVD-22 genotype under drought and well watered conditions, while the minimum P concentrations were observed in Giza-163 at 40, 60 DAS and in Ozcan at 80 DAS stage under both conditions. The results were in accordance with those obtained by **Elkholy**, ***et al***., (**2005**) and **Gunes**, ***et al***., (**2006**).

Table 4.6: Effect of drought stress on magnesium and phosphorous concentrations (%) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		Mg (%)			P (%)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal- 1	**Irrigated**	0.30 ± 0.00	0.30 ± 0.01	0.20 ± 0.01	0.45 ± 0.01	0.36 ± 0.02	0.17 ± 0.01
	Stress	0.29 ± 0.02	0.26 ± 0.03	0.13 ± 0.02	0.40 ± 0.03	0.30 ± 0.03	0.13 ± 0.00
	Average	0.30	0.28	0.17	0.43	0.33	0.15
Giza-163	**Irrigated**	0.24 ± 0.01	0.24 ± 0.01	0.18 ± 0.01	0.38 ± 0.03	0.31 ± 0.01	0.23 ± 0.02
	Stress	0.23 ± 0.01	0.23 ± 0.00	0.15 ± 0.01	0.37 ± 0.03	0.28 ± 0.02	0.14 ± 0.01
	Average	0.24	0.24	0.17	0.38	0.30	0.19
Ozcan	**Irrigated**	0.26 ± 0.02	0.23 ± 0.00	0.25 ± 0.00	0.41 ± 0.01	0.32 ± 0.01	0.16 ± 0.00
	Stress	0.25 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.38 ± 0.01	0.31 ± 0.02	0.12 ± 0.01
	Average	0.26	0.23	0.22	0.40	0.32	0.14
BVD -22	**Irrigated**	0.29 ± 0.01	0.27 ± 0.01	0.18 ± 0.02	0.45 ± 0.08	0.44 ± 0.03	0.35 ± 0.01
	Stress	0.27 ± 0.01	0.26 ± 0.02	0.13 ± 0.01	0.41 ± 0.03	0.39 ± 0.04	0.35 ± 0.01
	Average	0.28	0.27	0.16	0.43	0.42	0.35
Irrigated	**Average**	0.27	0.26	0.20	0.42	0.36	0.23
Stress	**Average**	0.26	0.24	0.15	0.39	0.32	0.19
Grand mean		0.27	0.25	0.18	0.41	0.34	0.21
l.s.d.	**Genotype**	0.01			0.02		
	Stage	0.01			0.02		
	Treatment	0.01			0.01		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.2.1.5 Effect of drought stress on sulphur (S) concentration (%)

Sulphur (S) it is a structural constituent of several coenzymes (**Astolfi**, ***et al***., **2004**). Similar to phosphorous results, sulphur concentrations were also decreased with age in all wheat genotypes (**Table 4.7**). In addition, sulphur concentrations in the shoots of the four wheat genotypes were decreased with imposition of water stress. Under drought stress conditions, Sahal-1, BVD-22, and Ozcan genotypes recorded same S concentrations at 40 DAS (0.32%), while at 60 DAS the maximum concentration was found in BVD-22 (0.32%). However, at 80 DAS stage S concentration was the highest in Giza-163 and Ozcan genotypes (0.22%). The results were in accordance with those reported by (**Nasri**, ***et al***., **2008**).

4.2.2.2 Effect of drought stress on micronutrients

Effects of drought stress on micronutrients availability are not as great as for macronutrients because plant requires only small quantities of these nutrients (**Hu** and **Schmidhalter**, **2005**).

4.2.2.2.1 Effect of drought stress on copper (Cu) concentration (mg kg^{-1})

Copper (Cu) is a structural element in regulatory proteins and participates in oxidative stress responses (**Marschner**, **1995**). Cu ions act as cofactors in many enzymes such as Cu/Zn superoxide dismutase (**Yruela**, **2005**). As shown in **Table 4.7**, Cu concentrations declined with age in all genotypes. Moreover, the drought stress reduced Cu concentrations in all wheat genotypes. The genotype Sahal-1 recorded the highest Cu concentrations at all growth stages and under all conditions.

4.2.2.2.2 Effect of drought stress on iron (Fe) concentration (mg kg^{-1})

Iron (Fe), functions as a cofactor and catalytic site of important enzymes. Some of these enzymes utilized in chlorophyll metabolism (**Davenport**, **1983**).

Table 4.7: Effect of drought stress on sulphur (%) and copper (ppm) concentrations of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		S (%)			Cu (ppm)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal- 1	**Irrigated**	0.36 ± 0.01	0.30 ± 0.02	0.25 ± 0.00	9.11 ± 0.5	7.76 ± 0.2	6.54 ± 0.1
	Stress	0.32 ± 0.02	0.27 ± 0.01	0.17 ± 0.00	8.80 ± 0.0	7.67 ± 0.2	5.91 ± 0.1
	Average	0.34	0.29	0.21	8.96	7.72	6.23
Giza-163	**Irrigated**	0.32 ± 0.01	0.29 ± 0.00	0.24 ± 0.00	8.65 ± 0.0	7.15 ± 0.1	5.03 ± 0.1
	Stress	0.28 ± 0.00	0.28 ± 0.01	0.22 ± 0.00	7.84 ± 0.1	6.90 ± 0.1	3.68 ± 0.0
	Average	0.30	0.29	0.23	8.25	7.03	4.36
Ozcan	**Irrigated**	0.33 ± 0.02	0.32 ± 0.02	0.26 ± 0.00	8.70 ± 0.3	7.19 ± 0.1	4.71 ± 0.1
	Stress	0.32 ± 0.02	0.31 ± 0.01	0.22 ± 0.01	8.31 ± 0.8	6.81 ± 0.5	3.88 ± 0.0
	Average	0.33	0.32	0.24	8.51	7.00	4.30
BVD- 22	**Irrigated**	0.37 ± 0.01	0.33 ± 0.03	0.25 ± 0.00	8.82 ± 0.5	7.41 ± 0.2	4.91 ± 0.1
	Stress	0.32 ± 0.02	0.32 ± 0.01	0.17 ± 0.00	8.64 ± 0.1	7.37 ± 0.1	3.69 ± 0.1
	Average	0.35	0.33	0.21	8.73	7.39	4.30
Irrigated	**Average**	0.35	0.31	0.25	8.82	7.38	5.30
Stress	**Average**	0.31	0.30	0.20	8.40	7.19	4.29
Grand mean		0.33	0.30	0.22	8.61	7.28	4.79
l.s.d.	**Genotype**	0.01			0.22		
	Stage	0.01			0.19		
	Treatment	0.01			0.16		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

Fe concentrations were decreased with age in all wheat genotypes except Ozcan. Furthermore, Fe was decreased in the plants of all genotypes due to water stress. The highest Fe concentrations were noted in Sahal-1 at 40, 60 DAS stages and in Ozcan at 80 DAS stage, under all conditions (**Table 4.8**). Fe concentrations were high under well-watered conditions because of its presence in more soluble forms (**Havlin**, ***et al***., **1999**). Similar results were demonstrated by **Gunes**, ***et al***., (**2006**).

4.2.2.2.3 Effect of drought stress on manganese (Mn) concentration (mg kg^{-1})

Manganese (Mn) plays an essential role in activation of several enzymes, such as isoenzymes of superoxide dismutase (**Campanella**, ***et al***., **2005**). It is also involved in scavenging of superoxide and hydrogen peroxide (**Ducic** and **Polle**, **2005**). The drought stress significantly reduced Mn concentrations in all wheat genotypes (**Table 4.8**). Under drought stress conditions, Mn accumulations were the highest in BVD-22 at 40 and 60 DAS, while at 80 DAS the highest concentration was observed in Giza-163 genotype. The results were in harmony with those achieved by **Gunes**, ***et al***., (**2006**) and **Hussein**, ***et al***., (**2009**).

4.2.2.2.4 Effect of drought stress on zinc (Zn) concentration (mg kg^{-1})

Zinc (Zn), protects plant cells from the damage effects that caused by ROS (**Cakmak**, **2000**), Zn also has a role in protection of chloroplasts from the photo-oxidation damages that occur by ROS (**Wang** and **Jin**, **2005**).The concentrations of Zn varied among wheat genotypes between 21.9 mg kg^{-1} (in Sahal-1 at 80 DAS under stress) and 70.5 mg kg^{-1} (in Sahal-1 at 40 DAS under irrigated conditions). The Zn concentrations were decreased due to drought in all genotypes (**Table 4.9**). Under drought stress, Sahal-1 at 40 DAS, BVD-22 at 60 DAS, and Ozcan at 80 DAS stage recorded the highest Zn concentrations, while the lowest concentrations were observed in Ozcan at 40, 60 DAS stages and in Sahal-1 at 80 DAS stage. The results were in parallel with those achieved by **Gunes**, ***et al***., **(2006**) and **Nasri**, ***et al***., (**2008**).

Table 4.8: Effect of drought stress on iron and manganese concentrations (ppm) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		Fe (ppm)			Mn (ppm)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal- 1	**Irrigated**	86.7 ± 11.6	78.6 ± 6.6	40.4 ± 0.2	109.0 ± 2.8	99.1 ± 2.4	73.9 ± 0.1
	Stress	71.3 ± 16.4	71.1 ± 0.9	30.6 ± 0.4	97.1 ± 3.9	92.1 ± 3.3	59.5 ± 0.1
	Average	79.0	74.9	35.5	103.0	95.6	66.7
Giza-163	**Irrigated**	67.9 ± 7.4	62.5 ± 0.3	58.5 ± 0.1	106.0 ± 3.0	105.0 ± 12.7	92.2 ± 2.0
	Stress	56.3 ± 5.3	52.7 ± 7.0	50.4 ± 0.1	97.5 ± 6.4	103.0 ± 5.9	66.7 ± 0.7
	Average	62.1	57.6	54.5	102.0	104.0	79.4
Ozcan	**Irrigated**	66.7 ± 1.6	67.0 ± 1.5	59.8 ± 20.7	99.3 ± 11.3	102.0 ± 3.2	91.2 ± 0.3
	Stress	66.7 ± 1.4	48.9 ± 1.5	51.0 ± 7.7	98.5 ± 9.5	100.0 ± 9.7	64.0 ± 1.4
	Average	66.7	58.0	55.4	98.9	101.0	77.6
BVD- 22	**Irrigated**	70.4 ± 0.3	68.5 ± 4.9	37.0 ± 0.4	109.0 ± 6.7	105.0 ± 0.1	69.1 ± 0.6
	Stress	68.4 ± 3.3	67.3 ± 3.4	30.5 ± 1.0	103.0 ± 9.6	105.0 ± 3.5	46.1 ± 0.3
	Average	69.4	67.9	33.8	106.0	105.0	57.6
Irrigated	**Average**	72.9	69.2	48.9	106.0	103.0	81.6
Stress	**Average**	65.7	60.0	40.7	99.0	100.0	59.1
Grand mean		69.3	64.6	44.8	102.0	102.0	70.3
l.s.d.	**Genotype**	5.8			4.8		
	Stage	5.0			4.1		
	Treatment	4.1			3.4		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.3 Effect of drought stress on proline content

Proline (Pro) acts as a compatible solute regulating and reducing water loss from plant cells, some stressed plants used it as a source of storage for carbon and nitrogen (**Samaras**, ***et al***., **1995**). There was a steep increase in proline content in all wheat genotypes when subjected to drought stress (**Table 4.9**). The proline contents in stressed plants were 17.34 times greater than irrigated plants. On the other hand, the proline content increased 29 fold in Sahal-1, 18.5 fold in Giza-163, 32.2 fold in Ozcan and 9.9 fold in BVD-22 as compared to the control plants.

Among all wheat genotypes BVD-22 showed the highest proline content under both irrigated and stressed conditions and at all growth stages, the highest being at 40 DAS stage (101.7 µ moles Pro/g FW) under irrigated conditions and at 60 DAS (599.4 µ moles Pro/g FW) under drought conditions. In contrast, the lowest proline content was obtained from leaves of Giza-163 genotype under stress conditions at all growth stages. The low Pro content of Giza-163 genotype under stress shows its susceptibility to drought. The results were in harmony with those achieved by **Yao**, ***et al***., (**2009**), and **Johari-Pireivatlou**, ***et al***., (**2010**).

4.2.4 Effect of drought stress on soluble carbohydrate content

High carbohydrate concentration, beside its role in maintaining protein structure and cell membrane stabilization (**Hoekstra**, ***et al***., **2001**), plays a significant role in osmotic adjustment (**Mohammadkhani** and **Heidari**, **2008**), and serve as signal molecule for sugar-responsive genes which enhancing the defense responses (**Smeekens**, **2000**). Generally, soluble carbohydrates showed a decrease with age in all genotypes under both drought and irrigated conditions, except BVD-22 genotype, which showed an increase with age under stress conditions (**Table 4.10**). Drought caused an increase in soluble carbohydrates content in all wheat genotypes. Among all genotypes, Sahal-1 maintained the highest SC content, under all conditions. Similar results were obtained by **Johari-Pireivatlou**, ***et al***., (**2010**).

Table 4.9: Effect of drought stress on zinc concentrations (ppm) and proline content (µ moles pro. / g FW), of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		Zn (ppm)			Pro (µ moles pro. /g FW)		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal- 1	**Irrigated**	70.5 ± 3.9	55.2 ± 3.5	33.6 ± 0.5	26.8 ± 1.6	14.6 ± 0.7	4.2 ± 1.3
	Stress	67.5 ± 3.2	49.0 ± 3.4	21.9 ± 0.1	377.8 ± 66.9	489.9 ± 43.6	459.0 ± 20.3
	Average	69.0	52.1	27.7	202.3	252.3	231.6
Giza-163	**Irrigated**	62.4 ± 4.7	53.6 ± 0.2	33.3 ± 3.7	30.3 ± 6.6	21.9 ± 1.0	5.4 ± 0.5
	Stress	60.0 ± 2.9	46.0 ± 1.8	24.6 ± 0.0	265.7 ± 11.3	468.5 ± 27.9	331.4 ± 4.5
	Average	61.2	49.8	28.9	148.0	245.2	168.4
Ozcan	**Irrigated**	44.5 ± 5.5	35.5 ± 4.5	31.6 ± 0.7	24.8 ± 1.0	8.9 ± 1.3	3.3 ± 1.0
	Stress	39.5 ± 7.3	32.5 ± 3.1	25.6 ± 0.4	350.3 ± 7.2	479.7 ± 19.5	359.8 ± 62.1
	Average	42.0	34.0	28.6	187.5	244.3	181.5
BVD- 22	**Irrigated**	65.6 ± 4.3	56.6 ± 1.1	35.3 ± 0.3	101.7 ± 33.8	47.1 ± 1.0	6.1 ± 0.5
	Stress	64.3 ± 0.8	55.0 ± 0.9	24.0 ± 1.7	445.0 ± 4.9	599.4 ± 78.2	490.1 ± 68.0
	Average	65.0	55.8	29.7	273.7	323.3	248.1
Irrigated	**Average**	60.8	50.2	33.5	45.9	23.1	4.7
Stress	**Average**	57.8	45.6	24.0	359.9	509.4	410.1
Grand mean		59.3	47.9	28.7	202.9	266.2	207.4
l.s.d.	**Genotype**	2.6			61.1		
	Stage	2.2			52.9		
	Treatment	1.9			43.2		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.5 Effect of drought stress on lipid peroxidation levels

Drought stress increases reactive oxygen species (ROS) accumulation, which cause oxidative damage to chloroplast membranes (**Cai**, ***et al***., **2007**) and lead to increase in the malondialdehyde level. As well as the generation of unsaturated fatty acids affects membrane structures, their properties, and leads to cellular damage to plant membranes (**Quan**, ***et al***., **2004**). Lipid peroxidation levels in wheat leaves were determined by measuring malondialdehyde (MDA) content (**Table 4.10**). All stressed genotypes showed high levels of MDA content when compared to the unstressed genotypes. The effect of drought stress was more pronounced in Giza-163 genotype at 40 and 80 DAS and in Ozcan genotype at 60 DAS stage. However, BVD-22 recorded the lowest MDA contents at all stages. These results were in accordance with those obtained by **Costa**, ***et al***., (**2010**).

4.2.6 Effect of drought stress on antioxidant enzymes activities

The ROS may react with proteins, membrane lipids and nucleic acids, causing oxidative damage and impairing the normal functions of cells, which in turn leads to cell death (**Mittler**, **2002**; **Mittler**, ***et al***., **2004**). The ability to reduce the damaging effects of ROS may be associated with drought tolerance. Plants use antioxidant defense mechanisms to prevent these damages (**Agarwal** and **Pandey**, **2003**). In the present study, the activities of antioxidant enzymes (except CAT) were increased under drought stress, compared to the control (unstressed) plants.

Table 4.10: Effect of drought stress on soluble carbohydrates content (mg/g DW), and malondialdehyde content (nmol ml^{-1}) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

		SC (mg/g DW)			MDA (nmol ml^{-1})		
Genotype	**Variant**	**40 DAS**	**60 DAS**	**80 DAS**	**40 DAS**	**60 DAS**	**80 DAS**
Sahal- 1	**Irrigated**	5.95 ± 0.9	5.76 ± 0.8	4.97 ± 0.8	0.25 ± 0.01	0.62 ± 0.04	0.86 ± 0.03
	Stress	7.40 ± 0.1	6.43 ± 0.8	5.95 ± 0.4	0.33 ± 0.02	1.73 ± 0.02	4.80 ± 0.04
	Average	6.68	6.10	5.46	0.29	1.18	2.83
Giza-163	**Irrigated**	5.47 ± 0.0	5.01 ± 0.9	4.76 ± 0.3	0.25 ± 0.00	0.29 ± 0.00	1.76 ± 0.02
	Stress	7.39 ± 0.4	5.30 ± 0.2	5.07 ± 1.0	0.43 ± 0.02	1.45 ± 0.01	6.13 ± 0.00
	Average	6.44	5.16	4.91	0.34	0.87	3.95
Ozcan	**Irrigated**	4.19 ± 0.0	3.97 ± 0.3	3.43 ± 0.3	0.23 ± 0.03	0.25 ± 0.02	0.71 ± 0.04
	Stress	6.21 ± 0.8	4.32 ± 0.1	3.65 ± 0.6	0.29 ± 0.03	1.89 ± 0.03	2.92 ± 0.04
	Average	5.20	4.14	3.54	0.26	1.07	1.82
BVD-22	**Irrigated**	4.46 ± 0.3	3.19 ± 0.4	4.29 ± 0.0	0.18 ± 0.02	0.21 ± 0.02	0.54 ± 0.03
	Stress	4.69 ± 1.4	5.15 ± 0.2	5.70 ± 0.2	0.25 ± 0.02	1.31 ± 0.01	2.65 ± 0.01
	Average	4.58	4.17	5.00	0.22	0.76	1.60
Irrigated	**Average**	5.02	4.48	4.36	0.23	0.34	0.97
Stress	**Average**	6.42	5.30	5.09	0.33	1.60	4.13
Grand mean		5.72	4.89	4.73	0.28	0.97	2.55
l.s.d.	**Genotype**	0.50			0.19		
	Stage	0.44			0.16		
	Treatment	0.36			0.13		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level).

4.2.6.1 Effect of drought stress on ascorbate peroxidase activity

Ascorbate peroxidase (AP) is an enzyme that catalyzes the conversion of H_2O_2 to water and O_2 (**Gratao**, ***et al***., **2005**). AP activities of shoots (expressed per mg protein), were affected by drought stress (**Table 4.11**). There was an increase in AP activity in all wheat genotypes when subjected to drought stress, except Ozcan at 60 DAS and Giza-163 at 40 and 80 DAS stages. The maximum increases in AP activities were observed in BVD-22 genotype at 40 DAS, and in Sahal-1 genotype at 60 and 80 DAS stages. These results were in parallel with those achieved by **Khanna-Chopra** and **Selote**, (**2007**).

4.2.6.2 Effect of drought stress on glutathione reductase activity

Glutathione reductase (GR) is essential for maintenance high concentrations of reduced glutathione, and involved in H_2O_2 detoxification (**Foyer**, ***et al***., **1994**). Similar to ascorbate peroxidase results, also glutathione reductase activity was affected by drought (**Table 4.11**). The activity of GR was increased under drought stress conditions in comparison to control conditions in all wheat genotypes except Ozcan and BVD-22 genotypes at 80 DAS stage, which showed the reverse trend. The increase in GR activities were pronounced in BVD-22 at 40 DAS, in Sahal-1 at 60 DAS, and in Giza-163 at 80 DAS. These results were in agreement with those obtained by **Renu** and **Devarshi**, (**2007**).

4.2.6.3 Effect of drought stress on superoxide dismutase content

Superoxide dismutase (SOD) plays a crucial role in antioxidant defense because it catalyzes conversion of the superoxide radical to molecular oxygen and H_2O_2 (**Costa**, ***et al***., **2010**). Also in the case of superoxide dismutase, drought increased the enzyme activity in all wheat genotypes, except Ozcan and Giza-163 genotypes at all growth stages, which showed the opposite trend (**Table 4.12**). The SOD contents were 9.62 and 8.56 U mg^{-1} protein in irrigated and non-irrigated plants, respectively. The highest activities of SOD were observed in BVD-22 at 40 DAS, and in Sahal-1 at 60 and 80 DAS stages. **Renu** and **Devarshi**, (**2007**), obtained similar results.

Table 4.11: Effect of drought stress on ascorbate peroxidase activity (µmol/mg protein/min.) and glutathione reductase activity (nmol/mg protein /min.) of four *T. aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

Genotype	Variant	AP (µmol mg^{-1} prt. $min.^{-1}$)			GR (nmol mg^{-1} prt. $min.^{-1}$)		
		40 DAS	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS
Sahal- 1	**Irrigated**	0.62 ± 0.04	0.13 ± 0.02	0.23 ± 0.03	96.9 ± 10.1	51.0 ± 7.7	86.9 ± 6.2
	Stress	0.62 ± 0.05	0.39 ± 0.04	0.46 ± 0.02	121.3 ± 10.9	99.6 ± 11.1	95.7 ± 7.7
	Average	0.62	0.26	0.34	109.1	75.3	91.3
	Differ. %	0.00	196.2	97.65	25.1	95.3	10.2
Giza-163	**Irrigated**	0.33 ± 0.03	0.32 ± 0.03	0.23 ± 0.02	79.1 ± 8.8	98.1 ± 5.5	63.6 ± 6.2
	Stress	0.31 ± 0.02	0.36 ± 0.04	0.23 ± 0.02	84.6 ± 9.7	123.3 ± 13.0	73.8 ± 8.5
	Average	0.32	0.34	0.23	81.8	110.7	68.7
	Differ. %	-4.60	13.33	-1.20	7.0	25.7	16.0
Ozcan	**Irrigated**	0.40 ± 0.02	0.71 ± 0.03	0.21 ± 0.02	71.4 ± 9.6	69.4 ± 9.6	131.2 ± 11.2
	Stress	0.45 ± 0.05	0.36 ± 0.05	0.37 ± 0.01	79.7 ± 9.2	109.3 ± 10.2	51.5 ± 5.0
	Average	0.43	0.54	0.29	75.5	89.3	91.3
	Differ. %	13.94	-49.64	76.82	11.6	57.6	-60.8
BVD -22	**Irrigated**	0.28 ± 0.03	0.29 ± 0.03	0.44 ± 0.03	78.7 ± 5.6	86.1 ± 3.2	108.0 ± 15.0
	Stress	0.36 ± 0.03	0.33 ± 0.04	0.46 ± 0.01	102.9 ± 4.4	135.6 ± 13.4	107.9 ± 16.0
	Average	0.32	0.31	0.45	90.8	110.8	107.9
	Differ. %	26.31	16.61	5.63	30.8	57.5	-0.2
Irrigated	**Average**	0.41	0.36	0.28	81.5	76.1	97.4
Stress	**Average**	0.44	0.36	0.38	97.1	116.9	82.2
	Differ. %	7.02	-0.23	36.66	19.1	53.6	-15.6
Grand mean		0.42	0.36	0.33	89.3	96.5	89.8
l.s.d.	**Genotype**	0.17			19.8		
	Stage	0.15			17.1		
	Treatment	0.12			13.9		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level), Differ.= values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

Table 4.12: Effect of drought stress on superoxide dismutase content (Unit/mg protein), and catalase activity (nmol/mg protein /min.) of four *Triticum aestivum* genotypes, the genotypes were exposed to drought stress at 40, 60, and 80 days after sowing (DAS).

Genotype	Variant	SOD (U mg^{-1} prt.)			CAT (nmol mg^{-1} prt. $min.^{-1}$)		
		40 DAS	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS
Sahal- 1	**Irrigated**	8.50 ± 0.4	2.70 ± 0.2	11.50 ± 0.6	128.10 ± 8.7	114.30 ± 11.3	169.30 ± 8.2
	Stress	9.90 ± 0.8	7.20 ± 0.5	13.00 ± 0.1	104.40 ± 5.4	53.60 ± 5.7	125.10 ± 10.4
	Average	9.20	4.94	12.27	116.25	83.96	147.19
	Differ. %	16.39	163.0	13.46	-18.50	-53.12	-26.10
Giza-163	**Irrigated**	7.59 ± 0.6	6.10 ± 0.6	9.80 ± 0.2	111.40 ± 11.9	132.90 ± 11.0	134.0 ± 10.8
	Stress	6.26 ± 0.4	6.10 ± 0.3	8.40 ± 0.2	71.50 ± 3.7	110.70 ± 10.6	64.40 ± 3.3
	Average	6.92	6.10	9.09	91.47	121.78	99.19
	Differ. %	-17.60	0.51	-14.17	-35.78	-16.70	-51.96
Ozcan	**Irrigated**	8.00 ± 0.0	9.40 ± 0.3	26.20 ± 0.1	123.30 ± 10.8	151.10 ± 15.9	212.30 ± 17.5
	Stress	7.70 ± 0.5	5.30 ± 0.6	8.10 ± 0.0	61.20 ± 5.6	63.80 ± 4.0	38.60 ± 4.5
	Average	7.85	7.35	17.13	92.26	107.44	125.47
	Differ. %	-4.66	-43.08	-69.13	-50.32	-57.79	-81.83
BVD -22	**Irrigated**	8.20 ± 1.2	5.20 ± 0.1	12.20 ± 0.9	137.70 ± 14.0	115.20 ± 8.6	169.90 ± 11.4
	Stress	10.10 ± 0.4	8.10 ± 0.6	12.70 ± 1.4	81.80 ± 8.5	109.40 ± 6.9	113.00 ± 17.0
	Average	9.14	6.66	12.44	109.73	112.29	141.46
	Differ. %	22.41	54.78	3.62	-40.60	-5.00	-33.51
Irrigated	**Average**	8.09	5.85	14.92	125.11	128.37	171.39
Stress	**Average**	8.47	6.68	10.54	79.74	84.37	85.26
	Differ. %	8.28	6.26	12.73	-36.26	-34.28	-50.25
Grand mean		8.30	6.30	12.70	102.43	106.37	128.33
l.s.d.	**Genotype**	1.94			27.11		
	Stage	1.68			23.48		
	Treatment	1.37			19.17		

*The data represent mean ± SD of three replicates, l.s.d.: least significant differences of means (5% level), Differ.:=values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

4.2.6.4 Effect of drought stress on catalase activity

The wheat genotypes responded to drought stress with a noticeable decrease in the activity of catalase with decreases of 36.3, 34.3, and 50.3% at 40, 60, and 80 DAS stages, respectively (**Table 4.12**). The lowest reduction in CAT activity was found in BVD-22 at 60 DAS, and in Sahal-1 at 40 and 80 DAS. The activity of CAT decreased from 115.2 to 109.4 nmol mg^{-1} prt.$min.^{-1}$ in BVD-22 at 60 DAS and from 128.1, 169.3 to 104.4, 125.1 nmol mg^{-1} prt.$min.^{-1}$ in Sahal-1 40 and 80 DAS respectively. In contrast, the highest reduction was observed in Ozcan genotype at all growth stages. The results were in parallel with those achieved by **Tayebeh** and **Hassan**, (**2010**).

4.2.7 Identification of drought responsive genes by mRNA differential display

The differential display technique is an important tool used to identify the differentially expressed genes (**Liang** and **Pardee**, **1992**). In addition, it used to obtain gene expression profiles (**Canli, 2007**). From the greenhouse experiment data, Sahal-1 and BVD-22 genotypes showed better performance under drought stress conditions compared with other genotypes (Ozcan and Giza-163). Therefore, the mRNA DD technique was used to isolate and identify the genes whose expression was changed in response to drought stress in both genotypes (Sahal-1 and BVD-22). Total RNA was isolated from plants that had been exposed to drought and irrigated conditions at 40 DAS stage, and after isolation, 2% agarose gel electrophoresis was used to check the quality of RNA samples (**Fig.4.5)**. The presence of sharp rRNA bands indicates that the RNA was not degraded. Furthermore, RNA content was measured at 260nm wavelength by using Nanodrop spectrophotometer.

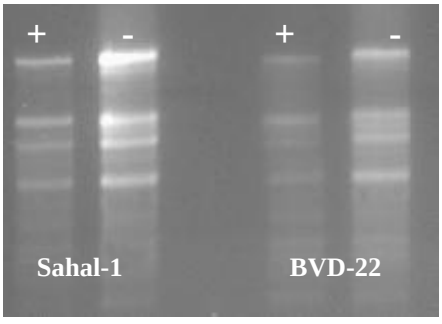
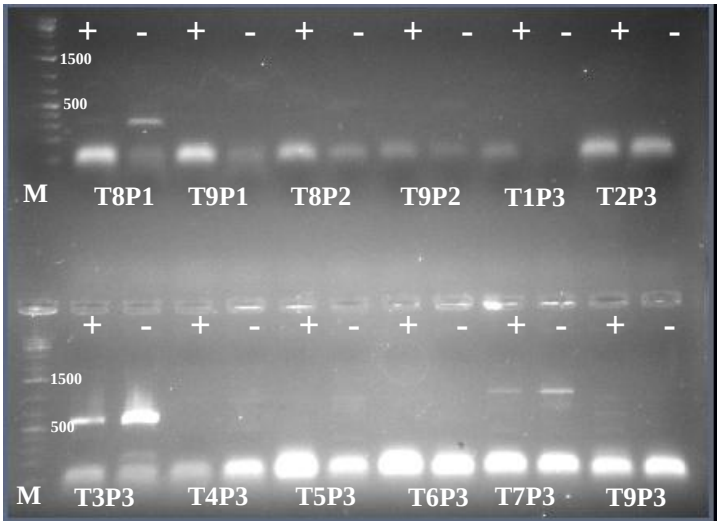
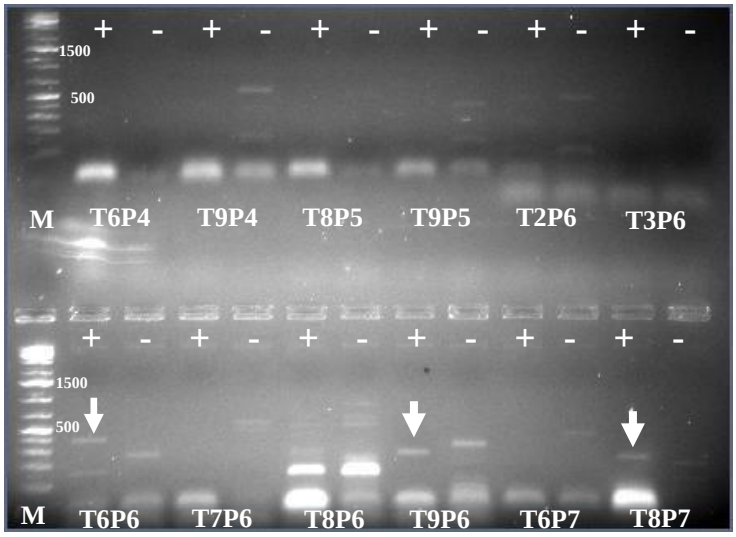


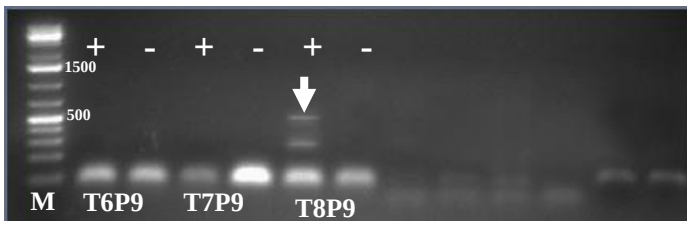
Fig. 4.5: Quality of RNA samples on 2% agarose gel, (+) = stress, (-) = irrigated.



(a)

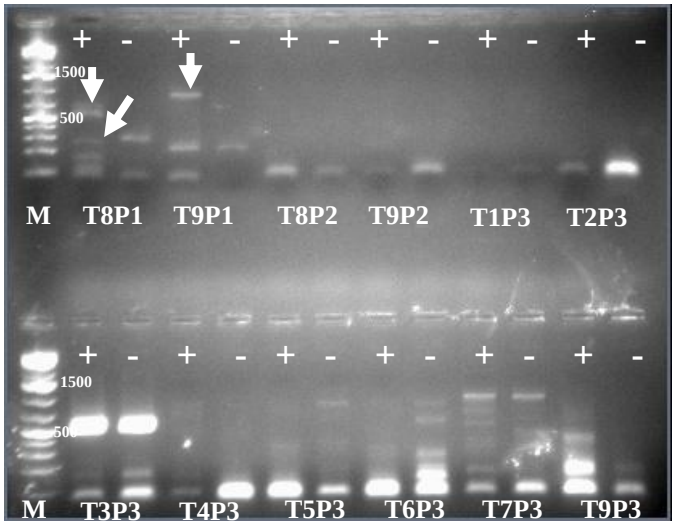


(b)

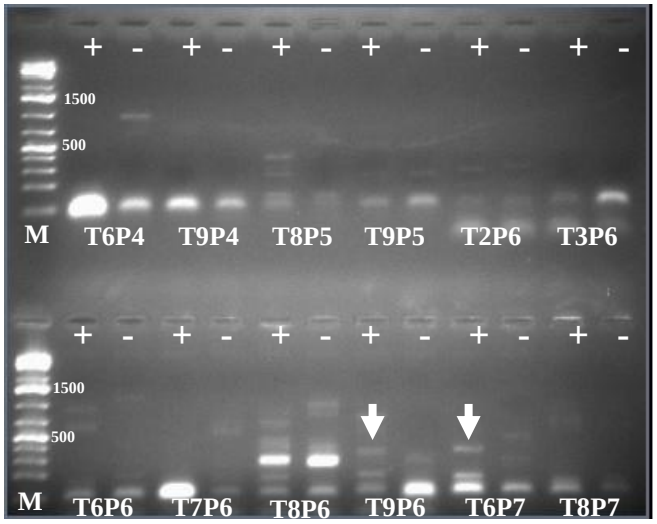


(c)

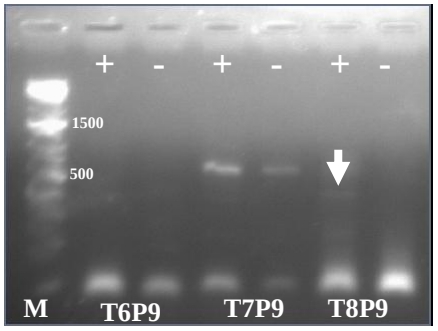
Fig. 4.6: Agarose gel electrophoresis pictures of mRNA differential display PCR products of Sahal-1 genotype before gel extraction. The genotype was exposed to drought stress 40 days after sowing (DAS). (a) PCR products obtained via T8P1, T9P1, T8P2, T9P2, T1P3, T2P3, T3P3, T4P3, T5P3, T6P3, T7P3, and T9P3 primers. (b) PCR products obtained via T6P4, T9P4, T8P5, T9P5, T2P6, T3P6, T6P6, T7P6, T8P6, T9P6, T6P7 and T8P7 primers. (c) PCR product obtained via T6P9, T7P9, and T8P9 primers. The fragments displayed with arrows were extracted from the gel for sequencing analysis, (+) = stress, (-) = irrigated.



(a)



(b)



(c)

Fig. 4.7: Agarose gel electrophoresis pictures of mRNA differential display PCR products of BVD-22 genotype before gel extraction. The genotype was exposed to drought stress 40 days after sowing (DAS). (a) PCR products obtained via T8P1, T9P1, T8P2, T9P2, T1P3, T2P3, T3P3, T4P3, T5P3, T6P3, T7P3 and T9P3 primers. (b) PCR products obtained via T6P4, T9P4, T8P5, T9P5, T2P6, T3P6, T6P6, T7P6, T8P6, T9P6, T6P7, and T8P7primers. (c) PCR product obtained via T6P9, T7P9 and T8P9 primers. The fragments displayed with arrows were extracted from the gel for sequencing analysis, (+) = stress, (-) = irrigated.

After isolation from wheat genotypes, the total RNA samples were used as a template for cDNA synthesis. In order to detect the expression profile of tolerant genotypes in response to drought stress, the cDNAs obtained from Sahal-1 and BVD-22 genotypes were subjected to mRNA differential display method with 72 different primer combinations were used in PCR reactions. The gel electrophoresis results of the fragments isolated from Sahal-1 and BVD-22 genotypes are shown in **Fig.4.6** and **4.7**.

A total of 30 cDNA fragments were found to be differentially expressed. Of these, 10 cDNAs whose levels of expression were significantly altered by drought stress were selected, identified and extracted from the agarose gel. Then these fragments were re-amplified with the same primer combinations for confirmation, and the products separated on a 1% agarose gel (**Fig.4.8)**. The fragment bands are designated as Sah-1, Sah-2, Sah-3, Sah-4, BV-1, BV-2, BV-3, BV-4, BV-5, and BV-6.

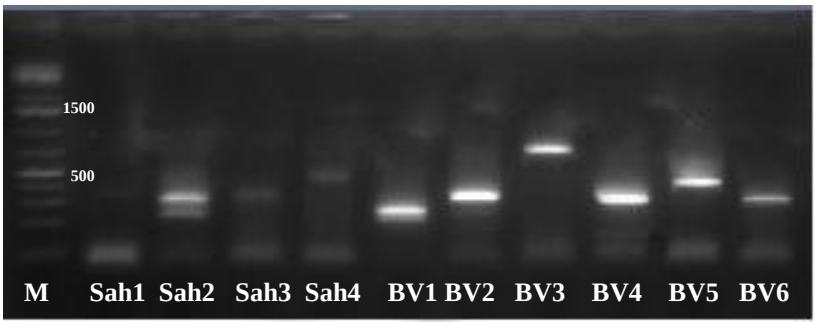


Fig. 4.8: After gel extraction and confirmation with the same primers

4.2.7.1 Sub cloning of drought genes in *E.coli* with pGEM-T Easy vector

After gel extraction and confirmation steps, the fragment bands were ligated into pGEM-T Easy vector, and then used to transform *E.coli* strain DH5α. The pGEM-T Easy vector contains a *Lac-Z* region, which encodes for the enzyme β-galactosidase, and is interrupted when the vector contains an insert. This allowed identification of positive colonies by blue-white selection, the white colonies being selected from the plates. To confirm whether the vectors in these white colonies contained the fragments of interest, colony PCR reactions were performed with the same primer combinations

and the PCR products were separated by 1% agarose gel electrophoresis (**Fig.4.9** and **4.10**).

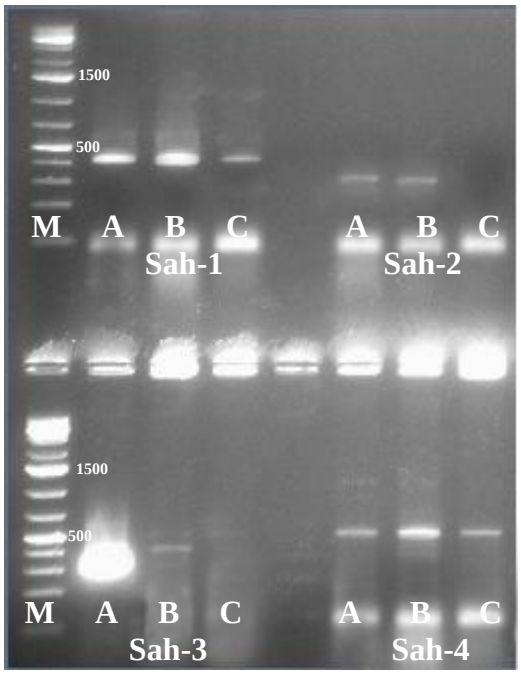


Fig. 4.9: Colony PCR reaction of clones Sah1, Sah2, Sah3 and Sah4 from Sahal-1 genotype.

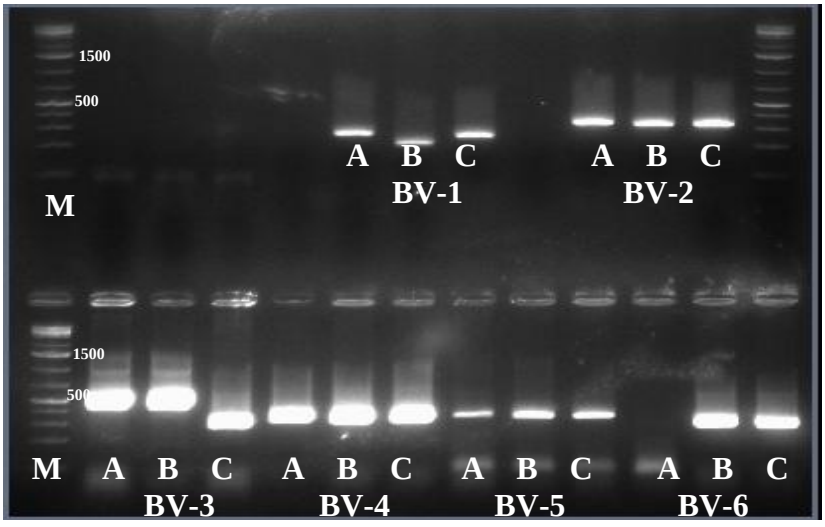


Fig. 4.10: Colony PCR reaction of clones BV-1, BV-2, BV-3, BV-4, BV-5 and BV-6 from BVD-22 genotype.

After colony PCR reactions, the plasmids were isolated from *E. coli* DH5α cells and successful plasmid isolation checked using 1% agarose gel electrophoresis for 40 min. at 100 V. (**Fig.4.11** and **4.12**).

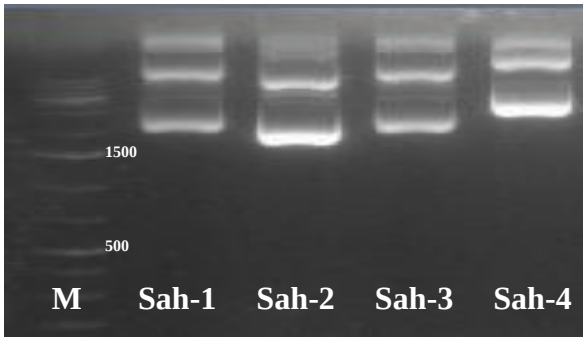


Fig. 4.11: Agarose gel analysis of minipreps for Sah1, Sah2, Sah3 and Sah4 from Sahal-1 genotype.

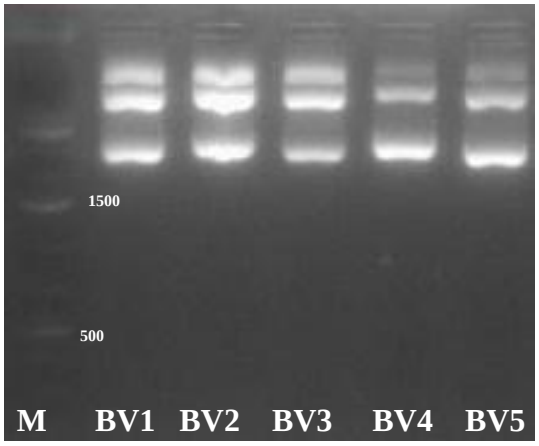


Fig. 4.12: Agarose gel analysis of minipreps for Bv1, Bv2, Bv3, Bv4 and Bv5 from BVD-22 genotype.

Isolation of differentially expressed cDNA fragments from the subcloning vector

The pGEM-T Easy vector has two *EcoR*I recognition sites, so to check whether the plasmids containing the required fragments, the plasmids were also digested with

*EcoR*I restriction enzyme. After digesting, the insert sizes were checked on 1% agarose gels (**Fig.** 4.13**Fig.4.13** and **4.14**).

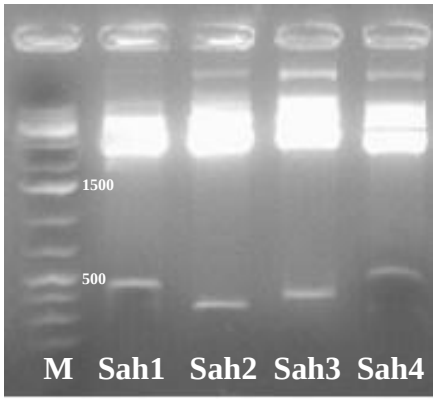


Fig. 4.13: Agarose gel showing digests, for Sahal-1 genotype.

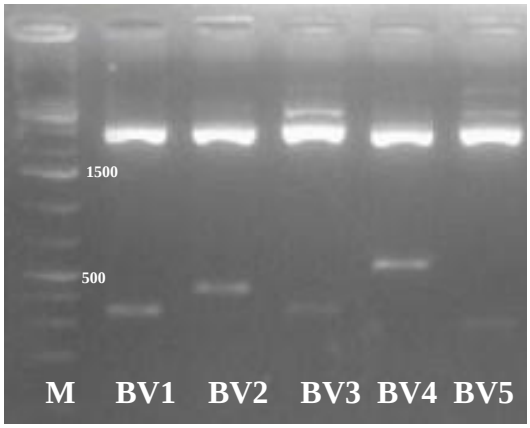


Fig. 4.14: Agarose gel showing digests, for BVD-22 genotype.

4.2.7.2 Sequencing and characterization of cDNA clones

The differentially expressed cDNA fragments were sequenced by using the M13 forward primer. To remove vector regions, the sequences were submitted to the VecScreen algorithm (www.ncbi.nlmn.nih.gov). The sequences of the fragments obtained from the mRNA DD study are depicted in **Tables 4.13** and **4.14**.

Table 4.13: Sequences of the isolated fragments from Sahal-1, sizes and the primer combinations that were used in mRNA DD.

	Primers	Size (bp)	Sequence
Sah-1	T6P6	415	TAAGGAAAGTAGTTCCCCCGTTAAATATTTTGGCCAACGTT TTTGCATTTGCTACACGACTCGGGGGGTACCCCCCCGCCTT TGGGAAAGGCTTCATTACCTTCCGGTTTCCAAAGATAAAG GGAAATTAAAAGATCCCCCAATGTTTGAAAAAAAAGGTTA TCTCCTTCGGCCCCCAACTTTTGGCAAAATATTTTGGGGCC CGTGTTTACTCCCTGGGGTTAGGGCCCTTGGAAATTTCTTT ATTGGGTGCCCCCCCAAAAGGGTTTTTTGGTTGGGGGGAG AACACAAATCCTTCAGAAAAGAGTGGGGGGCAACAGATTT TTCTCGCGGCCAACAGAAGAAACCACCCCAAAAAAATATA TTTTTTTACGAAGAACTTTGGGGCATATCATGTAATCATTC TGACGATACC
Sah-2	T9P6	337	GCTTGCGGTCCATGGCCGCTTTCCCAGCCGGGAATTCGGTC GGGCCAGTTGATTAATGGATCCGCCAAACGCGGGGGAAA GGCCGTTTGGCGTATGGGCGCTCTTTCCGTTCCCCCCCTAA TGAACCGCCGGCCCTGGTCGGTCGGGCGGCGCGGGGCGTA TCAGGTCCCTTCAAGGGCGTAATTCGGGTATTCCCCGGATC AGGGGATAACGGCAGGAAGGACCTGGGAAGAAAAGGGCC GGCAAAGGGCAAGAACCCTAAAAAGGCGCCGTGGTGGCC GTTTTTCCAAGGTCCGGCCCCCTGAACAAGCATTCCAAAA TTCGCGGCTCAAGTAGA
Sah-3	T8P7	362	CCATTTCGTCATCCAAAGTGCCTGACTCCCGTCGGGAAAA ACTACGAACGGGAGGCTTACCTCTGGCCCAGTGCTGAATG AACCCGGAGACCACGCTCCCGGTCCCAATTTTCCGAATAA ACCCCCCCCCGAAGGCCAAGCCAAAATGGCCCGAACTTAC CGCCCCCCCCGCCTATATTTGGCCGGAACCAAAAAAATTTC CCCTTAAATTTGGCAATGTTGTCCTTTCAAAATCCGGGGGA CCCCCTTTGGAGGGTCTTCCCCCGTCCCCAAATGGGGAATT TTCCCCGTTGGGAAAAAAGATTCTCCTGCCCAATTGGAAA AATGGCCGGGTTTCCCGGGGTGGGGCTGTTTTTTGGGTGC
Sah-4	T8P9	679	AAACCTGGCCCTTACCGTATCCCTGGCAGCTTTCTCCCTTC GGGAAGGGTGCGACTTTGCCAAGTTCCGGATGAAGGACCC CATTTCCGGTTAAGGACGTCCGTCCATTCTGGAATGTAAGC CGAACCCACCGTTATTCCGAACGGTGGGTCCATTCAGGTA AGATTTGCTTGAGAACCACCCGGTAAAACCAACTTTTCCC CTTGGCAGAAACCTTGGTAAAGGTTAACCAAAGAAGGATA GTATGCGGGCTCCAAAATTCCTAGGGGGGGGCAAACCCCG GACCCTAGAAAACAGATTTTGATTTCGCCTTTTTGAACCAT TTCCCTCCCAAAAAAAAGGATTCCTTTCCCGCCCAAAAAC CCCGTGGTCCCGGTAATTTTTTGGCCCCCAATTTACCCAAA CAAAAGGTGGACAATTTCTTTTTTTTCCCGGGGCGCCCCAA CCGAAAAAACACAAATGTGTTTCTGGGTTAAAAAAGGCCC CCACCCCCTTAAAAAATGTTATAACAAAACGCTACTTCCTC TTACCTCGGACTTATGGGGCCGAAACCGTTTTTTCTATCCG TTCACCCGAATCCACCCCCCCACCCTGTTCCCCCCCCCCGA TCAAGCCCCTCCCGGGCTTCCCTGCCCCAGAACACCATAA CACGCATTTCAAAATAGGGCGGTTCGACTTT

Table 4.14: Sequences of the isolated fragments from BVD-22, sizes and the primer combinations that were used in mRNA DD.

	Primers	Size (bp)	Sequence
BV-1	T8P1	488	CAAAGATATATAGAGAAAACTGGGCTGAAAGTTACCATG GTTAATCAGGGGGGCCCTTTTCACGGATTGGCTATTTCGT TACCCAAATGGCCGAACCCCCGCGGGGAAAAACCCCAAC CGGGGGGGTTACACTTGGGCCCAGGTGGAAAGAAACCCG GAACACACTCCCCGGCCCAAATTTACCAAAAAAACCCCCC GGGAGGGGCAGAGAAAAGTGTCCGCAATTTTCCCCCCCC ACCCATAATTTGGCGGGAAAAAAAAATTTCCCCTATATTT ACCACGGTTTCCTTCAAAACCTTGGGCCCCCCTTTTGGTTT TTCCTCTCTCCCACAAAAGAAATAACCCCTTGGGAAAAAA TACTCTCTCCCCCCAAAAAAAAGGGGGCTCCTTCAGGGGA ATTAATTTCCCCCCCCAATTTTTGGGGAAACACAAAAAAA GAGGGGCGCTCTCCGGAAGAAACCCAAAAATTTTATTTTA AGAACAATCTT
BV-2	T8P1	391	ACTAAAGGAAATAAGTTCCCCAGTTAAAAGGTTTGGCCA ACCGTTGTTGCCATTTGCCACACGGAATCTTGGGTGTCCA CCCTCCCCCGTTTTGGAAATGGGTCCAATCCACCTTCCCG GTTCCAAAAGGATAACGGGAAAGTTACTTAGATCCCCCA ATGTTTGGGAAAAAAAACGGGTTATCCCTCTCGCGGCCTC CCAAACTTTTTGGCAAAAAAAAATTTGGGGCCCCCGGGGT TAAACCCCTCTTGGGTTTAGGGGGCCCCCGGAAAATTTTC TCTCATTGTGGGGGCCCCCCCGAAAAAGGATTTTTTCGGG GGGGGGGGGGAAACCCCACCAACCTTCTCAGAAAAATGG GTGGGCGGCAAAAATTTCTTCTTCGCCGAGGGTT
BV-3	T9P1	1.119	CACCTGTGAACATAGCTCCCGCGCATGGCATGAATCGCTT GACTGTTCTATTGCGGCACCTATTTCCCTTGTTTCCGCCCC CCTGCCAACCTGCTTCCTGTGTGAAACTGTTATCCGCTCTG AATACCTCCCACATACCTATAGGGATACAAAGAGTGTCCC CCTGGCAGCGCCCACGGGCCCTCTTGTGTTCATTGTTGCC GCTTAGCTGATTGCCGGCTTCCTTTCCGCCTTCCTGTCTCG GCAGCTGTGTTATAGATCCCGCCGTCGCGCTGTCAGTTCC GCGTTGCGCATTGGGTCCTCTTCGGCTTGCTCGCTCGATG ACTCGATGCACTCGGTCGGCCGCATGCTATCAGCTAACTC AGCTCATGCAAACGTGGTAATAAGACTATACTTATCATCA TGGGATCACGCCAGTGAGAACATGATAACAAGAGGAAGG TATGTAGGCGGAGCTGCAGAGATCCTGAGTTGCTGGCCTG TTTCCATATACACTGCCACACTGAAAAATGTCAACTGCGC TCTGCTGAAGTCAGAAGCGTCGTACCTTGAGTTGATGAGT CTGTATCCCTTAATCACCCCATCGATGCTCCCTCGGGCGCT GTGGTGTTGCATGCACTGCATCATGCCGATAAACCTGAGC ATCTAACGATCATCCGTTAGATCCTTTTGATTTCTGCTAGA TGATCCGAGGCACAACTCTCACGTTTGTAGGATTTATGGT CTTTGAGATCATGATATGAGCTCAACCCCTCGTTCCTTTCA TACTGCTGCTGCATTATTTGAATCATTCTCATCGTTCTATC GAGCAACTAGGACCGACGTTTCGACATGTGTCATCAGCTG ATGCACCGATGCATTCAGAATCAGGCGATTTGTTACTCAT ATATCGCATGACTGCAGACTCGCCGTACACTACGATACGC GAAGAGCATGACGACTGACATCTTGGCCTCGAGTGAGCA GTAACCTCGAACAAAGCTCCAGTTCTGATCTAAACAAACA CGCAGTCGGAGGCTATTGTTAGACCTTGAACTCTCCTCAT

			CATGATCGTACTCATTCGGATTCAATGCCGTACATTGACA ATCTCGCATGGCCATGGTCAGGATACCGGAGACT
BV-4	T9P6	337	CTTTATGCTGAGTGATAGCTTTTTTTTTGGCAGCATTCATA CAGGTTATTTTCTTTAATAACATCCATTCGGCTCGTCTTCC ACGAAGAACATCCACACGGAACACATCTGCCGCGCATAC ACACAGGTGGCAGGACAGGTATCTACAAGCGACTGCGGT AGTAGAAAGGTAGCGGTTCGAAGTTTTCATAGTTGCATAT ACTAATATCTAATACCATGCTGAGCAGAAGGCAAGGCCG TACGACGACTGACGTCTCGCCGCATTACGCATATGGATCA CGCAACCACGAACGACCCAATATGCATGCGAGACACCCA TCATTTATGGAGGGTCAAT
BV-5	T6P7	415	CTTTATCCTCAGTGATTCTTTTTTTTTCGGGAATAAGTGGC TCTTGGCCATATGTGTGTTTTACATGTCCCTACATCCCAAC GATTTGGCCATATCCTGACCAATGTCTTGCCTACCGCATC ATTTGAGGAGTTCCGGTCCAGTCTTTGTGTCAACCCCGGT GCCCCTTCTCCTGAGGGGGGGGGGGTTGAGTACATGTGTT GTGCACCGGAACTTATTGGGCCCCCCTCCAAGTTACACTG TGGTCATGATGTCTGGGCCTTGTCGGGAACAACGGACATG CAAAACACAAATATGGCCAAAAGCCACCTATTCCCGAAA AAAAAAGATATCATTTAGCGAAGGGAAACAATCATGAAT TCACTCCCGCCTGCCTGTCGACCATATGGAAGAGCTCCCT CCCCGTTGGATGGAT
BV-6	T8P9	304	CATTATGCTGAGTGATATCTTTTTTTTTGCTGGTGTCGATG CATGAGCAGGTGCACATGCAAAATTGATTTTATTTAGACT GAACGGGTCACAAAGTGTGATATACTATGATACAGAAAC GTGCCGGCCGGTCTGCTCCACCGCCGTCACTCTGTCTCTG GCTGTGCATCCTCTCCGGCCGTCAATTGCCCCGAGACTGT TTCTTCTTCTTCTTCCTCTTCTTCGTCCTTGGCGGGAAGGC TCCTACAGCCGGCCTCCTCCATTATCAGCACCTGATGACC TGCCACATTTAGTGAGGGTTAAT

4.2.7.3 BLASTN results

The sequenced fragments were compared with nucleotide database using the BLASTN (EST database) algorithm at the NCBI (www.ncbi.nlm.nih.gov). The BLASTN results are shown in **Tables 4.15** and **4.16**.

4.2.7.3.1 BLASTN results of Sahal-1 genotype

A 416 bp cDNA fragment (Sah-1) obtained by using T6P6 primer combination, was found to be similar to (*Glycine max* cDNA clone genome systems clone ID: Gm-c1065-76685- similar to SW: Blat_Ecoli P00810 Beta-Lactamase Precursor; mRNA sequence), with an e-value of $3e^{-16}$, and to (*Vitis vinifera* cv. perlette LibB *Vitis vinifera* cDNA, mRNA sequence), with an e-value of $2e^{-07}$.

The primer combination T9P6 gave 337 bp cDNA fragment (Sah-2), that was found to be similar to (Drought stress (leaf) *Oryza sativa* indica group cDNA clone NL53_B03 (3-), mRNA sequence) with an e-value of $1e^{-46}$, and to (Coffee drought stressed leaf cDNA library *Coffea canephora* cDNA, mRNA sequence), with an e-value of $1e^{-45}$. A 362 bp cDNA fragment (Sah-3) obtained using primer combination T8P7 displayed similarity to (EST-1654 *Spartina alterniflora* root salinity induced expressed sequence tag (EST) *Spartina alterniflora* cDNA, mRNA sequence) with e = $5e^{-13}$.

A 679 bp cDNA fragment (Sah-4) amplified by primer combination T8P9 was found to be similar to (field drought stressed root cDNA library) with an e-value of $4e^{-11}$. It was also found to be similar to (Brassica seed development drought normalized FTYFDC *Brassica napus* cDNA 5', mRNA sequence) with the same e-value.

Table 4.15: BLASTN search results of drought stress cDNAs that were isolated by differential display from Sahal-1.

Fragment	BlastN Hit	NCBI Accession No.	e-value	Identity
Sah- 1	Sai84h06.y1 Gm-c1065 *Glycine max* cDNA clone genome systems clone ID: Gm-c1065-7668 5- similar to SW: BLAT_ECOLI P00810 BETA-LACTAMASE PRECURSOR, mRNA sequence.	BI972926	$3e^{-16}$	74%
	VV_PEb04h04.b1 *Vitis vinifera* cv. perlette LibB *Vitis vinifera* cDNA, mRNA sequence.	EV232910.1	$2e^{-07}$	70%
Sah-2	NL53_B03 Drought stress (leaf) *Oryza sativa* Indica Group cDNA clone NL53_B03 (3-), mRNA sequence.	GT284587	$1e^{-46}$	75%
	CC_09_UAS466 Coffee drought stressed leaf cDNA library *Coffea canephora* cDNA, mRNA sequence.	GW397408	$1e^{-45}$	75%
Sah-3	EST-1654 *Spartina alterniflora* root salinity induced expressed sequence tag (EST) *Spartina alterniflora* cDNA, mRNA sequence.	EH277618	$5e^{-13}$	81%
Sah-4	ICC4958_CD104_A07 ICC4958 field drought stressed root cDNA library.	GR398891.1	$4e^{-11}$	69%
	FTYFDC_UP_001_F11_14JAN2004_085 Brassica seed development drought normalized FTYFDC *Brassica napus* cDNA 5', mRNA sequence.	EE506138.1	$4e^{-11}$	69%

4.2.7.3.2 BLASTN results of BVD-22 genotype

The primer combination T8P1 gave a 391 bp cDNA fragment (BV-2) that was found to be similar to (*Brassica napus* Ex 20-Lib9 *Brassica napus* cDNA 5-, mRNA sequence) and (*Triticum aestivum* cDNA clone wle1n.pk0086.e115- end, mRNA sequence) with e-values of $3e^{-04}$ and $2e^{-05}$ respectively. A 1.119 bp cDNA fragment (BV-3) obtained using the T9P1 primer combination displayed similarity to (Salt-tolerant *Dunaliella salina* cDNA library *Dunaliella salina* cDNA, mRNA sequence), (field drought stressed root cDNA library *Cicer arietinum* cDNA clone ICC4958_CD104_A075-, mRNA sequence), (dehydration stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD69_B065-, mRNA sequence), and (dehydration stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD66_E115', mRNA sequence) with $2e^{-05}$, $2e^{-05}$, $5e^{-07}$, and $2e^{-05}$ e-values respectively.

The primer combination T9P6 gave a 337 bp cDNA fragment (BV-4) that was found to be similar to (*Triticum aestivum* developing seed heat stress reverse subtractive library *Triticum aestivum* cDNA clone Taw21-012-A07-A-049 3-, mRNA sequence), and similar to (*Triticum aestivum* flower heat stress forward subtractive library *Triticum aestivum* cDNA clone Tau21-004-G05-A-036 3-, mRNA sequence), with $4e^{-58}$ e-values for both, and also similar to (cDNA library of a compatible interaction between stripe rust (*Puccinia striiformis*) and wheat *Triticum aestivum* cDNA 5- similar to reticulon, mRNA sequence) with $1e^{-128}$ e-value. A 415 bp cDNA fragment (BV-5) provided by the T6P7 primer combination was found to be similar to (Dactylis leaf DDRT-cDNA *Dactylis glomerata* cDNA clone 2s10-t7 similar to *Leucine aminopeptidase*, mRNA sequence), (dehydration stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD69_A055-, mRNA sequence), (Slow drought stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD73_E125-, mRNA sequence), and similar to (field drought stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD111_F105-, mRNA sequence) with e-values of $1e^{-14}$, $9e^{-11}$, $1e^{-09}$, and $4e^{-09}$ respectively.

Table 4.16: BLASTN search results of drought stress cDNAs that were isolated by differential display from BVD-22.

Fragment	BlastN Hit	NCBI Accession No.	e-value	Identity
BV-2	EX20LIB9_UP_028_F08_25FEB2005_054 *Brassica napus* Ex 20-Lib9 *Brassica napus* cDNA 5-, mRNA sequence.	EE500043.1	$3e^{-04}$	73%
	wle1n.pk0086.e11 wle1n *Triticum aestivum* cDNA clone wle1n.pk0086.e11 5- end, mRNA sequence.	CA634431.1	$2e^{-05}$	71%
BV-3	Hust515T7 Salt-tolerant *Dunaliella salina* cDNA library *Dunaliella salina* cDNA, mRNA sequence.	GH611964.1	$2e^{-05}$	74%
	ICC4958_CD104_A07 ICC4958 field drought stressed root cDNA library *Cicer arietinum* cDNA clone ICC4958_CD104_A07 5-, mRNA sequence.	GR398891.1	$2e^{-05}$	74%
	ICC1882_CD69_B06 ICC1882 dehydration stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD69_B06 5-, mRNA sequence.	GR391334.1	$5e^{-07}$	73%
	ICC1882_CD66_E11 ICC1882 dehydration stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD66_E11 5', mRNA sequence.	GR391096.1	$2e^{-05}$	72%
BV-4	Taw21-02-A07-A-049.g *Triticum aestivum* developing seed heat stress reverse subtractive library *Triticum aestivum* cDNA clone Taw21-012-A07-A-049 3-, mRNA sequence.	GD188088.1	$4e^{-58}$	85%
	WRIC_123 cDNA library of a compatible interaction between stripe rust (*Puccinia striiformis*) and wheat *Triticum aestivum* cDNA 5-similar to reticulon, mRNA sequence.	GR302507.1	$1e^{-128}$	94%
	Tau21-004-G05-A-036.g *Triticum aestivum* flower heat stress forward subtractive library *Triticum aestivum* cDNA clone Tau21-004-G05-A-036 3-, mRNA sequence.	GD188648.1	$4e^{-58}$	85%
BV-5	EST0001 Dactylis leaf DDRT-cDNA *Dactylis glomerata* cDNA clone 2s10-t7 similar to *Leucine aminopeptidase*, mRNA sequence.	BG724444.1	$1e^{-14}$	79%
	ICC1882_CD69_A05 ICC1882 dehydration stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD69_A05 5-, mRNA sequence.	GR391321.1	$9e^{-11}$	86%
	ICC1882_CD73_E12 ICC1882 Slow drought stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD73_E12 5-, mRNA sequence.	GR394676.1	$1e^{-09}$	82%
	ICC1882_CD111_F10 ICC1882 field drought stressed root cDNA library *Cicer arietinum* cDNA clone ICC1882_CD111_F10 5-, mRNA sequence.	GR408968.1	$4e^{-09}$	85%
BV-6	CJ643812 Y.Ogihara unpublished cDNA library Wh_EMI *Triticum aestivum* cDNA clone whei14n01 5-, mRNA sequence.	CJ643812.1	$1e^{-134}$	100%
	CJ863865 Y. Ogihara unpublished cDNA library, whsctal *Triticum aestivum* cDNA clone whsctal20n07 3-, mRNA sequence.	CJ863865.1	$4e^{-127}$	99%

The primer combination T8P9 gave a 304 bp cDNA fragment (BV-6) was found to be similar to (cDNA library Wh_EMI *Triticum aestivum* cDNA clone whei14n01 5-,

mRNA sequence) and (cDNA library, whsctal *Triticum aestivum* cDNA clone whsctal20n07 3-, mRNA sequence) with e-values of $1e^{-134}$ and $4e^{-127}$ respectively.

4.2.7.4 BLASTX results

The sequenced fragments that were isolated from Sahal-1 and BVD-22 genotypes were analyzed and compared with protein databases (nr, swissprot, refseq_protein, and pdb) using BLASTX algorithm at the NCBI (www.ncbi.nlm.nih.gov).The BLASTX results indicated similarity to many different proteins identified in various organisms such as the proteins shown in **Tables 4.17** and **4.18**.

4.2.7.4.1 BLASTX results of Sahal-1 genotype

According to the results in **Table 4.17**, the fragment that was amplified with T6P6 primers in Sahal-1 (Sah-1) was found to have 42% similarity to plastid high chlorophyll fluorescence 136 precursor from *Zea mays* and Photosystem II stability/assembly factor HCF136, chloroplastic in *Oryza sativa*. The fragment amplified with T9P6 primers (Sah-2) showed 32% similarity to retrotransposon protein, putative, unclassified isolated from *Oryza sativa*. The fragment amplified with T8P7 primers (Sah-3) had 50% similarity to ATP binding / protein kinase/ protein serine/threonine kinase from *Arabidopsis thaliana* and the crystal structure of superoxide dismutase from *Potentilla atrosanguinea*. The fragment amplified with T8P9 primers (Sah-4) was found to have 51% similarity to proline-rich extensin-like receptor kinase 10 of *Arabidopsis thaliana*.

4.2.7.4.2 BLASTX results of BVD-22 genotype

For the six fragments that were isolated from BVD-22 genotype, BLASTX results are shown in **Table 4.18**. The fragment that was amplified with T8P1 primers (BV-1) showed 50% similarity to Glucose-6-phosphate 1-dehydrogenase from *Medicago sativa*. The other fragment that was amplified by the same primer combination (BV-2) was found to have 57% identity to Formin-like protein 20(AtFH20) in *Arabidopsis thaliana*.

Table 4.17: BLASTX search results of drought stress cDNAs that were isolated by differential display from Sahal-1.

Fragment No.	Primers	Database	BLASTX hit	Identity
Sah-1	T6P6	nr	ABQ53629. Plastid high chlorophyll fluorescence 136 precursor Zea *mays*.	42%
		swissprot	Q5Z5A8. Photosystem II stability/assembly factor HCF136, chloroplastic in *Oryza sativa*.	42%
Sah-2	T9P6	nr	ABA99784.retrotransposon protein, putative, unclassified from *Oryza sativa* (japonica cultivar-group).	32%
Sah-3	T8P7	refseq_protein	NP_177036.ATP binding / protein kinase/ protein serine/threonine kinase from *Arabidopsis thaliana*.	50%
		pdb	2Q2L_A.Chain A, crystal structure of superoxide dismutase from *Potentilla atrosanguinea*.	50%
Sah-4	T8P9	nr	NP_173940.2.PERK10(proline-rich extensin-like receptor kinase 10); ATP binding / protein kinase/ protein serine/threonine kinase/ protein tyrosine kinase in *Arabidopsis thaliana*	51%

Table 4.18: BLASTX search results of drought stress cDNAs that were isolated by differential display from BVD-22.

Fragment No.	Primers	Database	BLASTX hit	Identity
Bv-1	T8P1	swissprot	Q42919. Glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform; Short=G6PD from *Medicago sativa*.	50%
Bv-2	T8P1	swissprot	Q9FLQ7. Formin-like protein 20; Short=AtFH20 in *Arabidopsis thaliana*.	57%
Bv-3	T9P1	pdb	1ULK_A.Chain A, crystal structure of pokeweed lectin-C from *Phytolacca americana*.	40%
Bv-4	T9P6	swissprot	P48495. Trios phosphate isomerase, cytosolic; Short=TPI; Short=Trios-phosphate isomerase.	46%
Bv-5	T6P7	nr	ABA94365.retrotransposon protein, putative, unclassified in *Oryza sativa* (japonica cultivar-group).	54%
Bv-6	T8P9	refseq_protein	NP_001151147. Transferase family protein from *Zea mays*.	40%

The fragment that was amplified with T9P1 primers (BV-3) was found to be similar to crystal structure of pokeweed lectin-C from *Phytolacca americana*, with 40% identity. Meanwhile, the fragment that was amplified with T9P6 primers (BV-4) showed 46% similarity to Trios phosphate isomerase (TPI). Whereas the fragment that was amplified with T6P7 primers (BV-5) had 54% similarity to a retrotransposon protein, putative, unclassified from *Oryza sativa*. The fragment that was amplified with T8P9 primers (BV-6) showed 40% identity to a transferase family protein of *Zea mays*.

4.2.7.5 ORF regions of the obtained sequences

The open reading frames of the obtained sequences from Sahal-1 and BVD-22 were detected using ORF Finder algorithm of NCBI.

4.2.7.5.1 ORF regions of the obtained sequences from Sahal-1

The ORFs found for the fragments isolated from Sahal-1 were shown in **Table 4.19**. The fragment amplified with T6P6 primers (Sah-1) had a ninety amino acids long ORF in the frame two, a forty-two amino acids long ORF in the frame +3, and eighty-nine amino acids long ORF in the frame +3. The fragment amplified with T9P6 primers (Sah-2) had two ORFs in the frames -1 and -3 of 86 and 44 amino acids in length, respectively. The fragment amplified with T8P7 primers (Sah-3) had three ORFs in the frames +2, +3 and -1 of 95, 94 and 52 amino acids in length, respectively. However, the fragment amplified with T8P9 primers (Sah-4) had three ORFs in the frame +1, +3, and -2 of 62,41and 46 amino acids in length, respectively.

4.2.7.5.2 ORF regions of the obtained sequences from BVD-22

The ORFs found for the fragments isolated from BVD-22 were listed in **Table 4.20**. The fragment amplified with T8P1 primers (Bv-1) had two long ORFs in the frames +1 and +3 of 101 and 133 amino acids in length, respectively. The second fragment that amplified with the same primers (Bv-2) had a 77 amino acid long ORF in the frame +3, and 52 amino acids long ORF in the frame -2.

Table 4.19: ORFs of the sequences of Sahal-1 genotype.

	Primers	Frame	Sequence	Length (aa)
Sah-1	T6P6	+2	M F E K K G Y L L R P P T F G K I F W G P C L L P G V R A L G N F F I G C P P K R V F W L G G E H K S F R K E W G A T D F S R G Q Q K K P P Q K N I F F Y E E L W G I S C N H S D DT	90
		+3	R K V V P P L N I L A N V F A F A T R L G G Y P P A F G K G F I T F R F P K I K G N *	42
		+3	L K K K V I S F G P Q L L A K Y F G A R V Y S L G L G P L E I S L L G A P P K G F F G W G E N T N P S E K S G G Q Q I F L A A N R R N H P K K I Y F F T K N F G A Y H V I I L T I	89
Sah-2	T9P6	-1	M L V Q G A G P W K N G H H G A F L G F L P F A G P F L P R S F L P L S P D P G N T R I T P L K G P D T P R A A R P T R A G G S L G G E R K E R P Y A K R P F P R V W R I H *	86
		-3	Y L S R E F W N A C S G G R T L E K R P P R R L F R V L A L C R P F S S Q V L P A V I P *	44
Sah-3	T8P7	+2	L N E P G D H A P G P N F P N K P P P E G Q A K M A R T Y R P P A Y I W P E P K K F P L K F G N V V L S K S G G P P L E G L P P S P N G E F S P L G K K I L L P N W K N G R V S R G G A V F W V	95
		+3	M N P E T T L P V P I F R I N P P P K A K P K W P E L T A P P P I F G R N Q K N F P L N L A M L S F Q N P G D P L W R V F P R P Q M G N F P R W E K R F S C P I G K M A G F P G V G L F F G C	94
		-1	A P K K Q P H P G K P G H F S N W A G E S F F P T G K I P H L G T G E D P P K G V P R I L K G Q H C Q I *	52
Sah-4	T8P9	+1	M C F W V K K G P H P L K K C Y N K T L L P L T S D L W G R N R F F Y P F T R I H P P T L F P P P P D Q A P P G L P C P R T P *	62
		+3	M G P K P F F L S V H P N P P P H P V P P P R S S P S R A S L P Q N T I T R I S K *	41
		-2	K S N R P I L K C V L W C S G A G K P G R G L I G G G E Q G G G V D S G E R I E K T V S A P *	46

The fragment that was amplified with T9P1 primers (Bv-3) had two ORFs in the frames +3 and -1 that were 60 and 78 amino acids in length, respectively. In addition, the fragment that was amplified with T9P6 primers (Bv-4) had two ORFs in the frames +1 and -3 of 47 and 38 amino acids in length, respectively. The fragment amplified with T6P7 primers (Bv-5) had two ORFs in the frames +1 and +2 of 95 and 84 amino acids

in length, respectively. However, the fragment amplified with T8P9 primers (Bv-6) had two ORFs in the frame +2, and +3 of 79 and 61 amino acids in length, respectively.

Table 4.20: ORFs of the sequences of BVD-22 genotype.

	Primers	Frame	Sequence	Length (aa)
Bv-1	T8P1	+1	M V N Q G G P F H G L A I S L P K W P N P R G E K P Q P G G L H L G P G G K K P G T H S P A Q I Y Q K N P P G G A E K S V R N F P P P P I I W R E K K I S P I F T T V S F K T L G P P F G F S S L P Q K K *	101
		+3	M A E P P R G K T P T G G V T L G P R W K E T R N T L P G P N L P K K P P G R G R E K C P Q F S P P T H N L A G K K N F P Y I Y H G F L Q N L G P P F W F F L S P T K E I T P W E K I L S P P K K K G A P S G E L I S P P N F W G N T K K E G R S P E E T Q K F Y F K N N L	133
Bv-2	T8P1	+3	M F G K K N G L S L S R P P K L F G K K K F G A P G V K P L L G L G G P R K I F S H C G G P P E K G F F R G G G G N P T N L L R K M G G R Q K F L L R R G	77
		-2	L G D L S N F P V I L L E P G R W I G P I S K T G E G G H P R F R V A N G N N G W P N L L T G E L I S F S	52
Bv-3	T9P1	+3	M N R L T V L L R H L F P L F P P P C Q P A S C V K L L S A L N T S H I P I G I Q R V S P W Q R P R A L L C S L L P L S *	60
		-1	M L R F I G M M Q C M Q H H S A R G S I D G V I K G Y R L I N S R Y D A S D F S R A Q L T F F S V A V Y M E T G Q Q L R I S A A P P T Y L P L V I M F S L A *	78
Bv-4	T9P6	+1	L H I L I S N T M L S R R Q G R T T T D V S P H Y A Y G S R N H E R P N M H A R H P S F M E G Q	47
		-3	M R G R C V P C G C S S W K T S R M D V I K E N N L Y E C C Q K K S Y H S A *	38
Bv-5	T6P7	+1	M C V L H V P T S Q R F G H I L T N V L P T A S F E E F R S S L C V N P G A P S P E G G G V E Y M C C A P E L I G P P S K L H C G H D V W A L S G T T D M Q N T N M A K S H L F P K K K D I I *	95
		+2	M S C L P H H L R S S G P V F V S T P V P L L L R G G G L S T C V V H R N L L G P P P S Y T V V M M S G P C R E Q R T C K T Q I W P K A T Y S R K K K I S F S E G K Q S *	84
Bv-6	T8P9	+2	L I L F R L N G S Q S V I Y Y D T E T C R P V C S T A V T L S L A V H P L R P S I A P R L F L L L L P L L R P W R E G S Y S R P P P L S A P D D L P H L V R V N	79
		+3	M I Q K R A G R S A P P P S L C L W L C I L S G R Q L P R D C F F F F F L F F V L G G K A P T A G L L H Y Q H L M T C H I *	61

4.2.7.6 The known protein motifs of the obtained sequences from Sahal-1 and BVD-22

The protein sequences were searched for known protein motifs using the Motif Scan algorithm by searching against PROSITE patterns, PROSITE patterns (frequent match producers), PROSITE profiles, Prefile (more profiles), Pfam HMMs (local models), and Pfam HMMs (global models) databases.

The longest amino acid sequence found for Sahal-1 was that obtained by T8P7 primers (Sah-3), which had 95 amino acids (**Table 4.19**). The sequence was searched for conserved motifs using Motif Scan algorithm and was found to have a putative Amidation site in the region 73-76 (LGKK), Protein kinase C phosphorylation site in the region 28-30 (TYR). Interestingly, it also showed a proline-rich region profile in the region 4-66 (**Fig.4.15**).

Fig. 4.15: The motif predicted for the ninety-five amino acids long ORF sequence of the fragment amplified with T8P7 primers in the Sahal- 1 genotype found by Motif Scan algorithm

On the other hand, the longest amino acid sequence found for BVD-22, obtained using T8P1 primers (Bv-1) had 133 amino acids (**Table 4.20**). The sequence was searched for conserved motifs and was found to have a putative Amidation site in the region 55-58 (AGKK), ATP/GTP-binding site in the region 2-9 (AEPPRGKT), Casein kinase II phosphorylation site in the region 121-124 (SPEE), and Protein kinase C phosphorylation site in the region 125-127 (TQK).

4.2.7.7 Pairwise alignment of the fragments that were amplified with the same primer sets

There were two primer sets that amplified a fragment both for Sahal-1 and BVD-22 genotypes. These fragments aligned using ClustalW algorithm of EBI. The pairwise alignments of the fragments were shown in **Fig.4.16** and **4.17**.

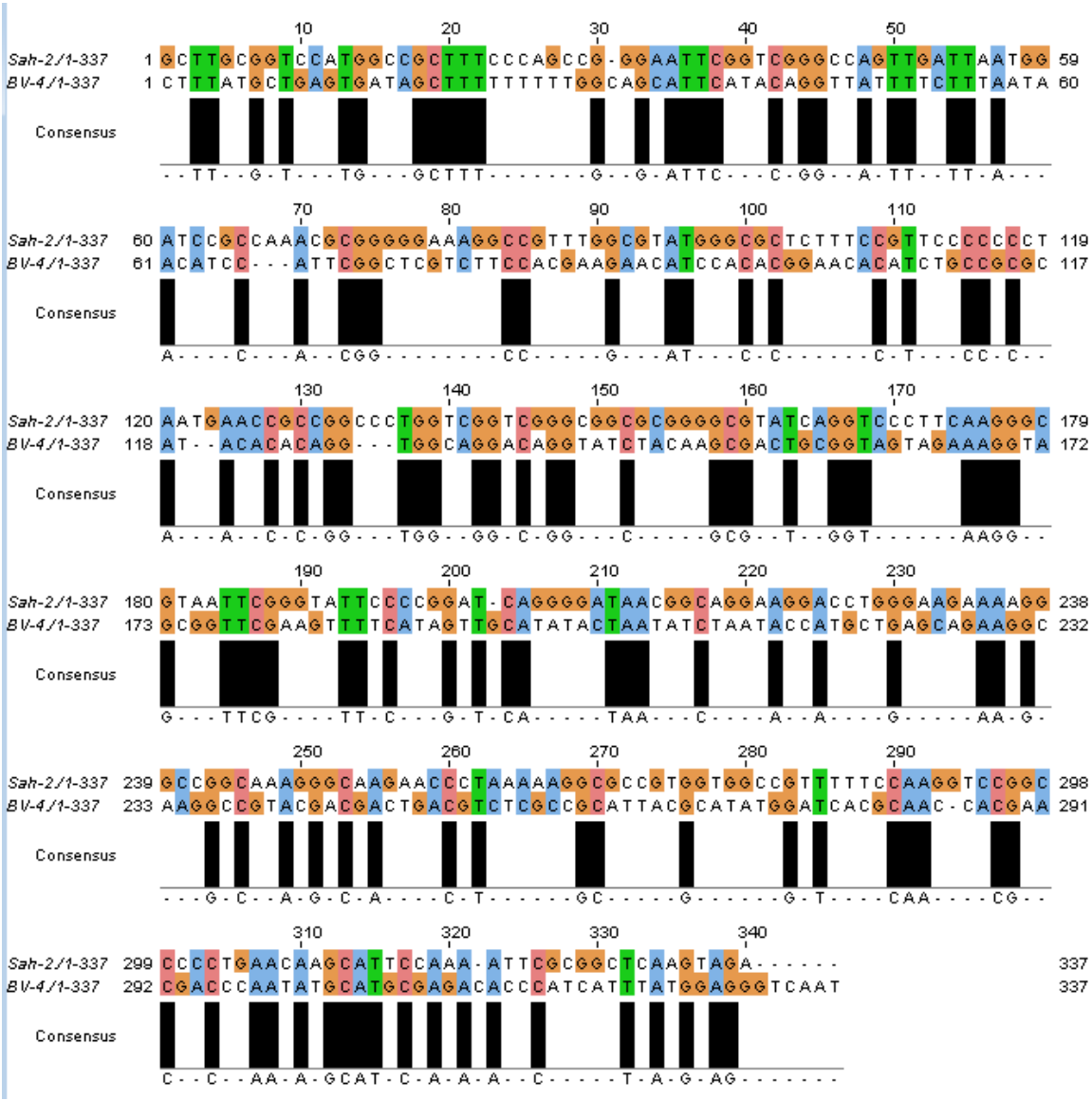


Fig. 4.16: Pairwise alignment of the fragments amplified with T9P6 primers both in Sahal-1 and BVD-22 genotypes

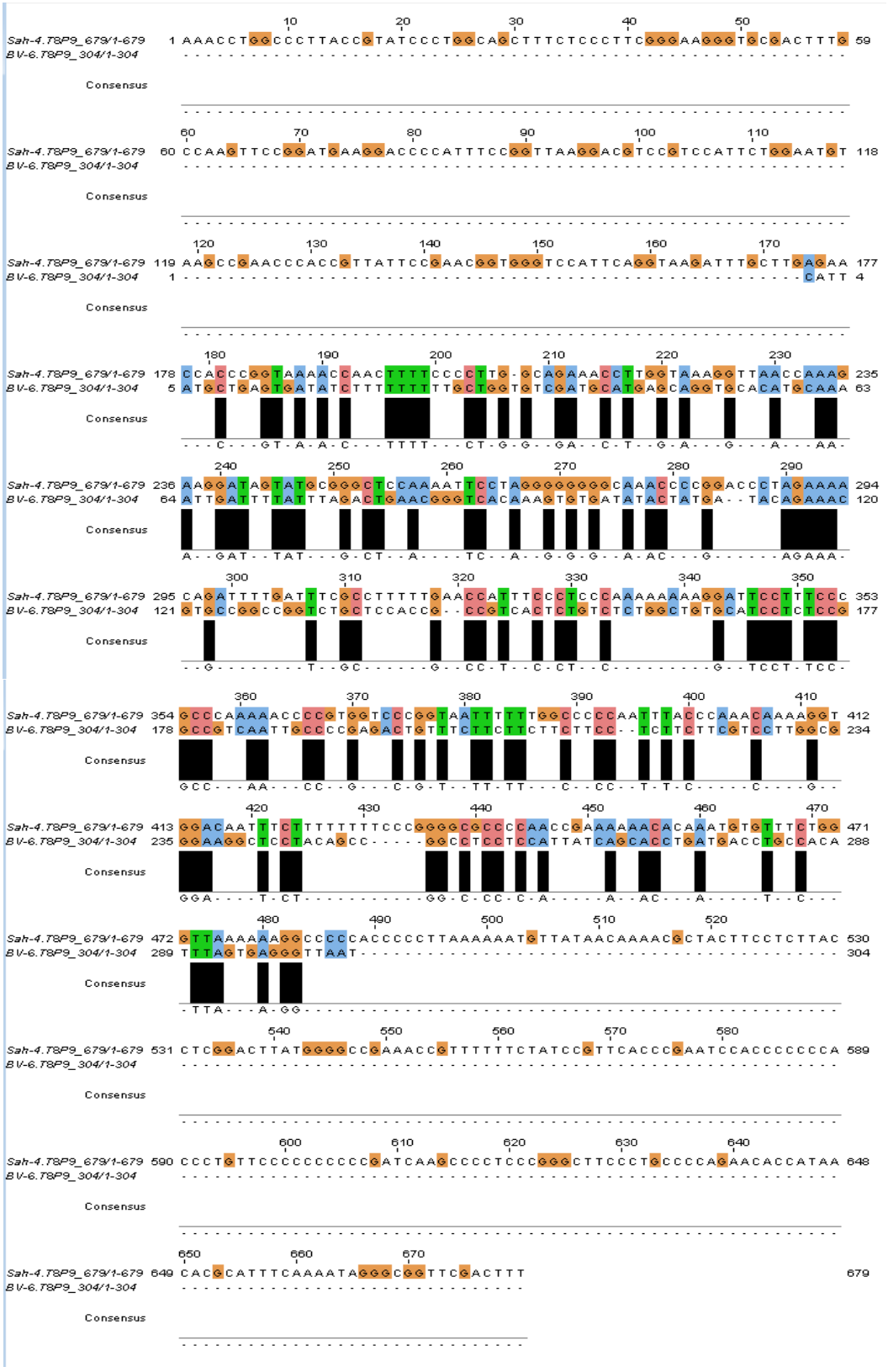
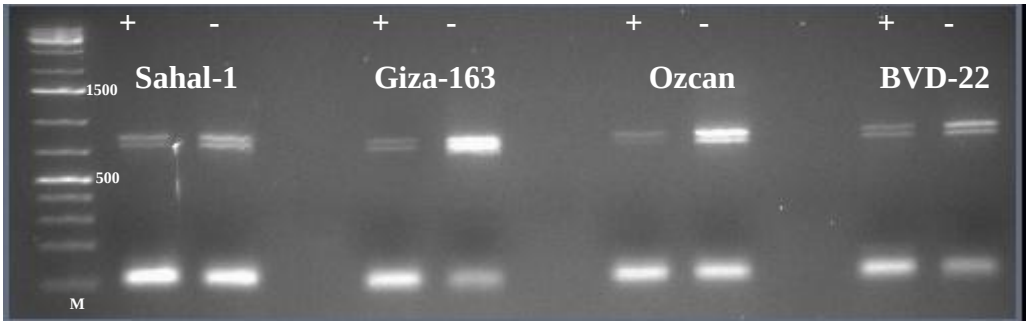


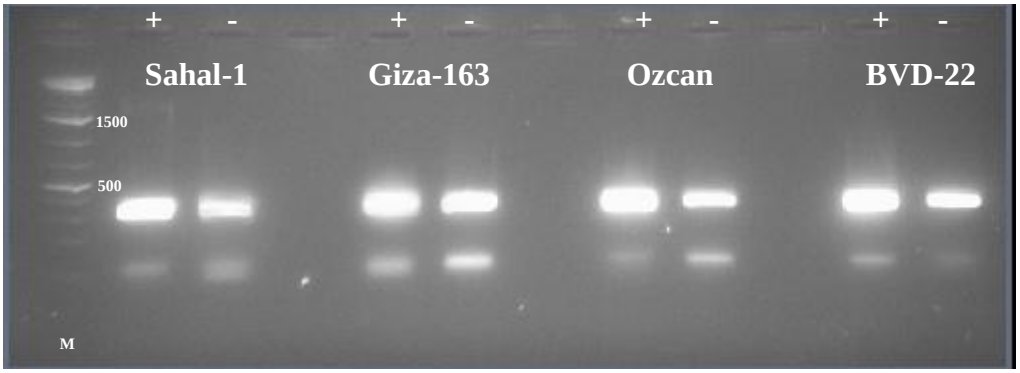
Fig. 4.17: Pairwise alignment of the fragments amplified with T8P9 primers both in Sahal-1 and BVD-22 genotypes

4.2.7.8 DREB genes

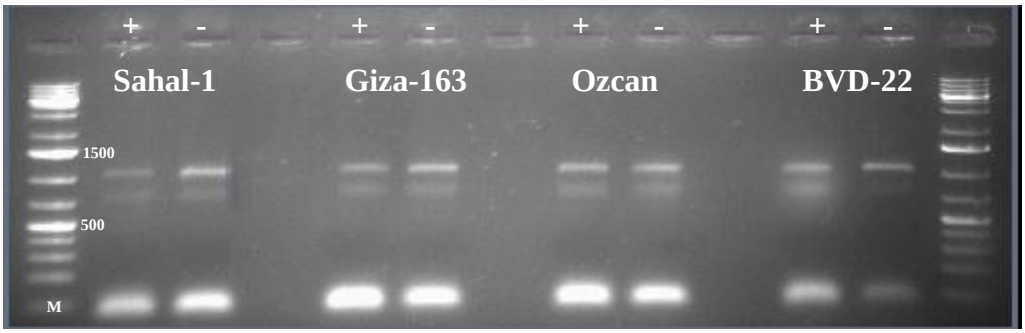
The transcription factors are binds to specific sequence of promoter region of the target genes, which will be, activate as a response to drought. Dehydration-responsive element binding (DREB) proteins constitute a large family of transcription factors that are involved in a biotic stress tolerance. DREBs regulate many functional genes related to drought stress (**Ito**, ***et al***., **2006**).



(a)



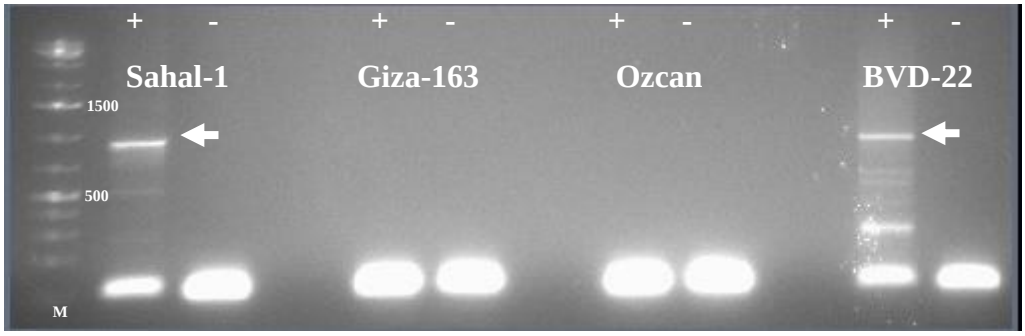
(b)



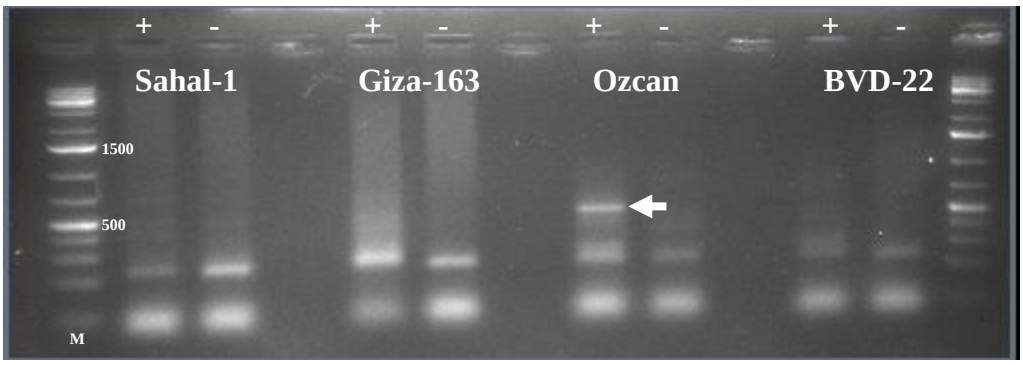
(c)

Fig. 4.18: Agarose gel electrophoresis pictures of PCR products of Sahal-1,Giza-163, Ozcan and BVD-22 genotypes. The genotypes were exposed to drought stress 40 days after sowing (DAS). (a) PCR products obtained via Dreb 1 primer (annealing temp. was 56.5 °C). (b) PCR products obtained via Dreb R13A primer (annealing temp. was 51.8 °C). (c) PCR products obtained via Dreb 3a primer (annealing temp. was 52 °C), (+) =Stress, (-) =Irrigated.

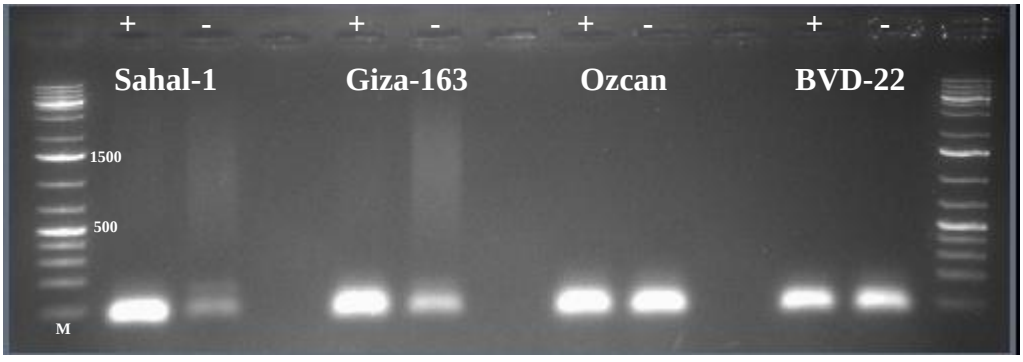
DREB genes consist of two subclasses, 1) DREB gene1, which induced by cold stress, and 2) DREB gene2, which induced by dehydration stress (**Choi**, ***et al.***, **2002**). As shown in **Fig.4.18**, Dreb-1s and Dreb 3a were expressed in all genotypes under all conditions. Although Dreb R1-3A expressed in all genotypes under drought and irrigated conditions, the level of expression under drought stress was higher than control.



(a)



(b)



(c)

Fig. 4.19: Agarose gel electrophoresis pictures of PCR products of Sahal-1, Giza-163, Ozcan and BVD-22 genotypes. The genotypes were exposed to drought stress 40 days after sowing (DAS). (a) PCR products obtained via Dreb R2 1A (annealing temp. was 51 °C). (b) PCR products obtained via Dreb R12B primer (annealing temp. was 47.7 °C). (c) PCR products obtained via Dreb R1 2A primer (annealing temp. was 46°C), (+) =Stress, (-) =Irrigated.

As shown in **Fig.4.19**, Dreb R2 1A was expressed in both Sahal-1 and BVD-22 genotypes only under drought stress conditions. Dreb R1 2B was expressed only in Ozcan genotype under drought stress conditions. Dreb R1 2A expression was not detected in any genotype under all conditions.

4.3 Field experiment

For a study closer to agricultural conditions, we evaluated the response of forty-nine bread wheat genotypes to drought stress in the open field.

4.3.1 Effect of irrigation system on growth and morphological characteristics

4.3.1.1 Effect on plant height (cm)

Plant height was measured on 26 June 2009, before harvesting. The effect of water stress on plant height was clear (**Table 4.21**). Almost all genotypes had produced good plant height under well-watered conditions, while the plant height was significantly reduced under water stress, except Katia, Momtchill, and Seval genotypes, which showed equal values under both drought and well-watered conditions.

The plant height of Hawk, Kirgiz95, Zitnica, Es84-24/seri//seri and Kutluk94 genotypes was the most affected by drought stress with percentage decreases of 25, 23.8, 21.2, 20.8, and 16.3%, while Bolal2973, Mufitbey, Pastor, Flamura85, Weston, Es00-ke3, Dagdas, Jagger, Kirkpinar79, Ks82w422, Ktk/ye2453, Gerek gm, F12.71/coc//prl"s", Izgi01, Pyn/bau, Bayraktar, and Gerek79 genotypes showed the lowest reduction. These results were in accordance with those reported by **Mirakhori**, ***et al***., (**2009**) and **Moayedi**, ***et al***., (**2010**).

4.3.1.2 Effect on heading date

Generally, drought stress delayed the heading date in most genotypes compared to the well-watered conditions except Flamura85 and Stk52/trumbull, genotypes which showed the opposite trend (**Table 4.22**), while Bayraktar, F12.71/coc//prl"s", Izgi01,

Katia, Momtchill, Pastor, and Suzen97 genotypes recorded the same heading date under both drought and well watered conditions. Under drought stress, the delay of heading date ranged from 1 days (Kirkpinar79, Century, Ekg15//tast, Es00-ke3, Gerek gm, Seval, Soyer, Tosunbey, and Jagger genotypes) to 4 days (Es84-24/seri//seri, Aytin98, Ca8055/krc66, Es84-24//ks82w409, and Gerek79 genotypes).The results were in parallel with those achieved by **Bayoumi**, ***et al***., (**2008**).

Table 4.21: The effect of irrigation systems on plant height (cm) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Plant height (cm)						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	113.0 ± 2.0	124.0 ± 8.5	-8.9	Kirgiz95	99.0 ± 5.6	130.0 ± 3.1	-23.8
Aytin98	91.0 ± 6.6	98.0 ± 9.6	-7.1	Kirkpinar79	89.0 ± 3.6	92.0 ± 4.4	-3.3
Bayraktar	109.0 ± 1.2	110.0 ± 9.5	-0.9	Krc/bez	117.0 ± 2.5	135.0 ± 1.0	-13.3
Bezostaya1	105.0 ± 3.0	114.0 ± 9.1	-7.9	Ks82w422	87.0 ± 1.5	90.0 ± 3.5	-3.3
Bolal2973	109.0 ± 3.2	116.0 ± 9.3	-6.0	Ktk/ye2453	124.0 ± 4.4	128.0 ± 6.5	-3.1
Ca8055/krc66	111.0 ± 5.5	121.0 ± 5.6	-8.3	Kutluk94	113.0 ± 3.1	135.0 ± 3.0	-16.3
Century	91.0 ± 2.5	105.0 ± 8.4	-13.3	Lov/bll/	92.0 ± 9.8	105.0 ± 6.4	-12.4
Dagdas	123.0 ± 0.6	129.0 ± 2.3	-4.7	Mnch/5/	93.0 ± 4.2	110.0 ± 8.7	-15.5
Ekg15//tast	100.0 ± 5.0	116.0 ± 7.4	-13.8	Momtchill	101.0 ± 3.8	101.0 ± 0.0	0.0
Es00-ke3	113.0 ± 2.6	119.0 ± 2.0	-5.0	Momtchill/gun	107.0 ± 4.6	119.0 ± 6.8	-10.1
Es84-24//ks82w409	79.0 ± 5.1	91.0 ± 3.1	-13.2	Mufitbey	112.0 ± 7.5	119.0 ± 1.5	-5.9
Es84-24/seri//seri	84.0 ± 3.2	106.0 ± 4.0	-20.8	Pastor	95.0 ± 4.7	101.0 ± 4.0	-5.9
F12.71/coc//kauz	91.0 ± 2.1	106.0 ± 2.3	-14.2	Pyn/bau	98.0 ± 5.2	99.0 ± 2.0	-1.0
F12.71/coc//prl"s"	115.0 ± 6.4	118.0 ± 3.0	-2.5	Seval	102.0 ± 7.8	102.0 ± 3.5	0.0
Flamura85	86.0 ± 9.0	91.0 ± 6.6	-5.5	Sonmez01	94.0 ± 4.0	106.0 ± 3.6	-11.3
Gerek gm	98.0 ± 2.6	101.0 ± 3.8	-3.0	Soyer	90.0 ± 4.2	98.0 ± 1.2	-8.2
Gerek79	110.0 ± 6.2	111.0 ± 0.6	-0.9	Stk52/trumbull	86.0 ± 6.0	95.0 ± 4.5	-9.5
Gun91	102.0 ± 5.3	116.0 ± 10.7	-12.1	Suzen97	113.0 ± 1.5	121.0 ± 1.7	-6.6
Harmankaya99	84.0 ± 1.2	95.0 ± 4.6	-11.6	Tosunbey	92.0 ± 6.2	105.0 ± 3.6	-12.4
Hawk	75.0 ± 2.1	100.0 ± 6.6	-25.0	Vona//no57	98.0 ± 2.0	105.0 ± 5.7	-6.7
Ikizce96	113.0 ± 5.6	121.0 ± 5.5	-6.6	Vorona/kauz	99.0 ± 4.7	107.0 ± 2.5	-7.5
Izgi01	92.0 ± 5.7	94.0 ± 6.7	-2.1	Weston	110.0 ± 4.0	116.0 ± 6.0	-5.2
Jagger	96.0 ± 4.0	100.0 ± 2.9	-4.0	Zitnica	82.0 ± 2.5	104.0 ± 6.0	-21.2
Karahan	108.0 ± 2.1	122.0 ± 3.5	-11.5	**Grand mean**	100.0	110.0	-9.1
Katia	102.0 ± 2.9	102.0 ± 2.6	0.0	**l.s.d Genotype**	5.7		
Kirac66	102.0 ± 3.2	119.0 ± 3.2	-14.3	**Treatment**	1.2		
				G x T	8.0		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

Table 4.22: The effect of irrigation systems on heading date of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Heading date						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	27 ± 1.0	25 ± 1.0	8.0	Kirgiz95	24 ± 0.6	21 ± 1.7	14.0
Aytin98	24 ± 1.7	20 ± 0.6	20.0	Kirkpinar79	30 ± 0.6	29 ± 0.0	3.0
Bayraktar	16 ± 0.6	16 ± 0.6	0.0	Krc/bez	26 ± 0.6	24 ± 0.6	8.0
Bezostaya1	27 ± 1.2	24 ± 0.0	13.0	Ks82w422	23 ± 0.6	20 ± 0.6	15.0
Bolal2973	24 ± 1.0	21 ± 0.0	14.0	Ktk/ye2453	25 ± 0.0	22 ± 1.2	14.0
Ca8055/krc66	24 ± 2.6	20 ± 1.2	20.0	Kutluk94	30 ± 1.0	27 ± 1.5	11.0
Century	24 ± 1.0	23 ± 1.2	4.0	Lov/bll/	31 ± 0.0	29 ± 0.0	7.0
Dagdas	26 ± 1.5	24 ± 0.6	8.0	Mnch/5/	23 ± 0.6	21 ± 1.7	10.0
Ekg15//tast	25 ± 0.0	24 ± 0.6	4.0	Momtchill	20 ± 1.5	20 ± 0.6	0.0
Es00-ke3	24 ± 0.6	23 ± 0.6	4.0	Momtchill/gun	25 ± 2.3	22 ± 1.2	14.0
Es84-24//ks82w409	24 ± 1.2	20 ± 1.0	20.0	Mufitbey	27 ± 1.0	25 ± 1.5	8.0
Es84-24/seri//seri	28 ± 0.0	24 ± 0.0	17.0	Pastor	19 ± 0.0	19 ± 1.5	0.0
F12.71/coc//kauz	28 ± 1.7	26 ± 0.0	8.0	Pyn/bau	26 ± 1.2	24 ± 1.2	8.0
F12.71/coc//prl"s"	23 ± 0.6	23 ± 1.2	0.0	Seval	22 ± 1.7	21 ± 0.0	5.0
Flamura85	19 ± 0.6	20 ± 0.6	-5.0	Sonmez01	23 ± 1.0	20 ± 0.0	15.0
Gerek gm	21 ± 1.5	20 ± 0.6	5.0	Soyer	22 ± 2.1	21 ± 0.6	5.0
Gerek79	24 ± 1.0	20 ± 0.6	20.0	Stk52/trumbull	23 ± 1.2	24 ± 0.0	-4.0
Gun91	29 ± 0.6	26 ± 0.6	12.0	Suzen97	25 ± 0.0	25 ± 0.6	0.0
Harmankaya99	22 ± 3.8	20 ± 1.0	10.0	Tosunbey	20 ± 1.2	19 ± 1.0	5.0
Hawk	22 ± 1.7	20 ± 0.6	10.0	Vona//no57	21 ± 1.7	19 ± 0.6	11.0
Ikizce96	21 ± 0.6	19 ± 0.6	11.0	Vorona/kauz	24 ± 0.0	21 ± 0.0	14.0
Izgi01	18 ± 1.5	18 ± 1.2	0.0	Weston	28 ± 0.6	25 ± 1.2	12.0
Jagger	19 ± 0.6	18 ± 0.6	6.0	Zitnica	23 ± 0.6	20 ± 1.2	15.0
Karahan	25 ± 0.0	22 ± 1.7	14.0	**Grand mean**	24	22	9
Katia	20 ± 0.6	20 ± 0.6	0.0	**l.s.d Genotype**	1.2		
Kirac66	26 ± 0.0	24 ± 0.0	8.0	**Treatment**	0.3		
				G x T	1.7		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions. ** The heading was at May 2009.

4.3.1.3 Effect on biomass (kg m^{-2})

Total biomass of all genotypes were suppressed by drought stress except Lov/bll and Flamura85 genotypes which showed the opposite of that with percentage increases of 6 and 10.4% (**Table 4.23**). Biomass under rain fed conditions, ranged between 0.9 kg m^{-2} (Es84-24//ks82w409, Seval, Vona//no57) and 1.5 kg m^{-2} (Momtchill/gun). The biomasses of Vona//no57, Hawk, Harmankaya99, F12.71/coc//kauz, and Soyer

genotypes were the most affected with percentage decreases of 42.5, 41.2, 37.9, 37.3, and 37.1%, while Altay2000, Kutluk94, Gun91, Ekg15//tast, Century, Ikizce96, Suzen97, Weston, Katia, Aytin98, Momtchill, Vorona/kauz, Ks82w422, Momtchill/gun, Krc/bez, Bayraktar, Mnch/5/ and Pastor, genotypes showed the lowest reduction under drought stress. Similar results were demonstrated by **Sangtarash**, (**2010**).

Table 4.23: The effect of irrigation systems on biomass (kg m^{-2}) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Biomass (kg m^{-2})						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	1.1 ± 0.2	1.5 ± 0.2	-23.3	Kirgiz95	1.1 ± 0.2	1.5 ± 0.1	-25.2
Aytin98	1.1 ± 0.1	1.3 ± 0.3	-15.0	Kirkpinar79	1.1 ± 0.2	1.6 ± 0.1	-31.7
Bayraktar	1.3 ± 0.1	1.5 ± 0.1	-10.7	Krc/bez	1.4 ± 0.1	1.5 ± 0.0	-11.1
Bezostaya1	1.0 ± 0.1	1.3 ± 0.1	-28.4	Ks82w422	1.2 ± 0.1	1.3 ± 0.3	-12.2
Bolal2973	1.2 ± 0.2	1.8 ± 0.2	-32.6	Ktk/ye2453	1.0 ± 0.2	1.5 ± 0.2	-28.8
Ca8055/krc66	1.1 ± 0.0	1.6 ± 0.2	-30.6	Kutluk94	1.2 ± 0.1	1.5 ± 0.0	-21.9
Century	1.2 ± 0.1	1.5 ± 0.2	-18.4	Lov/bll/	1.2 ± 0.1	1.2 ± 0.1	6.0
Dagdas	1.3 ± 0.1	1.8 ± 0.1	-31.5	Mnch/5/	1.2 ± 0.1	1.3 ± 0.3	-7.5
Ekg15//tast	1.2 ± 0.0	1.5 ± 0.3	-20.8	Momtchill	1.4 ± 0.2	1.6 ± 0.0	-13.4
Es00-ke3	1.3 ± 0.1	1.7 ± 0.1	-24.4	Momtchill/gun	1.5 ± 0.2	1.7 ± 0.2	-11.4
Es84-24//ks82w409	0.9 ± 0.1	1.5 ± 0.1	-35.9	Mufitbey	1.2 ± 0.2	1.8 ± 0.1	-33.9
Es84-24/seri//seri	1.1 ± 0.0	1.5 ± 0.0	-24.0	Pastor	1.1 ± 0.0	1.2 ± 0.1	-1.7
F12.71/coc//kauz	1.0 ± 0.1	1.6 ± 0.1	-37.3	Pyn/bau	1.2 ± 0.1	1.8 ± 0.2	-33.5
F12.71/coc//prl"s"	1.0 ± 0.2	1.3 ± 0.0	-23.7	Seval	0.9 ± 0.2	1.3 ± 0.2	-28.3
Flamura85	1.4 ± 0.1	1.3 ± 0.1	10.4	Sonmez01	1.2 ± 0.2	1.6 ± 0.2	-28.2
Gerek gm	1.2 ± 0.1	1.5 ± 0.1	-24.3	Soyer	1.1 ± 0.2	1.7 ± 0.1	-37.1
Gerek79	1.1 ± 0.0	1.4 ± 0.2	-23.7	Stk52/trumbull	1.0 ± 0.1	1.3 ± 0.1	-24.6
Gun91	1.3 ± 0.1	1.6 ± 0.1	-21.5	Suzen97	1.3 ± 0.0	1.5 ± 0.1	-15.8
Harmankaya99	1.1 ± 0.1	1.7 ± 0.4	-37.9	Tosunbey	1.2 ± 0.1	1.7 ± 0.2	-33.3
Hawk	1.0 ± 0.0	1.7 ± 0.2	-41.2	Vona//no57	0.9 ± 0.0	1.6 ± 0.0	-42.5
Ikizce96	1.2 ± 0.1	1.5 ± 0.1	-16.3	Vorona/kauz	1.3 ± 0.0	1.5 ± 0.2	-12.8
Izgi01	1.0 ± 0.2	1.4 ± 0.1	-27.1	Weston	1.1 ± 0.1	1.3 ± 0.3	-15.8
Jagger	1.0 ± 0.2	1.4 ± 0.1	-29.8	Zitnica	1.0 ± 0.0	1.4 ± 0.1	-26.2
Karahan	1.3 ± 0.1	1.9 ± 0.3	-32.3	**Grand mean**	1.2	1.5	-23.8
Katia	1.3 ± 0.1	1.5 ± 0.1	-15.4	**l.s.d Genotype**	0.2		
Kirac66	1.0 ± 0.0	1.4 ± 0.0	-27.5	**Treatment**	0.0		
				G x T	0.2		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

4.3.1.4 Effect on harvest index (%)

Harvest index is the proportion of the biological yield, which forms the economic yield. As shown in **Table 4.24**, the controls had the highest HI and water stressed plants had the lowest. The harvest index was affected by drought stress; a mean reduction of 2.4% was recorded in HI for all genotypes. Zitnica, Pyn/bau, Izgi01, Gerek gm, Ikizce96, and Mufitbey genotypes recorded the minimum reductions of 2.2, 2.3, 2.3, 2.4, 2.5, and 2.7%.

Table 4.24: The effect of irrigation systems on harvest index (%) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Harvest index (%)						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	0.41 ± 0.01	0.40 ± 0.00	2.5	Kirgiz95	0.38 ± 0.02	0.42 ± 0.01	-9.5
Aytin98	0.42 ± 0.01	0.44 ± 0.04	-4.5	Kirkpinar79	0.42 ± 0.00	0.46 ± 0.02	-8.7
Bayraktar	0.40 ± 0.02	0.43 ± 0.01	-7.0	Krc/bez	0.37 ± 0.00	0.36 ± 0.03	2.8
Bezostaya1	0.42 ± 0.01	0.41 ± 0.01	2.4	Ks82w422	0.38 ± 0.03	0.41 ± 0.02	-7.3
Bolal2973	0.38 ± 0.01	0.38 ± 0.02	0.0	Ktk/ye2453	0.38 ± 0.03	0.37 ± 0.02	2.7
Ca8055/krc66	0.42 ± 0.01	0.42 ± 0.00	0.0	Kutluk94	0.39 ± 0.01	0.39 ± 0.01	0.0
Century	0.46 ± 0.02	0.46 ± 0.01	0.0	Lov/bll/	0.37 ± 0.01	0.40 ± 0.05	-7.5
Dagdas	0.37 ± 0.03	0.36 ± 0.00	2.8	Mnch/5/	0.43 ± 0.01	0.43 ± 0.01	0.0
Ekg15//tast	0.37 ± 0.00	0.37 ± 0.02	0.0	Momtchill	0.43 ± 0.02	0.42 ± 0.01	2.4
Es00-ke3	0.45 ± 0.00	0.42 ± 0.02	7.1	Momtchill/gun	0.40 ± 0.02	0.42 ± 0.02	-4.8
Es84-24//ks82w409	0.43 ± 0.03	0.45 ± 0.02	-4.4	Mufitbey	0.36 ± 0.01	0.37 ± 0.01	-2.7
Es84-24/seri//seri	0.43 ± 0.00	0.45 ± 0.00	-4.4	Pastor	0.47 ± 0.03	0.43 ± 0.01	9.3
F12.71/coc//kauz	0.40 ± 0.00	0.43 ± 0.03	-7.0	Pyn/bau	0.42 ± 0.01	0.43 ± 0.02	-2.3
F12.71/coc//prl"s"	0.40 ± 0.04	0.43 ± 0.02	-7.0	Seval	0.46 ± 0.00	0.45 ± 0.03	2.2
Flamura85	0.41 ± 0.01	0.46 ± 0.01	-10.9	Sonmez01	0.44 ± 0.01	0.42 ± 0.01	4.8
Gerek gm	0.40 ± 0.05	0.41 ± 0.00	-2.4	Soyer	0.47 ± 0.03	0.45 ± 0.00	4.4
Gerek79	0.38 ± 0.01	0.38 ± 0.02	0.0	Stk52/trumbull	0.33 ± 0.04	0.43 ± 0.02	-23.3
Gun91	0.38 ± 0.01	0.42 ± 0.02	-9.5	Suzen97	0.42 ± 0.01	0.42 ± 0.02	0.0
Harmankaya99	0.46 ± 0.03	0.48 ± 0.01	-4.2	Tosunbey	0.46 ± 0.03	0.43 ± 0.01	7.0
Hawk	0.44 ± 0.01	0.44 ± 0.03	0.0	Vona//no57	0.49 ± 0.01	0.40 ± 0.01	22.5
Ikizce96	0.39 ± 0.03	0.40 ± 0.02	-2.5	Vorona/kauz	0.43 ± 0.00	0.46 ± 0.04	-6.5
Izgi01	0.43 ± 0.03	0.44 ± 0.01	-2.3	Weston	0.38 ± 0.01	0.40 ± 0.02	-5.0
Jagger	0.46 ± 0.01	0.46 ± 0.02	0.0	Zitnica	0.45 ± 0.05	0.46 ± 0.02	-2.2
Karahan	0.40 ± 0.04	0.39 ± 0.03	2.6	**Grand mean**	0.41	0.42	-2.4
Katia	0.40 ± 0.04	0.47 ± 0.00	-14.9	**l.s.d Genotype**	0.02		
Kirac66	0.37 ± 0.02	0.36 ± 0.03	2.8	**Treatment**	0.01		
				G x T	0.03		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

On the other hand, the genotypes, Bolal2973, Ca8055/krc66, Century, Ekg15//tast, Gerek79, Hawk, Jagger, Kutluk94, Mnch/5/, and Suzen97 recorded equal HI values under both drought and well watered conditions. In contrast, a maximum increase in HI of 22.5% was in Vona//no57 followed by Pastor, Es00-ke3, Tosunbey, Sonmez01, Soyer, Krc/bez, Kirac66, Dagdas, Ktk/ye2453, Karahan, Altay2000, Momtchill, Bezostaya1, and Seval genotypes with maximum increases of 9.3, 7.1, 7, 4.8, 4.4, 2.8, 2.8, 2.8, 2.7, 2.6, 2.5, 2.4, 2.4, and 2.2% respectively. The reduction in harvest index in wheat genotypes under drought stress was reported by **Nouri-Ganbalani**, ***et al***., (**2009**), and **Moayedi**, ***et al***., **(2010**).

4.3.1.5 Effect of irrigation on normalized difference vegetation index (NDVI)

Plant stress can be quantified with NDVI (**Johnsen**, ***et al***., **2009**). Generally, values of NDVI were greater under irrigated than under rain fed conditions except Momtchill (NDVI value was similar to control), and also except Es84-24//ks82w409, Es84-24/seri//seri, Gerek gm, Harmankaya99, Ikizce96, Izgi01, Jagger, Karahan, Katia, Ks82w422, Pyn/bau, Seval, Sonmez01, Suzen97, and Vorona/kauz genotypes (NDVI values were higher than controls (**Table 4.25**).

Under rain fed conditions, the NDVI values ranged from 0.37 in Stk52/trumbull to 0.64 in Gun91. The lowest reduction of NDVI following water stress was found in Bayraktar, Mnch/5, Hawk, Gun91, and Ktk/ye2453 genotypes. However, Kirac66, Weston, Soyer, Aytin98, and Century genotypes recorded the highest reduction. Similar results were demonstrated by **Baghzouz**, ***et al***., (**2006**) who found that the NDVI was decreased with decreasing tissue water content in tall fescue and annual ryegrass.

Table 4.25: The effect of irrigation systems on NDVI values of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	NDVI						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	0.54 ± 0.03	0.60 ± 0.00	-10.3	Kirgiz95	0.55 ± 0.01	0.60 ± 0.04	-8.2
Aytin98	0.48 ± 0.02	0.59 ± 0.04	-18.5	Kirkpinar79	0.52 ± 0.02	0.56 ± 0.03	-7.0
Bayraktar	0.44 ± 0.00	0.45 ± 0.03	-2.5	Krc/bez	0.50 ± 0.02	0.53 ± 0.01	-6.6
Bezostaya1	0.52 ± 0.01	0.56 ± 0.02	-8.0	Ks82w422	0.46 ± 0.00	0.38 ± 0.02	20.7
Bolal2973	0.45 ± 0.02	0.51 ± 0.05	-12.2	Ktk/ye2453	0.54 ± 0.01	0.55 ± 0.01	-1.3
Ca8055/krc66	0.53 ± 0.01	0.57 ± 0.05	-5.9	Kutluk94	0.53 ± 0.01	0.59 ± 0.02	-9.4
Century	0.48 ± 0.04	0.58 ± 0.03	-16.9	Lov/bll/	0.57 ± 0.01	0.59 ± 0.04	-4.0
Dagdas	0.56 ± 0.03	0.62 ± 0.04	-9.6	Mnch/5/	0.43 ± 0.01	0.44 ± 0.05	-2.1
Ekg15//tast	0.50 ± 0.01	0.52 ± 0.07	-3.3	Momtchill	0.53 ± 0.05	0.53 ± 0.04	0.0
Es00-ke3	0.55 ± 0.01	0.58 ± 0.03	-5.4	Momtchill/gun	0.53 ± 0.01	0.59 ± 0.05	-10.2
Es84-24//ks82w409	0.51 ± 0.02	0.49 ± 0.03	4.8	Mufitbey	0.57 ± 0.02	0.63 ± 0.00	-8.3
Es84-24/seri//seri	0.55 ± 0.01	0.51 ± 0.04	7.6	Pastor	0.40 ± 0.01	0.46 ± 0.03	-11.6
F12.71/coc//kauz	0.49 ± 0.03	0.54 ± 0.01	-10.3	Pyn/bau	0.55 ± 0.04	0.54 ± 0.02	0.2
F12.71/coc//prl"s"	0.44 ± 0.03	0.50 ± 0.04	-11.2	Seval	0.41 ± 0.05	0.38 ± 0.01	7.5
Flamura85	0.41 ± 0.04	0.44 ± 0.03	-6.4	Sonmez01	0.51 ± 0.02	0.49 ± 0.00	4.8
Gerek gm	0.47 ± 0.06	0.44 ± 0.03	7.8	Soyer	0.51 ± 0.01	0.63 ± 0.04	-18.7
Gerek79	0.50 ± 0.02	0.55 ± 0.03	-9.5	Stk52/trumbull	0.37 ± 0.04	0.43 ± 0.03	-15.3
Gun91	0.64 ± 0.01	0.65 ± 0.05	-1.5	Suzen97	0.52 ± 0.01	0.51 ± 0.04	2.3
Harmankaya99	0.57 ± 0.01	0.57 ± 0.05	0.5	Tosunbey	0.47 ± 0.01	0.53 ± 0.02	-11.9
Hawk	0.45 ± 0.01	0.46 ± 0.05	-2.1	Vona//no57	0.45 ± 0.03	0.50 ± 0.04	-11.2
Ikizce96	0.53 ± 0.04	0.51 ± 0.06	3.3	Vorona/kauz	0.50 ± 0.03	0.46 ± 0.05	8.8
Izgi01	0.48 ± 0.02	0.43 ± 0.00	11.6	Weston	0.53 ± 0.04	0.66 ± 0.02	-18.8
Jagger	0.48 ± 0.01	0.46 ± 0.04	4.5	Zitnica	0.51 ± 0.01	0.61 ± 0.06	-15.9
Karahan	0.56 ± 0.03	0.55 ± 0.02	0.3	**Grand mean**	0.50	0.53	-4.19
Katia	0.47 ± 0.01	0.44 ± 0.02	6.1	**l.s.d Genotype**	0.03		
Kirac66	0.48 ± 0.03	0.61 ± 0.02	-22.1	**Treatment**	0.01		
				G x T	0.05		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions, *** NDVI was measured at different dates during **8 and 17 June**, and the average was taken.

4.3.1.6 Effect of irrigation systems on flag leaf chlorophyll content

The soil plant analysis development (SPAD) meter was used to measure chlorophyll content of flag leaves. Generally, drought had significant negative effects on chlorophyll content of flag leaves of all wheat genotypes except Bayraktar, Bolal2973, Ca8055/krc66, Dagdas, Es00-ke3, F12.71/coc//kauz, F12.71/coc//prl"s"/ Gerek gm,

Gerek79, Gun91, Harmankaya99, Jagger, Katia, Kutluk94, Sonmez01, Suzen97, and Zitnica genotypes, which showed high SPAD values under drought (**Table 4.26**). Under drought stress, the lowest reduction in SPAD values were observed in Es84-24//ks82w409, Izgi01, Lov/bll/, Kirgiz95, Pastor, Pyn/bau, Kirkpinar79, Mufitbey, Krc/bez, Ks82w422, Ktk/ye2453, Hawk, Ekg15//tast, and Weston genotypes. The obtained results are in agreement with those obtained by **Balouchi**, ***et al***., (**2009**).

Table 4.26: The effect of irrigation systems on SPAD values of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	SPAD						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	45.55 ± 3.1	50.65 ± 2.7	-10.1	Kirgiz95	41.40 ± 0.6	42.93 ± 0.2	-3.6
Aytin98	27.63 ± 2.3	35.98 ± 4.6	-23.2	Kirkpinar79	46.65 ± 0.8	48.08 ± 1.0	-3.0
Bayraktar	29.80 ± 2.2	29.43 ± 2.9	1.3	Krc/bez	44.35 ± 2.5	45.50 ± 1.0	-2.5
Bezostaya1	42.00 ± 1.8	47.03 ± 2.1	-10.7	Ks82w422	33.13 ± 0.6	33.88 ± 1.9	-2.2
Bolal2973	39.35 ± 2.6	39.35 ± 1.2	0.0	Ktk/ye2453	34.55 ± 0.5	34.93 ± 0.4	-1.1
Ca8055/krc66	48.75 ± 2.0	40.05 ± 3.1	21.7	Kutluk94	38.55 ± 1.5	37.55 ± 1.7	2.7
Century	36.83 ± 1.5	46.13 ± 3.1	-20.2	Lov/bll/	45.80 ± 2.3	48.45 ± 0.7	-5.5
Dagdas	47.05 ± 1.3	44.88 ± 1.5	4.8	Mnch/5/	38.30 ± 3.0	48.15 ± 4.0	-20.5
Ekg15//tast	42.35 ± 1.7	42.65 ± 1.2	-0.7	Momtchill	39.40 ± 4.2	44.63 ± 1.4	-11.7
Es00-ke3	43.65 ± 0.9	42.25 ± 4.1	3.3	Momtchill/gun	41.15 ± 3.4	44.93 ± 1.5	-8.4
Es84-24//ks82w409	50.15 ± 0.9	54.28 ± 2.4	-7.6	Mufitbey	47.50 ± 1.7	48.75 ± 0.7	-2.6
Es84-24/seri//seri	44.90 ± 3.5	51.23 ± 1.4	-12.3	Pastor	36.60 ± 0.6	37.88 ± 2.9	-3.4
F12.71/coc//kauz	46.18 ± 3.4	43.25 ± 2.3	6.8	Pyn/bau	48.43 ± 1.6	49.95 ± 0.3	-3.1
F12.71/coc//prl"s"	43.48 ± 1.7	40.78 ± 1.1	6.6	Seval	26.95 ± 2.9	41.03 ± 1.2	-34.3
Flamura85	29.83 ± 1.7	32.85 ± 2.4	-9.2	Sonmez01	45.35 ± 3.0	44.15 ± 5.0	2.7
Gerek gm	38.93 ± 0.5	24.03 ± 2.4	62.0	Soyer	40.63 ± 1.7	47.18 ± 3.4	-13.9
Gerek79	27.38 ± 4.0	25.43 ± 0.4	7.7	Stk52/trumbull	20.98 ± 2.8	42.88 ± 0.4	-51.1
Gun91	46.48 ± 2.2	43.38 ± 1.8	7.1	Suzen97	53.50 ± 1.6	50.58 ± 1.3	5.8
Harmankaya99	52.60 ± 1.3	50.15 ± 4.9	4.9	Tosunbey	39.63 ± 1.2	43.73 ± 4.0	-9.4
Hawk	28.78 ± 3.0	29.03 ± 0.6	-0.9	Vona//no57	37.70 ± 2.1	42.75 ± 0.4	-11.8
Ikizce96	29.23 ± 3.8	36.78 ± 1.6	-20.5	Vorona/kauz	37.35 ± 1.8	45.60 ± 3.7	-18.1
Izgi01	29.95 ± 1.5	32.03 ± 0.3	-6.5	Weston	46.05 ± 1.4	46.28 ± 0.4	-0.5
Jagger	30.95 ± 3.6	26.10 ± 0.6	18.6	Zitnica	37.95 ± 0.9	37.08 ± 3.6	2.4
Karahan	37.20 ± 2.1	42.00 ± 2.8	-11.4	**Grand mean**	39.51	41.59	-1.6
Katia	37.53 ± 1.2	34.53 ± 0.9	8.7	**l.s.d Genotype**	2.37		
Kirac66	37.68 ± 3.8	46.73 ± 1.1	-19.4	**Treatment**	0.48		
				G x T	3.35		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions, *** SPAD was measured at different dates during **9 and 16 June** and the average was taken.

Sairam and **Saxena**, (**2000**) reported that the total chlorophyll contents decreased with age, under stress and non-stress conditions. Water stress resulted in an accelerated chlorophyll breakdown starting in the wheat leaves (**Fig.4.20**). Meanwhile, the leaf senescence was delayed in Pyn/bau, Ca8055/krc66, Dagdas, Kirkpinar79, F12.71/coc//kauz, Es84-24//, Harmankaya99, and Suzen97 genotypes (**Table 4.27**). The ability to maintain the functionality of the photosynthetic machinery under drought stress is important in drought tolerance mechanisms. It may be possible to enhance drought tolerance by delaying senescence induced by drought (**Rivero**, ***et al***., **2007**).

Table 4.27: The effect of irrigation systems on SPAD (stay green) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	SPAD values under water stressed						
	9-Jun	16-Jun	Grand mean	Genotypes	9-Jun	16-Jun	Grand mean
Altay2000	48.1 ± 3.8	43.0 ± 2.5	45.6	Kirac66	46.2 ± 4.7	29.2 ± 2.9	37.7
Aytin98	43.3 ± 3.9	12.0 ± 0.7	27.6	Kirgiz95	46.4 ± 0.4	36.4 ± 0.8	41.4
Bayraktar	44.1 ± 0.9	15.6 ± 3.6	29.8	Kirkpinar79	46.7 ± 0.1	46.6 ± 1.6	46.7
Bezostaya1	47.2 ± 1.8	36.9 ± 1.8	42.0	Krc/bez	45.9 ± 2.2	42.8 ± 2.8	44.4
Bolal2973	47.9 ± 1.8	30.8 ± 3.5	39.4	Ks82w422	50.2 ± 0.5	16.1 ± 0.6	33.1
Ca8055/krc66	51.1 ± 1.6	46.5 ± 2.5	48.8	Ktk/ye2453	41.1 ± 0.5	28.0 ± 0.5	34.6
Century	46.7 ± 0.3	27.0 ± 2.8	36.8	Kutluk94	46.7 ± 0.3	30.4 ± 2.7	38.6
Dagdas	48.7 ± 0.7	45.4 ± 1.9	47.1	Lov/bll/	48.3 ± 1.3	43.4 ± 3.3	45.8
Ekg15//tast	47.8 ± 0.1	36.9 ± 3.4	42.4	Mnch/5/	50.4 ± 3.3	26.3 ± 2.7	38.3
Es00-ke3	47.5 ± 0.0	39.8 ± 1.8	43.7	Momtchill	49.0 ± 0.5	29.9 ± 7.9	39.4
Es84-24//ks82w409	53.4 ± 0.6	47.0 ± 1.2	50.2	Momtchill/gun	48.9 ± 0.4	33.4 ± 6.5	41.2
Es84-24/seri//seri	50.1 ± 0.5	39.8 ± 6.6	44.9	Mufitbey	51.5 ± 0.3	43.5 ± 3.1	47.5
F12.71/coc//kauz	45.4 ± 6.5	47.0 ± 0.2	46.2	Pastor	47.2 ± 1.0	26.0 ± 0.2	36.6
F12.71/coc//prl"s"	50.3 ± 0.7	36.7 ± 2.7	43.5	Pyn/bau	51.3 ± 2.6	45.6 ± 0.7	48.4
Flamura85	49.1 ± 1.3	10.6 ± 2.2	29.8	Seval	35.9 ± 0.0	18.0 ± 5.9	27.0
Gerek gm	44.2 ± 0.8	33.7 ± 0.3	38.9	Sonmez01	50.2 ± 0.8	40.5 ± 5.3	45.4
Gerek79	38.4 ± 3.1	16.4 ± 4.9	27.4	Soyer	47.6 ± 1.8	33.7 ± 1.7	40.6
Gun91	49.5 ± 0.6	43.5 ± 3.7	46.5	Stk52/trumbull	31.6 ± 4.5	10.4 ± 1.0	21.0
Harmankaya99	58.9 ± 0.1	46.3 ± 2.5	52.6	Suzen97	54.8 ± 0.3	52.3 ± 3.0	53.5
Hawk	42.7 ± 1.7	14.9 ± 4.4	28.8	Tosunbey	49.6 ± 0.3	29.7 ± 2.2	39.6
Ikizce96	39.1 ± 6.9	19.4 ± 0.8	29.2	Vona//no57	42.5 ± 1.4	32.9 ± 2.9	37.7
Izgi01	47.4 ± 2.0	12.5 ± 1.1	30.0	Vorona/kauz	45.8 ± 1.8	29.0 ± 1.8	37.4
Jagger	48.7 ± 1.8	13.3 ± 5.5	31.0	Weston	49.0 ± 0.6	43.2 ± 2.3	46.1
Karahan	49.4 ± 1.1	25.0 ± 3.2	37.2	Zitnica	45.4 ± 0.8	30.6 ± 1.0	38.0
Katia	49.7 ± 0.9	25.4 ± 1.4	37.5	**Grand mean**	47.2	31.9	39.5

*The data represent mean ± SD of three replicates. *** SPAD measured at different dates during 9 and 16 June and the average was taken.

4

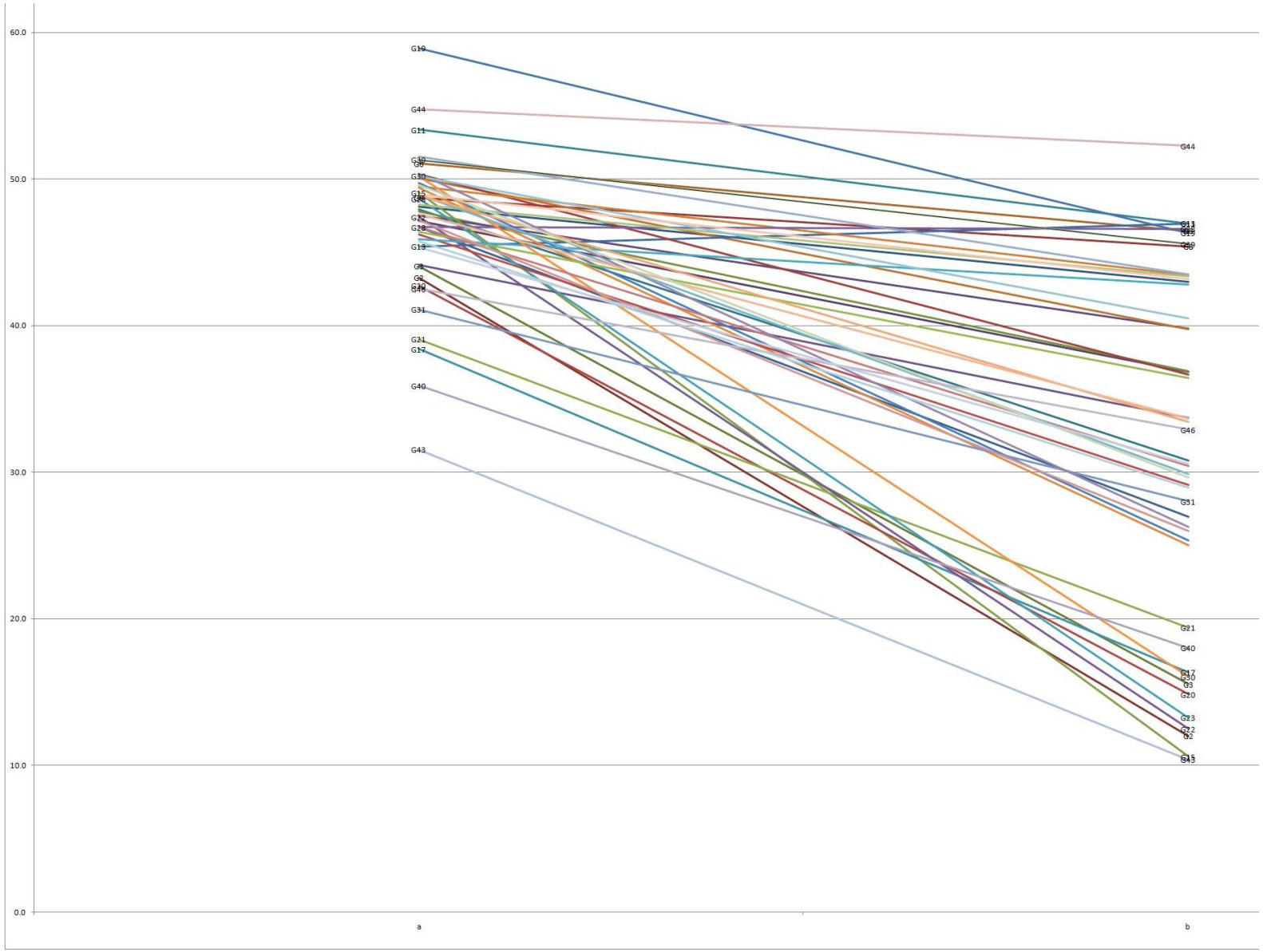


Fig. 4.20: Chlorophyll breakdown in the wheat leaves

4.3.1.7 Effect of irrigation systems on canopy temperature (CT)

Canopy temperature was measured using handheld infrared thermometer on sunny days. The results are presented in **Table 4.28**.

Table 4.28: The effect of irrigation systems on canopy temperature (°C) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	CT						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	28.1 ± 0.9	26.9 ± 1.3	4.5	Kirgiz95	28.6 ± 0.5	27.8 ± 0.8	2.6
Aytin98	28.5 ± 1.2	26.3 ± 1.3	8.4	Kirkpinar79	28.3 ± 0.9	27.2 ± 0.3	3.8
Bayraktar	27.9 ± 0.6	27.8 ± 1.0	0.6	Krc/bez	27.5 ± 0.8	27.1 ± 0.8	1.5
Bezostaya1	28.6 ± 0.8	26.8 ± 0.5	6.7	Ks82w422	30.0 ± 0.9	28.1 ± 0.6	6.9
Bolal2973	28.1 ± 1.1	27.8 ± 0.6	1.1	Ktk/ye2453	27.1 ± 0.6	27.1 ± 0.8	0.0
Ca8055/krc66	27.0 ± 0.3	28.4 ± 1.5	-5.0	Kutluk94	27.1 ± 0.5	26.2 ± 0.6	3.3
Century	28.3 ± 0.6	26.8 ± 1.2	5.6	Lov/bll/	27.1 ± 0.6	27.2 ± 0.6	-0.2
Dagdas	26.9 ± 0.3	26.7 ± 0.5	0.9	Mnch/5/	27.8 ± 0.4	27.4 ± 0.7	1.6
Ekg15//tast	27.2 ± 0.6	27.1 ± 0.7	0.5	Momtchill	28.0 ± 0.4	27.3 ± 0.6	2.8
Es00-ke3	27.1 ± 0.4	27.3 ± 0.7	-0.6	Momtchill/gun	27.6 ± 0.9	27.3 ± 0.7	1.1
Es84-24//ks82w409	28.2 ± 0.4	27.7 ± 1.3	1.7	Mufitbey	27.9 ± 0.6	26.9 ± 0.4	3.7
Es84-24/seri//seri	28.5 ± 0.5	27.2 ± 0.3	5.0	Pastor	28.2 ± 0.6	28.8 ± 0.5	-2.1
F12.71/coc//kauz	28.8 ± 0.6	27.8 ± 0.4	3.6	Pyn/bau	27.7 ± 1.2	27.1 ± 0.2	2.3
F12.71/coc//prl"s"	27.7 ± 0.6	27.3 ± 0.4	1.7	Seval	27.7 ± 0.9	27.7 ± 0.5	0.1
Flamura85	29.1 ± 0.7	29.0 ± 0.6	0.3	Sonmez01	27.6 ± 0.6	27.6 ± 0.4	0.1
Gerek gm	27.7 ± 0.3	28.6 ± 0.4	-3.3	Soyer	28.3 ± 1.0	27.0 ± 1.2	4.9
Gerek79	28.3 ± 0.7	27.0 ± 0.3	4.8	Stk52/trumbull	29.1 ± 0.7	27.7 ± 0.7	4.9
Gun91	26.8 ± 0.8	27.8 ± 0.5	-3.6	Suzen97	27.8 ± 0.2	27.7 ± 0.5	0.1
Harmankaya99	28.8 ± 0.3	28.7 ± 1.2	0.5	Tosunbey	28.9 ± 0.7	27.0 ± 0.6	7.0
Hawk	28.7 ± 0.6	29.3 ± 0.7	-1.8	Vona//no57	28.4 ± 0.3	27.1 ± 1.0	4.9
Ikizce96	27.4 ± 1.1	27.7 ± 0.3	-1.1	Vorona/kauz	27.7 ± 0.4	27.5 ± 0.6	0.7
Izgi01	27.8 ± 0.4	27.4 ± 0.9	1.3	Weston	27.4 ± 0.5	27.8 ± 0.8	-1.3
Jagger	29.2 ± 0.7	28.7 ± 0.6	1.5	Zitnica	28.0 ± 0.5	26.5 ± 0.3	5.4
Karahan	27.1 ± 0.9	26.8 ± 0.4	1.2	**Grand mean**	28.0	27.5	1.9
Katia	28.0 ± 0.8	27.9 ± 1.2	0.2	**l.s.d Genotype**	0.66		
Kirac66	28.9 ± 0.9	27.6 ± 0.6	4.6	**Treatment**	0.13		
				G x T	0.93		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions, *** Canopy temperature was measured at **2** and **8** June and the average was taken.

Generally, drought stressed plants displayed higher canopy temperature compared to well water plants, although some genotypes recorded the opposite trend, with the temperature under drought stress less than under well-watered conditions (Ca8055/krc66, Es00-ke3, Gerek gm, Gun91, Hawk, Ikizce96, Lov/bll, Pastor, and Weston). Meanwhile, Ktk/ye2453 genotype recorded equal canopy temperature under both conditions.

The genotypes, Seval, Sonmez01, Suzen97, Katia, Flamura85, Ekg15//tast, Harmankaya99, Bayraktar, Vorona/kauz, Dagdas, Bolal2973, Momtchill/gun, Karahan, and Izgi01 recorded a slight increase in canopy temperature under drought stress conditions. On the other hand, Ca8055/krc66, Dagdas, Ekg15//tast, Es00-ke3, Gun91, Ikizce96, Karahan, Ktk/ye2453, Kutluk94, Lov/bll//, and Weston were the coldest genotypes under rain fed conditions. The results were in accordance with those reported by **Siddique**, ***et al***., (**2000**) and **Olivares-Villegas**, ***et al***., (**2007**).

4.3.2 Effect of irrigation system on yield and its components

The wheat grain yield can be assessed in terms of three yield components, namely: 1) number of spikes per unit area, 2) number of kernels per spike and 3) kernel weight (**Moayedi**, ***et al***., **2010**).

4.3.2.1 Effect on number of spikes per m^2 (NSM)

Drought caused reduction in number of spikes m^{-2} (**Table 4.29**), of all wheat genotypes except Altay2000, Ekg15//tast, Flamura85, Ikizce96, Katia, Ks82w422, Soyer, Stk52/trumbull, Suzen97, and Weston genotypes which showed the opposite trend. The lowest reduction was found in Kirkpinar79, Tosunbey, Ca8055/krc66, Century, Es00-ke3, Lov/bll/, Bolal2973, Kirac66, Sonmez01, and Vorona/kauz genotypes. The results were in harmony with those achieved by **Sangtarash**, (**2010**) and **Moayedi**, ***et al***., **(2010**).

Table 4.29: The effect of irrigation systems on number of spikes m^{-2} of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Number of spikes m^{-2}						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	475.2 ± 24	430.1 ± 21	10.5	Kirgiz95	427.7 ± 92	626.4 ± 18	-31.7
Aytin98	603.2 ± 45	678.9 ± 99	-11.2	Kirkpinar79	429.1 ± 33	461.6 ± 10	-7.0
Bayraktar	609.0 ± 54	692.6 ± 35	-12.1	Krc/bez	424.6 ± 62	561.0 ± 14	-24.3
Bezostaya1	417.8 ± 11	454.1 ± 8	-8.0	Ks82w422	605.3 ± 48	502.9 ± 52	20.4
Bolal2973	646.3 ± 61	670.1 ± 24	-3.6	Ktk/ye2453	401.4 ± 75	502.2 ± 67	-20.1
Ca8055/krc66	451.1 ± 19	483.7 ± 78	-6.7	Kutluk94	474.5 ± 73	563.3 ± 48	-15.8
Century	703.2 ± 74	753.5 ± 7	-6.7	Lov/bll/	423.4 ± 17	443.6 ± 13	-4.6
Dagdas	394.3 ± 8	508.1 ± 26	-22.4	Mnch/5/	489.0 ± 58	526.6 ± 18	-7.1
Ekg15//tast	528.6 ± 40	470.2 ± 50	12.4	Momtchill	421.8 ± 99	496.2 ± 37	-15.0
Es00-ke3	527.3 ± 14	562.1 ± 55	-6.2	Momtchill/gun	451.9 ± 69	622.4 ± 82	-27.4
Es84-24//ks82w409	358.5 ± 43	492.1 ± 34	-27.1	Mufitbey	430.4 ± 36	565.1 ± 67	-23.8
Es84-24/seri//seri	452.8 ± 20	510.7 ± 23	-11.3	Pastor	427.2 ± 65	576.6 ± 61	-25.9
F12.71/coc//kauz	465.1 ± 41	640.0 ± 32	-27.3	Pyn/bau	444.6 ± 44	571.8 ± 53	-22.2
F12.71/coc//prl''s''	411.9 ± 48	484.4 ± 41	-15.0	Seval	385.3 ± 17	477.3 ± 39	-19.3
Flamura85	664.8 ± 71	464.4 ± 18	43.2	Sonmez01	484.2 ± 25	498.9 ± 73	-2.9
Gerek gm	524.8 ± 64	737.3 ± 8	-28.8	Soyer	509.9 ± 54	424.2 ± 14	20.2
Gerek79	651.5 ± 64	769.0 ± 59	-15.3	Stk52/trumbull	558.4 ± 47	542.5 ± 34	2.9
Gun91	500.0 ± 25	546.0 ± 61	-8.4	Suzen97	485.6 ± 75	453.7 ± 25	7.0
Harmankaya99	444.7 ± 40	539.0 ± 9	-17.5	Tosunbey	493.5 ± 44	529.3 ± 68	-6.8
Hawk	583.8 ± 5	656.4 ± 30	-11.1	Vona//no57	449.3 ± 37	550.9 ± 25	-18.4
Ikizce96	658.1 ± 52	603.5 ± 1	9.0	Vorona/kauz	715.6 ± 8	729.5 ± 12	-1.9
Izgi01	430.8 ± 57	472.6 ± 52	-8.8	Weston	450.3 ± 69	413.0 ± 57	9.0
Jagger	371.5 ± 8	601.1 ± 56	-38.2	Zitnica	401.7 ± 18	434.2 ± 6	-7.5
Karahan	511.9 ± 5	787.6 ± 49	-35.0	**Grand mean**	496.1	554.5	-10.5
Katia	545.9 ± 29	482.5 ± 54	13.1	**l.s.d Genotype**	53.3		
Kirac66	590.9 ± 62	609.9 ± 38	-3.1	**Treatment**	10.8		
				G x T	75.4		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

4.3.2.2 Effect on number of grains per spike (NGS)

All wheat genotypes showed a mean decrease of 1.6% for number of grains $spike^{-1}$ under rain fed conditions (**Table 4.30**). Generally, drought stress decreased the NGS in most genotypes compared to the well-watered conditions except Vorona/kauz, Bezostaya1, Flamura85, Kirgiz95, Kutluk94, Momtchill, Ca8055/krc66, Pastor, Es84-

24/seri//seri, Hawk, Mufitbey, Dagdas, Gerek gm, Bayraktar, Bolal2973, Vona//no57, Jagger, and Harmankaya99 genotypes, which showed an increase in the grains number per spike under rain fed conditions. And except also Ekg15//tast and Karahan genotypes which recorded the same number of grains spike^{-1} under both conditions.

Table 4.30: The effect of irrigation systems on number of grains spike^{-1} of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Number of grains spike^{-1}						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	32.6 ± 0.8	36.3 ± 1.9	-10.2	Kirgiz95	26.5 ± 2.6	25.5 ± 1.3	3.9
Aytin98	27.4 ± 2.3	28.2 ± 2.2	-2.8	Kirkpinar79	40.6 ± 1.2	42.9 ± 0.1	-5.4
Bayraktar	26.9 ± 0.9	22.1 ± 1.9	21.7	Krc/bez	22.4 ± 1.2	26.3 ± 0.7	-14.8
Bezostaya1	30.7 ± 1.8	29.8 ± 0.1	3.0	Ks82w422	26.5 ± 1.0	29.6 ± 2.3	-10.5
Bolal2973	29.3 ± 3.3	23.5 ± 0.6	24.7	Ktk/ye2453	28.2 ± 3.0	28.8 ± 3.2	-2.1
Ca8055/krc66	29.0 ± 2.7	26.7 ± 3.0	8.6	Kutluk94	27.2 ± 0.6	25.8 ± 1.2	5.4
Century	28.7 ± 1.2	33.5 ± 3.8	-14.3	Lov/bll/	28.4 ± 0.6	35.3 ± 2.1	-19.5
Dagdas	32.8 ± 2.9	29.2 ± 1.6	12.3	Mnch/5/	33.0 ± 2.1	37.6 ± 2.3	-12.2
Ekg15//tast	28.1 ± 0.7	28.1 ± 2.7	0.0	Momtchill	29.3 ± 1.1	27.0 ± 0.2	8.5
Es00-ke3	28.6 ± 0.4	30.4 ± 1.6	-5.9	Momtchill/gun	33.6 ± 2.1	34.5 ± 2.0	-2.6
Es84-24//ks82w409	37.2 ± 1.3	39.3 ± 1.5	-5.3	Mufitbey	29.5 ± 0.6	26.5 ± 3.2	11.3
Es84-24/seri//seri	38.0 ± 1.0	34.7 ± 1.8	9.5	Pastor	34.8 ± 1.9	31.9 ± 1.6	9.1
F12.71/coc//kauz	30.6 ± 0.9	31.5 ± 0.6	-2.9	Pyn/bau	32.6 ± 1.8	38.1 ± 0.9	-14.4
F12.71/coc//prl"s"	28.1 ± 1.6	28.4 ± 0.5	-1.1	Seval	31.3 ± 0.3	32.3 ± 2.7	-3.1
Flamura85	30.7 ± 0.4	29.8 ± 0.2	3.0	Sonmez01	29.7 ± 0.8	32.9 ± 0.6	-9.7
Gerek gm	27.8 ± 0.5	23.8 ± 1.0	16.8	Soyer	33.5 ± 2.1	39.3 ± 3.7	-14.8
Gerek79	22.4 ± 0.7	28.1 ± 2.8	-20.3	Stk52/trumbull	28.7 ± 3.3	35.3 ± 2.5	-18.7
Gun91	30.0 ± 1.1	30.8 ± 1.0	-2.6	Suzen97	29.0 ± 2.2	33.8 ± 0.2	-14.2
Harmankaya99	45.2 ± 2.0	32.9 ± 1.4	37.4	Tosunbey	32.1 ± 1.4	36.7 ± 1.4	-12.5
Hawk	28.4 ± 0.7	25.9 ± 2.4	9.7	Vona//no57	36.9 ± 2.1	29.6 ± 1.9	24.7
Ikizce96	25.6 ± 2.4	27.7 ± 1.6	-7.6	Vorona/kauz	32.1 ± 1.9	31.7 ± 1.4	1.3
Izgi01	27.1 ± 1.0	36.5 ± 2.9	-25.8	Weston	27.8 ± 1.1	28.1 ± 1.6	-1.1
Jagger	40.1 ± 3.8	31.2 ± 0.9	28.5	Zitnica	31.8 ± 2.9	33.4 ± 2.9	-4.8
Karahan	24.5 ± 1.3	24.5 ± 2.4	0.0	**Grand mean**	30.5	31.0	-1.6
Katia	36.8 ± 0.7	37.3 ± 0.8	-1.3	**l.s.d Genotype**	2.1		
Kirac66	23.8 ± 0.3	25.6 ± 0.4	-7.0	**Treatment**	0.4		
				G x T	3.0		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

The genotypes Izgi01, Gerek79, Lov/bll/, and Stk52/trumbull recorded the highest reduction in number of grains spike^{-1}, while Gun91, Momtchill/gun, Ktk/ye2453, Katia, F12.71/coc//prl"s", and Weston genotypes maintained the lowest reduction under stress conditions. The results were in harmony with those achieved by **Mirbahar**, ***et al***., (**2009**), and **Moayedi**, ***et al***., **(2010**).

4.3.2.3 Effect on thousand-grain weight (TGW)

Effect of irrigation systems on thousand-grain weight of forty-nine wheat genotypes are presented in **Table 4.31**. In general, drought resulted in reduction of TGW for all genotypes. A mean average decrease of 15.3% was recorded for TGW across all genotypes. Among all genotypes, the highest 1000 grains weight was recorded in Momtchill under both well-watered (46g) and water stressed (41.2g) and the lowest was observed in Stk52/trumbull under both irrigated (28g) and rain fed (19.5g). The minimum decreases of 1.3, 3.6, 3.7, 3.9, 4.6, 6.5, 6.6, 6.6, and 6.9% were recorded for Kirkpinar79, Aytin98, Izgi01, Dagdas, Bezostaya1, Es84-24//ks82w409, Sonmez01, Ktk/ye2453, and Momtchill/gun respectively, while the maximum decrease of 33.7, 30.4, 30.4, 29.6, and 29.2% was observed in Vorona/kauz, Stk52/trumbull, Hawk, Jagger, and Ks82w422 genotypes. The results were in harmony with those achieved by **Johari-Pireivatlou**, ***et al***., (**2010**) and **Moayedi**, ***et al***., **(2010**).

4.3.2.4 Effect on grain yield (t/ha)

Selection for yield under drought stress is effective and very important in breeding for drought-tolerance. Drought stress causes a great reduction in grain production of rained wheat in arid and semi-arid regions (**Bhutta**, ***et al***., **2006**). As expected, maximum yield was achieved under well-watered conditions in almost all wheat genotypes (**Table 4.32**), but Flamura85 and Pastor showed the opposite of that with percentage increases of 1.4 and 3.8%.

Table 4.31: The effect of irrigation systems on 1000-grain weight (g) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Thousand-grain weight (g)						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	35.5 ± 1.2	41.1 ± 0.8	-13.6	Kirgiz95	32.3 ± 1.0	35.9 ± 3.2	-10.0
Aytin98	29.4 ± 1.6	30.5 ± 3.8	-3.6	Kirkpinar79	30.0 ± 1.1	30.4 ± 3.6	-1.3
Bayraktar	30.4 ± 2.1	39.5 ± 3.0	-23.0	Krc/bez	34.8 ± 1.1	38.0 ± 2.5	-8.4
Bezostaya1	37.7 ± 0.2	39.5 ± 2.0	-4.6	Ks82w422	22.1 ± 2.9	31.2 ± 3.0	-29.2
Bolal2973	30.5 ± 1.2	34.8 ± 1.4	-12.4	Ktk/ye2453	33.9 ± 0.9	36.3 ± 1.0	-6.6
Ca8055/krc66	30.2 ± 2.3	37.3 ± 1.5	-19.0	Kutluk94	34.6 ± 2.5	40.5 ± 1.2	-14.6
Century	27.2 ± 1.1	29.6 ± 1.7	-8.1	Lov/bll/	33.0 ± 0.5	37.9 ± 1.3	-12.9
Dagdas	39.3 ± 1.8	40.9 ± 1.6	-3.9	Mnch/5/	27.2 ± 0.7	30.4 ± 0.4	-10.5
Ekg15//tast	30.9 ± 2.2	34.0 ± 0.4	-9.1	Momtchill	41.2 ± 0.7	46.0 ± 0.8	-10.4
Es00-ke3	38.4 ± 2.1	41.9 ± 1.8	-8.4	Momtchill/gun	36.2 ± 0.3	38.9 ± 0.5	-6.9
Es84-24//ks82w409	31.5 ± 1.8	33.7 ± 1.9	-6.5	Mufitbey	33.2 ± 0.8	44.9 ± 1.5	-26.1
Es84-24/seri//seri	29.2 ± 1.4	34.7 ± 1.6	-15.9	Pastor	29.7 ± 3.1	33.3 ± 2.1	-10.8
F12.71/coc//kauz	23.4 ± 0.9	29.6 ± 1.2	-20.9	Pyn/bau	30.9 ± 1.2	34.7 ± 3.6	-11.0
F12.71/coc//prl"s"	27.0 ± 1.6	35.3 ± 1.0	-23.5	Seval	28.7 ± 2.0	34.3 ± 0.2	-16.3
Flamura85	32.7 ± 2.0	42.9 ± 1.2	-23.8	Sonmez01	35.2 ± 0.8	37.7 ± 3.1	-6.6
Gerek gm	28.9 ± 3.2	34.7 ± 3.5	-16.7	Soyer	35.0 ± 0.6	42.8 ± 0.7	-18.2
Gerek79	25.4 ± 0.5	31.6 ± 4.2	-19.6	Stk52/trumbull	19.5 ± 2.0	28.0 ± 2.1	-30.4
Gun91	33.2 ± 2.2	36.8 ± 0.8	-9.8	Suzen97	35.2 ± 3.5	38.0 ± 1.4	-7.4
Harmankaya99	32.1 ± 0.2	41.9 ± 2.1	-23.4	Tosunbey	29.5 ± 1.6	37.3 ± 1.0	-20.9
Hawk	27.0 ± 1.6	38.8 ± 1.1	-30.4	Vona//no57	28.7 ± 1.1	39.6 ± 0.4	-27.5
Ikizce96	25.7 ± 2.3	34.4 ± 3.1	-25.3	Vorona/kauz	25.2 ± 1.2	38.0 ± 3.5	-33.7
Izgi01	33.9 ± 2.4	35.2 ± 2.1	-3.7	Weston	33.6 ± 3.7	37.6 ± 1.1	-10.6
Jagger	26.1 ± 0.8	37.1 ± 2.0	-29.6	Zitnica	39.4 ± 0.6	44.9 ± 1.6	-12.2
Karahan	25.4 ± 1.7	33.6 ± 1.1	-24.4	**Grand mean**	30.9	36.5	-15.3
Katia	27.9 ± 1.6	30.1 ± 3.7	-7.3	**l.s.d Genotype**	2.2		
Kirac66	28.0 ± 1.6	34.8 ± 2.6	-19.5	**Treatment**	0.5		
				G x T	3.2		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions

Yields (calculated as ton ha^{-1}) in well-watered plants varied from, 5.01 in Stk52/trumbull to 7.36 ton ha^{-1} in Pyn/bau and Tosunbey genotypes. In contrast, under drought stress conditions they varied from 3.11 in Stk52/trumbull to 6 ton ha^{-1} in Momtchill genotype. Under rain fed conditions, the poorest yields were recorded in Stk52/trumbull, F12.71/coc//kauz, Kirac66, Izgi01, and Seval genotypes, with yield values of 3.11, 3.49, 3.72, 3.74, and 4.03 t/ha, respectively.

On the other hand, Pastor, Zitnica, Harmankaya99, Krc/bez, Vorona/kauz, Pyn/bau, Tosunbey, Katia, Momtchill/gun, Flamura85, Es00-ke3, and Momtchill genotypes showed the highest yield with yield values of 5.25, 5.26, 5.27, 5.36, 5.38, 5.41, 5.43, 5.59, 5.86, 5.88, 5.95, and 6 t/ha, respectively.

Table 4.32: The effect of irrigation systems on grain yield (t/ha) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Grain yield (t/ha)						
	Water stressed	Well watered	Differ. %	Genotypes	Water stressed	Well watered	Differ. %
Altay2000	4.79 ± 0.7	5.74 ± 0.6	-16.6	Kirgiz95	4.49 ± 0.5	5.26 ± 0.5	-14.6
Aytin98	4.71 ± 0.4	6.67 ± 0.6	-29.4	Kirkpinar79	4.75 ± 0.4	6.57 ± 0.4	-27.7
Bayraktar	4.83 ± 0.4	6.23 ± 0.1	-22.5	Krc/bez	5.36 ± 0.1	5.6 ± 0.1	-4.3
Bezostaya1	4.14 ± 0.3	5.5 ± 0.1	-24.7	Ks82w422	4.36 ± 0.1	5.05 ± 0.5	-13.7
Bolal2973	5.15 ± 0.5	6.32 ± 0.3	-18.5	Ktk/ye2453	4.58 ± 0.3	5.44 ± 0.4	-15.8
Ca8055/krc66	4.73 ± 0.1	6.63 ± 0.4	-28.7	Kutluk94	4.71 ± 0.3	5.94 ± 0.2	-20.7
Century	4.89 ± 0.2	7.07 ± 0.4	-30.8	Lov/bll/	4.5 ± 0.4	5.44 ± 0.5	-17.3
Dagdas	4.7 ± 0.1	6.37 ± 0.2	-26.2	Mnch/5/	4.95 ± 0.3	5.99 ± 0.1	-17.4
Ekg15//tast	4.41 ± 0.2	5.52 ± 1	-20.1	Momtchill	6 ± 0.5	6.52 ± 0.1	-8
Es00-ke3	5.95 ± 0.2	6.79 ± 0.8	-12.4	Momtchill/gun	5.86 ± 0.4	6.87 ± 0.5	-14.7
Es84-24//ks82w409	4.33 ± 0.3	5.94 ± 0.1	-27.1	Mufitbey	4.39 ± 0.5	6.72 ± 0.6	-34.7
Es84-24/seri//seri	4.68 ± 0	6.51 ± 0.1	-28.1	Pastor	5.25 ± 0.4	5.06 ± 0.4	3.8
F12.71/coc//kauz	3.49 ± 0.4	6.04 ± 0.3	-42.2	Pyn/bau	5.41 ± 0.2	7.36 ± 0.5	-26.5
F12.71/coc//prl"s"	4.1 ± 0.3	5.8 ± 0.3	-29.3	Seval	4.03 ± 0.9	5.47 ± 0.2	-26.3
Flamura85	5.88 ± 0.3	5.8 ± 0.5	1.4	Sonmez01	5.19 ± 0.6	6.66 ± 0.2	-22.1
Gerek gm	4.16 ± 0.4	6.37 ± 0.2	-34.7	Soyer	4.7 ± 0.5	7.04 ± 0.3	-33.2
Gerek79	4.12 ± 0.3	6.05 ± 0.5	-31.9	Stk52/trumbull	3.11 ± 0.5	5.01 ± 0.5	-37.9
Gun91	5.06 ± 0.4	5.75 ± 0.4	-12	Suzen97	4.75 ± 0.4	5.95 ± 0.1	-20.2
Harmankaya99	5.27 ± 0.2	7.32 ± 0.2	-28	Tosunbey	5.43 ± 0.2	7.36 ± 0.1	-26.2
Hawk	4.29 ± 0.3	6.66 ± 0.4	-35.6	Vona//no57	4.34 ± 0.2	6.39 ± 0.2	-32.1
Ikizce96	4.79 ± 0	5.49 ± 0.3	-12.8	Vorona/kauz	5.38 ± 0.2	6.71 ± 0.3	-19.8
Izgi01	3.74 ± 0.2	6.09 ± 0.2	-38.6	Weston	4.25 ± 0.4	5.16 ± 0.8	-17.6
Jagger	4.76 ± 0.8	6.31 ± 0.2	-24.6	Zitnica	5.26 ± 0.1	6.98 ± 0.6	-24.6
Karahan	4.87 ± 0.7	6.52 ± 0.3	-25.3	**Grand mean**	4.74	6.17	-23.2
Katia	5.59 ± 0.2	6.71 ± 0.2	-16.7	**l.s.d Genotype**	0.45		
Kirac66	3.72 ± 0.1	5.38 ± 0.3	-30.9	**Treatment**	0.09		
				G x T	0.63		

*The data represent mean ± SD of three replicates, **Differ = values represent percent decrease (-) or increase (+) as compared to normal irrigated conditions.

The maximum reduction in yield was found in F12.71/coc//kauz, Izgi01, Stk52/trumbull, and Hawk genotypes. However, the genotypes Katia, Altay2000, Ktk/ye2453, Momtchill/gun, Kirgiz95, Ks82w422, Ikizce96, Es00-ke3, Gun91, Momtchill, and Krc/bez recorded the minimum reduction in grain yield. The results were in parallel with those achieved by **Sangtarash**, (**2010**) and **Moayedi**, ***et al***., **(2010**).

4.3.2.5 Drought susceptibility index (*DSI*)

The results indicated that DSI ranged from –0.16 in Pastor to 1.82 in F12.71/coc//kauz. The wheat genotypes Pastor, Flamura85, Krc/bez, Momtchill, Gun91, Es00-ke3, Ikizce96, Ks82w422, Kirgiz95, Momtchill/gun, Ktk/ye2453, Altay2000, Katia, Lov/bll/, Mnch/5/, Weston, Bolal2973, Ekg15//tast, Vorona/kauz, Suzen97, Kutluk94, Sonmez01, and Bayraktar expressed the lowest DSI, while the genotypes Soyer, Gerek gm, Mufitbey, Hawk, Stk52/trumbull, Izgi01 and F12.71/coc//kauz had highest DSI values (**Table 4.33**). The obtained results are in agreement with those obtained by **Bayoumi**, ***et al***., (**2008**).

4.3.2.6 Relative grain yield (RY)

The mean relative yield in case of water stress was less than that of well watered. The mean relative grain yields values under water stress and well-watered conditions were 0.79 and 0.84, respectively **(Table 4.33**). Altay2000, Bayraktar, Ikizce96, Century, Karahan, Mnch/5, Gun91, Bolal2973, Pastor, Sonmez01, Harmankaya99, Zitnica, Krc/bez, Pyn/bau, Tosunbey, Vorona/kauz, Katia, Flamura85, Momtchill/gun, Es00-ke3, and Momtchill genotypes, were relatively high yielding under water stress (**RY > mean RY**), while other genotypes were relatively low yielding (**RY < mean RY**). The results were in agreement with those obtained **Ahmad, *et al***., (**2003**).

Table 4.33: Drought susceptibility index (DSI) and relative grain yield (RY) of forty-nine wheat (*Triticum aestivum*) genotypes.

Genotypes	Yd	Yw	DSI	RY_S	RY_w	Genotypes	Yd	Yw	DSI	RY_S	RY_w
Altay2000	479.3	573.6	0.71	0.80	0.78	Kirac66	372.4	537.8	1.33	0.62	0.73
Aytin98	470.7	666.5	1.27	0.78	0.91	Kirgiz95	448.6	526.3	0.64	0.75	0.71
Bayraktar	482.8	622.9	0.97	0.80	0.85	Kirkpinar79	474.9	657.3	1.20	0.79	0.89
Bezostaya1	414.4	550.2	1.07	0.69	0.75	Krc/bez	535.7	560.1	0.19	0.89	0.76
Bolal2973	515.2	632.3	0.80	0.86	0.86	Ks82w422	435.6	505.3	0.60	0.73	0.69
Ca8055/krc66	473.1	662.9	1.24	0.79	0.90	Ktk/ye2453	458.1	543.6	0.68	0.76	0.74
Century	488.9	707.3	1.33	0.81	0.96	Kutluk94	471.3	594.3	0.89	0.79	0.81
Dagdas	469.7	636.9	1.13	0.78	0.87	Lov/bll/	449.6	544.0	0.75	0.75	0.74
Ekg15//tast	441.2	551.6	0.86	0.74	0.75	Mnch/5/	494.9	599.3	0.75	0.82	0.81
Es00-ke3	594.7	678.8	0.53	0.99	0.92	Momtchill	599.9	652.4	0.35	1.00	0.89
Es84-24//ks82w409	433.2	594.0	1.17	0.72	0.81	Momtchill/gun	585.6	687.3	0.64	0.98	0.93
Es84-24/seri//seri	467.6	651.2	1.22	0.78	0.88	Mufitbey	438.9	672.4	1.50	0.73	0.91
F12.71/coc//kauz	349.4	604.3	1.82	0.58	0.82	Pastor	524.5	505.5	-0.16	0.87	0.69
F12.71/coc//prl"s"	409.9	580.2	1.27	0.68	0.79	Pyn/bau	540.8	736.1	1.15	0.90	1.00
Flamura85	588.2	579.7	-0.06	0.98	0.79	Seval	402.6	547.2	1.14	0.67	0.74
Gerek gm	416.1	637.2	1.50	0.69	0.87	Sonmez01	519.3	665.8	0.95	0.87	0.90
Gerek79	411.6	604.8	1.38	0.69	0.82	Soyer	470.1	703.8	1.43	0.78	0.96
Gun91	505.7	574.7	0.52	0.84	0.78	Stk52/trumbull	311.2	500.9	1.64	0.52	0.68
Harmankaya99	527.0	732.3	1.21	0.88	0.99	Suzen97	474.8	595.4	0.87	0.79	0.81
Hawk	428.8	665.9	1.54	0.71	0.90	Tosunbey	542.8	735.8	1.13	0.90	1.00
Ikizce96	479.2	548.9	0.55	0.80	0.75	Vona//no57	433.9	639.4	1.39	0.72	0.87
Izgi01	373.6	609.2	1.67	0.62	0.83	Vorona/kauz	537.7	670.9	0.86	0.90	0.91
Jagger	476.1	631.1	1.06	0.79	0.86	Weston	424.6	515.6	0.76	0.71	0.70
Karahan	486.8	651.7	1.09	0.81	0.89	Zitnica	526.3	698.1	1.06	0.88	0.95
Katia	559.2	671.4	0.72	0.93	0.91	**Grand mean**	473.8	616.6	0.99	0.79	0.84

* RY_W = Relative grain yield under well water; RY_S = Relative grain yield under water stress condition.
** DSI= Drought susceptibility index.

4.3.3 Correlation coefficient analysis under drought stress conditions

The correlation coefficient analysis indicated that, there was significant positive correlations between grain yield and (biomass, 1000-grain weight) (r = 0.70, $P \leq 0.0001$ for biomass; r = 0.46, $P \leq 0.001$ for TGW). In addition, grain yield was significant positively correlated, but to a lesser extent, with harvest index (**Table 4.34**Table 4.34).

In our studies, we found weak positive correlations between plant height and (NDVI, biomass, 1000-grain weight). On the other hand, it had significant negative correlations with (harvest index, canopy temperature, number of grains spike^{-1}). The heading date was negatively associated with (harvest index, canopy temperature, number of grains spike^{-1}, number of spikes m^{-2}, and grain yield). These results were in parallel with those achieved by **Kilic** and **Yagbasanlar**, (**2010**) who found a negative correlation between grain yield and number of days to heading.

Table 4.34: Correlation coefficients between all traits of examined forty-nine wheat (*Triticum aestivum*) genotypes under drought stress.

	HD	BM	HI	NDVI	SPAD	CT	NGS	NSM	TGW	GY
Ph	0.19	0.31	-0.40	0.29	0.16	-0.61	-0.40	-0.08	0.29	0.09
	(0.184)	(0.029)	(0.004)	(0.043)	(0.264)	(0.000)	(0.004)	(0.566)	(0.042)	(0.545)
HD		0.01	-0.42	0.59	0.53	-0.31	-0.08	-0.20	0.15	-0.18
		(0.947)	(0.003)	(0.000)	(0.000)	(0.029)	(0.607)	(0.160)	(0.290)	(0.222)
BM			-0.25	0.26	0.17	-0.32	-0.19	0.31	0.29	0.70
			(0.088)	(0.072)	(0.250)	(0.025)	(0.185)	(0.031)	(0.043)	(0.000)
HI				-0.16	0.03	0.19	0.54	-0.14	0.12	0.32
				(0.258)	(0.859)	(0.187)	(0.000)	(0.334)	(0.412)	(0.024)
NDVI					0.64	-0.48	0.04	-0.28	0.49	0.23
					(0.000)	(0.000)	(0.763)	(0.049)	(0.000)	(0.105)
SPAD						-0.33	0.32	-0.46	0.46	0.22
						(0.021)	(0.026)	(0.001)	(0.001)	(0.121)
CT							0.24	0.21	-0.43	-0.21
							(0.093)	(0.145)	(0.002)	(0.144)
NGS								-0.37	0.06	0.24
								(0.009)	(0.689)	(0.090)
NSM									-0.45	0.09
									(0.001)	(0.559)
TGW										0.46
										(0.001)

* Numbers in parentheses indicate probability levels. Ph= plant height, HD= heading date, BM= biomass, HI= harvest index, NDVI= normalized difference vegetation index, CT= canopy temperature, NGS= number of grains spike^{-1}, NSM= number of spikes m^{-2}, TGW= thousand-grain weight, GY=grain yield.

Biomass showed significant positive correlations with number of spikes m^{-2}, and 1000-grain weight. On the other hand, there were no significant correlations between biomass and (heading date, harvest index, NDVI, SPAD, and number of grains spike^{-1}). These obtained results are in agreement with those obtained by **Kruse**, ***et al***., (**2005**).

Canopy temperature was positively associated with (harvest index, number of grains spike^{-1}, and number of spikes m^{-2}), while other characters (1000-grain weight, plant height, heading date, biomass, NDVI, and SPAD showed significant negative correlations with canopy temperature. Similar results were demonstrated by **Fenstemaker-Shaulis**, ***et al***., (**1997**) who reported that there was a negative correlation between NDVI and canopy temperature.

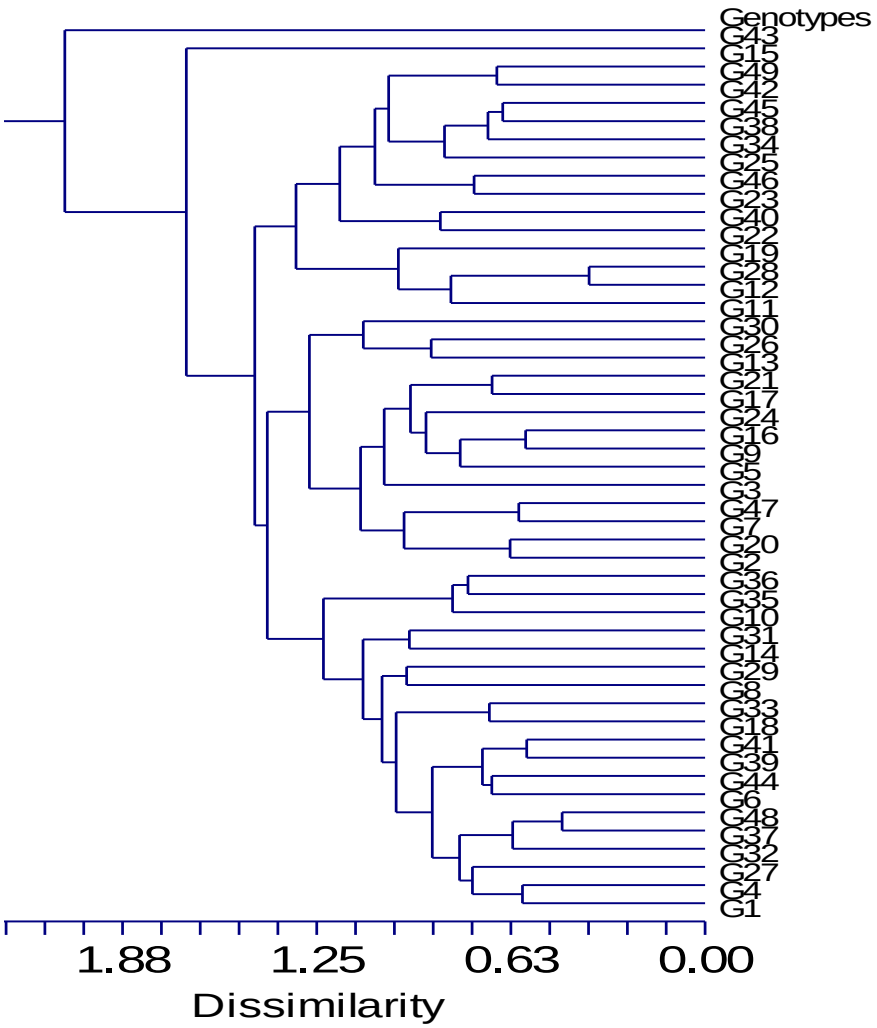
In our studies we found significant positive correlations between NDVI and (heading date, SPAD, and 1000-grain weight) (r = 0.59, $P \leq 0.000$ for heading date; r = 0.64, $P \leq 0.000$ for SPAD; r = 0.49, $P \leq 0.000$ for TGW). In addition, NDVI was significant negatively associated with canopy temperature and number of spikes m^{-2}.

SPAD observed positive significant correlations with (heading date, NDVI, number of grains spike^{-1}, and 1000-grain weight), whereas canopy temperature and number of spikes m^{-2} were negatively associated with SPAD. Among the existing yield components, the number of grains spike^{-1} showed the strongest significant positive correlation with harvest index. There was significant negative correlation between number of grains spike^{-1} and number of spike m^{-2}. In addition, number of spike m^{-2} was negatively associated with 1000-grain weight.

4.3.4 Dendrogram cluster analysis under drought stress conditions

The hierarchical cluster analysis grouped based on all traits the wheat genotypes into 30 groups at the 60% level. Group 1: Bezostaya1 and Altay2000; group 2: Kirgiz95; group 3: Kutluk94; group 4: Mufitbey and Weston; group 5: Ca8055/krc66 and Suzen97; group 6: Pyn/bau and Sonmez01; group 7: Gun91 and Lov/bll; group 8: Dagdas and Krc/bez; group 9: F12.71/coc//prl"s" and Ktk/ye2453; group 10: Es00-ke3; group 11: Momtchill and Momtchill/gun; group 12: Aytin98 and Hawk; group 13: Century and Vorona/kauz;group 14: Bayraktar; group 15: Bolal2973; group 16: Ekg15//tast and Gerek gm; group 17: Karahan; group 18: Gerek79 and Ikizce96; group 19: F12.71/coc//kauz and Kirac66; group 20: Ks82w422 and Es84-24//ks82w409; group 21: Kirkpinar79 and Es84-24/seri; group 22: Harmankaya99; group 23: Izgi01 and Seval; group 24: Jagger and Vona//no57; group 25: Katia; group 26: Mnch/5; group 27:

Pastor and Tosunbey; group 28: Soyer and Zitnica; group 29: Flamura85; group 30: Stk52/trumbull (**Fig.4.21**).



Cophenetic Correlation	0.658
Delta(0.5)	0.163
Delta(1.0)	0.205

Fig. 0.1: The hierarchical cluster analysis grouped the wheat genotypes into 31 groups of 49 Turkish genotypes.

5 DISCUSSION

Drought stress is the most damaging of a biotic stresses, which has a severe effects on plant functions, and leads to sharp decrease in productivity (**Tian** and **Lei**, **2007**). Wheat responds to drought stress in the form of changes to various morphological, physiological and biochemical processes. These changes could be attributed to the deleterious effects of drought stress on important metabolic processes. In this study, the growth of some wheat genotypes under drought stress was assessed by studying the effects of drought on morphological, physiological and molecular characteristics of some bread wheat genotypes at different levels:- laboratory, greenhouse, and open field levels.

5.1 Laboratory experiment

The suppressive effects of PEG6000 on ten wheat seeds development were recorded. The polyethylene glycol substance was used by other researchers to create osmotic shock in wheat plants (**Landjeva**, ***et al***., **2008**). The wheat genotypes differed in their response to drought stress induced by PEG 6000. PEG decreased shoot and root length, shoot and root weight (fresh and dry). Among all Egyptian genotypes, Sahal-1 showed the best performance; in contrast, Giza-163 recorded the worst performance under stress conditions. Among the Turkish genotypes, Ozcan recorded the greatest reduction in all traits, while BVD-22 recorded the lowest reduction. The reduction in shoot and the root length could be due to inhibition of cell division and elongation as a response to drought (**Bayoumi**, ***et al***., **2008**).

5.2 Greenhouse experiment

Sahal-1, Giza-163, Ozcan and BVD-22 have been selected from the laboratory experiment and were grown in greenhouse under controlled environmental conditions.

5.2.1 Effect of drought stress on plant height

Plant height plays an important role in photosynthesis (**Mirbahar**, ***et al***., **2009**). In the present study drought dramatically decreased, plant height of all wheat genotypes at all growth stages. Sahal-1 and Giza-163 were the tallest plants under drought stress; in contrast, Ozcan was the shortest one. The decrease in plant height probably related to inhibition of cell division, cell elongation, and expansion, which is a result of interruption of water flow from the xylem to the surrounding elongating cells (**Nonami**, **1998**). These results were in agreement with those obtained by **Nouri-Ganbalani**, ***et al***., (**2009**).

5.2.2 Effect of drought stress on relative water content

Maintenance of favorable plant water levels is vital for the development of drought resistance (**Passioura**, **2002**). Obviously, water-stressed genotypes had lower relative water content than non-stressed ones. Similar results were demonstrated by **Tambussi**, ***et al***., (**2000**) who reported that the water stressed wheat plants showed a significant decrease in RWC, with percentage decreases of 85 and 55% after 6 and 8 days of withholding water, respectively. The genotypes Sahal-1 and BVD-22, maintained better leaf water levels in terms of RWC compared to the other genotypes. The variation in RWC may be due to differences in the ability to absorb water from the soil or the ability to control water loss through the stomata. It may also be due to differences in the ability to accumulate and adjust osmotically to maintain tissue turgor and hence physiological activities (**Bayoumi**, ***et al***., **2008**). **Schonfeld**, ***et al***., (**1988**) showed that the wheat cultivars that had high RWC were more resistant to drought. The results are in agreement with the findings of **Tatar** and **Gevrek**, (**2008**).

5.2.3 Effect of drought stress on number of leaves per plant

Among all wheat genotypes, Sahal-1 had the lowest number of leaves per plant under drought stress conditions, with seven leaves. This could suggest that Sahal-1 tried

to decrease transpiration by decreasing the surface area as a drought avoidance mechanism. Similar results were obtained by **Fuzhong**, ***et al***., (**2008**).

5.2.4 Effect of drought stress on shoot fresh and dry mass

When wheat genotypes were subjected to drought, the shoot fresh and dry mass were significantly reduced. The reduction in SFM and DFM was more pronounced in Ozcan than in other genotypes, this lowest biomass of Ozcan shows its susceptibility to drought stress. The reduction in biomass might be due to negative effects of drought on important metabolic processes and photosynthesis, which decreased dry matter accumulation (**Nagarajan**, ***et al***., **1999**). Drought stress negatively affects net photosynthesis by decreasing photosynthetic rate. **Mirakhori**, ***et al***., (**2009**) reported that 90% of plant dry weight is resulted from CO_2 assimilation during photosynthesis. The drought-tolerant genotype (BVD-22) showed lesser reduction of both traits than those of drought-sensitive genotype (Ozcan). This could suggest that BVD-22 may have better adaptive mechanisms such as the control of stomata and stability of organelles within plant cell (**Setter** and **Flannigan**, **2001**). The results were in agreement with those found by **Tatar** and **Gevrek**, (**2008**).

5.2.5 Effect of drought stress on soil water content

In this study, Sahal1 recorded the lowest SWC compared with other genotypes under stress condition. This could suggest that Sahal1 has a deep root system as a drought avoidance mechanism, maintaining water uptake to sustain high tissue water potential. It is possible to improve the plant stress tolerance, through genes transformation, by transfer genes that function in water uptake and transport such as aquaporins and ion transporters (**Blumwald**, **2000**; **Wang**, ***et al***., **2003**).

5.2.6 Effect of drought stress on nutrient accumulation

Plant resistance to drought depends on plants-nutrient status (**Marschner**, **1995**). The present study clearly indicates that drought significantly reduced nutrient uptake and thus nutrient accumulation in all wheat genotypes. This reduction could be due to reduction in transpiration rate and stomatal closure, which impairs active transport from roots to shoots and reduces membrane permeability (**Alam**, **1999**). Also, it may be due to decreased diffusion rate of nutrients in the soil to be absorbed by root surface (**Alam**, **1999**) and decreased water availability which results in reduced total nutrient uptake (**Baligar**, ***et al***., **2001**).

Under stress treatment the genotypes Sahal-1 and BVD-22 at vegetative growth stages (i.e. 40 and 60 DAS) accumulated more P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn in shoots than other genotypes. This could suggest that both genotypes have good mechanisms to absorb nutrients from soil, also could be due to activation of genes that function in water uptake and ion transporters (**Blumwald**, **2000**). **Gunes**, ***et al***., (**2006**) concluded that the drought tolerant genotypes translocate nutrients from roots into shoots more than the susceptible genotypes. While at 80 DAS (i.e. seed development stage), the nutrient concentrations in shoots of both genotypes (Sahal-1 and BVD-22) were lower than other genotypes, it may be due to translocation from senescing leaves to the seeds being sped up at that stage (**Sangtarash**, **2010**). The results were in accordance with those reported by **Brown**, ***et al***., (**2006**).

5.2.7 Effect of drought stress on proline content

Among all amino acids, the accumulation of proline after drought was recognized as a beneficial drought tolerance indicator, it plays a significant role in minimizing the damages in plants subjected to drought (**Mohammadkhani** and **Heidari**, **2008**). In this study, the increases in the concentration of proline in all genotypes were found to be remarkable during drought stress; this increase was in accordance with the findings of **Johari-Pireivatlou**, ***et al***., (**2010**). Proline accumulation was 17 times higher in plants subjected to drought than in control plants. This accumulation might be attributed to two pathways: first, increased expression of proline synthetic enzymes and thus increase in

proline biosynthesis, and secondly, inhibition of proline oxidation and proline degradation (**Delauney** and **Verma**, **1990**; **Peng**, ***et al.***, **1996**) thus increases Pro accumulation. **Vendruscolo**, ***et al***., (**2007**) stated that proline might confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than as an osmotic adjustment.

The genotype BVD-22 showed maximum accumulation of proline content under drought stress among the four genotypes studied which is in line with observations of **Chandrasekar**, ***et al***., (**2000**) in wheat who observed that the highest proline content was in drought resistant genotype and the lowest was in sensitive genotype. The high Pro content of BVD-22 may be a result from calcium accumulation. BVD-22 recorded the highest Ca^{2+} concentration at 60 DAS stage. **Sadiqov**, ***et al***., (**2002**) reported that Ca participates in the signalling mechanisms of drought-induced proline accumulation. In contrast, the low Pro content of Ozcan and Giza-163 genotypes under drought stress conditions shows its susceptibility to drought. The results were in harmony with those achieved by **Yao**, ***et al***., (**2009**).

5.2.8 Effect of drought stress on soluble carbohydrate content

Carbohydrate synthesis under drought stress can be considered as a promising sign for drought tolerance. The accumulation of carbohydrate in response to drought is quite well documented (**Mohammadkhani** and **Heidari**, **2008**). It has a key role in drought tolerance (**Johari-Pireivatlou**, ***et al***., **2010**). The present work indicates that, the concentration of soluble carbohydrate increased as a response to drought in the four wheat genotypes. On the other hand, Sahal-1 maintained the highest SC content, under both drought and control conditions. This may be a result from Zinc accumulation. Sahal-1 at 40 DAS, recorded the highest Zn concentration. Zn is involved in carbohydrate metabolism through its effects on photosynthesis and sugar transformations (**Coruh**, **2007**). The sharp increase in SC under drought conditions may be a result from starch degradation and conversion of it into soluble sugars (**Fischer** and **Holl**, **1991**). Starch depletion was noted by (**Patakas** and **Noitsakis**, **2001**) in response to drought. It may be also due to low sugar utilization under stress conditions.

5.2.9 Effect of drought stress on lipid peroxidation level

Lipid peroxidation level was measured as the concentration of malondialdehyde (MDA). In the present study, a significant increase was observed in MDA level of all genotypes under drought stress. The lowest MDA contents at all growth stages, was observed in BVD-22 genotype, while Giza-163 and Ozcan recorded the highest contents under stress conditions. Drought increases reactive oxygen species accumulation, which cause oxidative damage to chloroplast membranes (**Cai**, ***et al***., **2007**) and lead to increases in MDA level. As well as the generation of unsaturated fatty acids affects membrane structures their properties, and leads to cellular damage to plant membranes (**Quan**, ***et al***., **2004**).

The increasing of MDA content in Giza-163 and Ozcan genotypes indicates that detoxification by their antioxidant systems was insufficient to prevent this damage. However, the reduction in MDA production in BVD-22 suggests more protection from oxidative damage to the cell membrane integrity of this genotype and a more efficient anti-oxidative system. Furthermore, the lowest MDA contents in BVD-22 may be a result from phosphorus accumulation. P is the key component of phospholipids and phosphor-proteins (**Hu** and **Schmidhalter**, **2005**). BVD-22, showed the highest values of P in the shoots under stressed conditions. Several reports have suggested that P has positive effects on plant growth under stress conditions, these positive effects could be due to it is role in increasing cell-membrane stability (**Sawwan**, ***et al***., **2000**).

5.2.10 Effect of drought stress on antioxidant enzymes activities

Drought stress increases accumulation of ROS within plant cells, which may react with proteins, lipids and nucleic acids, causing oxidative damages to plant cells (**Yao** and **Liu**, **2007**). The ability to reduce such damages may correlate with drought tolerance (**Tsugane**, ***et al***., **1999**). The tolerant cells activate their enzymatic antioxidant system, in order to protect from such damages. According to the result of the present study, antioxidant enzymes activities except catalase were positively affected by drought stress.

An enhancement of AP and SOD was observed in Sahal-1 genotype at 60 and 80 DAS stages. While, at 40 DAS stage the activities of both enzymes (AP and SOD) were the highest in BVD-22. Conversely, in BVD-22 an enhancement of GR was noticed at 40 DAS stage. While in Sahal-1, the maximum increase of GR activities was observed at 60 DAS stage. This could suggest that the plant response to drought may vary and depending on growth stage and genotype in terms of the types of enzyme that used in the antioxidant defense mechanism. **Jung**, (**2004**) concluded that the developmental stages of leaves might contribute to the differential prevention of oxidative damage in plants exposed to drought.

The enhancement of SOD enzyme in Sahal-1 at 60 and 80 DAS stages may be a result from cupper accumulation. Sahal-1 recorded the highest Cu accumulation under well-watered and stressed conditions. Cu ions act as cofactors in many enzymes such as Cu/Zn superoxide dismutase (**Yruela**, **2005**). On the other hand, BVD-22 recorded the highest SOD activity at 40 DAS stage. This may be a result from manganese accumulation. BVD-22 at 40 DAS, recorded the highest Mn accumulation. Mn plays an essential role in activation of several enzymes, such as isoenzymes of superoxide dismutase (**Campanella**, ***et al***., **2005**). Mn also involved in scavenging of superoxide and hydrogen peroxide (**Ducic** and **Polle**, **2005**).

The increase in the activity of APO, GR and SOD, as a response to drought may be due to increases in gene expression (**Costa**, ***et al***., **2010**). In contrast, CAT activity was particularly decreased in all genotypes and this is consistent with previous work by **Tayebeh** and **Hassan**, (**2010**). This decrease in CAT activity could be attributed to inhibition of enzyme synthesis or change in the assembly of enzyme subunits under drought stress. It may also due to the photo-inactivation of this enzyme (**Jung**, **2004**). These results indicate that CAT is highly sensitive enzyme to drought stress.

5.2.11 mRNA differential display

From the greenhouse experiment data, Sahal-1 and BVD-22 genotypes showed better performance under drought stress conditions compared with other genotypes. Therefore, the mRNA DD technique was used to isolate and identify the genes whose

expression was changed in response to drought stress in both genotypes. By using mRNA DD technique, we identified ten differentially expressed drought responsive transcripts, four in Sahal-1 and six in BVD-22. Except for a few sequences, the transcripts displayed similarity to previously identified proteins from NCBI protein database. The BLASTN results indicated that, the fragments identified in this study were important transcripts due to their similarity to different nucleotide sequences that were related to drought stressed leaf in *Oryza sativa*, drought stressed leaf cDNA library from *Coffea canephora*, dehydration stressed root cDNA library from *Cicer arietinum, Spartina alterniflora* root salinity induced expressed sequence tag, field drought stressed root cDNA library, Brassica seed development drought normalized, *Triticum aestivum* developing seed heat stress reverse subtractive library, and similar to *Triticum aestivum* flower heat stress forward subtractive library.

According to the BLASTX results, the fragment Sah-1 was found to have 42% similarity to photo-system II stability/assembly factor HCF136, chloroplastic in *Oryza sativa*. During oxygenic photosynthesis, the solar energy is converted into chemical energy. The photochemical functions are performed by two photo systems: Photosystem I and II (**Iwata** and **Barber**, **2004**). Light-stimulated steps of photosynthesis are facilitated by the PSI together with PSII. These photo systems are multisubunit complexes consist of protein and non-protein components, and drive light-dependent electron transfer reactions, resulting in the formation of high-energy products such as ATP and NADPH. On the other hand, the fragment Sah-2 showed 32% similarity to a putative retrotransposon protein isolated from *Oryza sativa.* Plant retrotransposons have been found to be activated by abiotic and biotic stresses (**Wessler**, **1996**; **Grandbastien**, **1998**). Retrotransposons are a class of mobile genetic elements that are ubiquitous in the genomes of many eukaryotic organisms (**Bennetzen**, **2000**). They function by allowing their sequence to be transcribed into RNA. The fragment Sah-3 had 50% similarity to crystal structure of superoxide dismutase from *Potentilla atrosanguinea*. SOD is an enzyme that plays a crucial role in antioxidant defense because it catalyzes the conversion of the superoxide radical to molecular oxygen and H_2O_2 (**Costa**, ***et al***., **2010**). Superoxide dismutases (SODs), a group of metalloenzymes, considered the first defense against ROS.

The fragment Sah-4 was found to have 51% similarity to proline-rich extensin-like receptor kinase 10 (PERK 10) of *Arabidopsis thaliana*. The diverse group of cell surface receptor-like protein kinases (RLKs), in plant, plays an important role in signal transduction mechanisms (**Stone** and **Walker**, **1995**; **Lease**, ***et al***., **1998**). These membrane-spanning proteins perceive the initial stimulus and transmit the information intra-cellularly through a signaling cascade, which ultimately results in the appropriate cellular responses (**Silva** and **Goring**, **2002**). The plasma membrane-associated proline-rich extensin-like receptor kinase 4, is a novel regulator of Ca^{+} signaling, and plays fundamental role in ABA responses in *Arabidopsis thaliana* (**Bai**, ***et al***., **2009**).

The fragment BV-1 showed 50% similarity to glucose-6-phosphate dehydrogenase from *Medicago sativa*. G6PDH, is a cytosolic enzyme, present in different parts of plant tissues, it is mainly found in the cytosol and plastids (**Corpas**, ***et al***., **1998**; **Esposito**, ***et al***., **2001**; **Knight**, ***et al***., **2001**). G6PDH determines NADPH level via oxidative pentose phosphate pathway (**Williams**, **1980**; **Copeland** and **Turner**, **1987**). The main function of OPPP is production of reducing power (as NADPH) for fatty acid synthesis. One of the uses of NADPH in the cell is to prevent oxidative damages by reducing glutathione via glutathione reductase, which converts reactive H_2O_2 into H_2O. Therefore, plant defense could benefit from improved NADPH availability due to increased G6PD activity (**Scharte**, ***et al***., **2009**). **Liu**, ***et al***., (**2007**) reported that the over-expression of G6PDH could decrease ROS accumulation.

The fragment BV-2 was found to have 57% identity to formin-like protein 20 in *Arabidopsis thaliana*. Formins are large multidomain proteins that have been found in all eukaryotes examined and are required for multiple actin-related processes, such as cytokinesis (**Wasserman**, **1998**). The actin cytoskeleton is required for many cellular processes in plant cells (**Yi**, ***et al***., **2005**), like cytoplasmic streaming, and tip growth (**Volkmann** and **Baluska**, **1999**; **Staiger**, ***et al***., **2000**). The fragment BV-3 was found to be similar to the crystal structure of pokeweed lectin-C, with 40% identity. Lectins are class of carbohydrate-binding proteins (**Jiang** and **Ramachandran**, **2010**). The lectin genes are involved in biotic/abiotic stress regulation. Each member of this gene super family may play specialized roles in a specific stress condition and function as a regulator of various environmental factors such as drought.

The fragment BV-4 showed 46% similarity to trios-phosphate isomerase. TPI is a cytoplasmic enzyme of carbohydrate metabolism that catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP) (**Henze**, ***et al***., **1994**; **Dorion**, ***et al***., **2005)**. It is a key enzyme in the Calvin cycle. **Umeda**, ***et al***., (**1994**) demonstrated that TPIs were induced by water stress in rice cells; a coordinated induction of these enzymes for the production of energy to maintain the homeostasis in stressed cells was supposed. TPI was induced by drought in rice and maize (**Salekdeh**, ***et al***., **2002**; **Riccardi**, ***et al***., **1998**). The fragment BV-5 had 54% similarity to a retrotransposon protein, putative, unclassified from *Oryza sativa*, while fragment BV-6 showed 40 % identity to a transferase family protein of *Zea mays*. The molecular control mechanisms of drought stress tolerance based on expression of specific stress-related genes, which involved in water uptake and transport such as aquaporins and ion transporters (**Blumwald**, **2000**). The result of mRNADD method clearly indicated that, mRNA DD is an efficient approach to identify drought responsive genes in Sahal-1 and BVD-22 genotypes.

5.2.12 DREB genes

Dehydration-responsive element binding (DREB) proteins constitute a large family of transcription factors that are involved in a biotic stress tolerance. DREBs regulate many functional genes related to drought stress (**Ito**, ***et al***., **2006**). In the present study, the Dreb R1-3A was expressed in all genotypes under all conditions, but the expression under drought was higher than under control. On the other hand, Dreb R2 1A was expressed in Sahal-1 and BVD-22 genotypes only under drought stress conditions. DREB genes consist of two subclasses, 1) DREB gene1, which induced by cold stress, and 2) DREB gene2, which induced by dehydration stress (**Choi**, ***et al***., **2002**). It is possible to engineer stress tolerance in transgenic plants by manipulating the expression of these genes (**Agarwal**, ***et al***., **2006**). **Ito**, ***et al***., (**2006**) concluded that DREB1-type genes are useful to improvement the stress tolerance.

5.3 Open field experiment

For a study closer to agricultural conditions, we evaluated the response of forty-nine bread wheat genotypes to drought stress in the open field. The effects of drought stress on all the traits measured across the wheat genotypes were clear. Almost all the wheat genotypes had produced good plant height, biomass, harvest index and yield components under well-watered conditions, while all these parameters were significantly reduced under stress conditions.

5.3.1 Effect of irrigation systems on plant height (cm)

Results from the field experiment showed that the drought stress significantly reduced plant height of all genotypes except Katia, Momtchill, and Seval, which showed equal values under all conditions. The plant height of Bolal2973, Mufitbey, Pastor, Flamura85, Weston, Es00-ke3, Dagdas, Jagger, Kirkpinar79, Ks82w422, Ktk/ye2453, Gerek gm, F12.71/coc//prl"s", Izgi01, Pyn/bau, Bayraktar, and Gerek79 genotypes were the lowest affected by drought stress. The reduction in plant height could be due to inhibition of cell division or length of cells by drought stress (**Sarvestani**, ***et al***., **2008**). In addition, it may be due to decrease in relative turgidity and dehydration of protoplasm, which is associated with a loss of turgor and reduced expansion of cells and cell division. **Daneshian** and **Jonobi**, (**2001**) introduced plant height as a drought tolerance index in soybean plants. These results were in agreement with those obtained by **Moayedi**, ***et al***., (**2010**).

5.3.2 Effect of irrigation systems on heading date

Drought stress delayed the heading date of most genotypes compared to control conditions except Flamura85 and Stk52/trumbull genotypes, which showed the opposite trend and flowered earlier. They exhibited drought escape as a drought tolerance mechanism, and so have the ability to complete their life earlier (**Bayoumi**, ***et al***., **2008**). Drought escape is highly heritable but it is associated with lower yields (**Wortmann**, **1998**). Bayraktar, F12.71/coc//prl"s", Izgi01, Katia, Momtchill, Pastor,

and Suzen97 genotypes recorded the same heading date under both conditions. On the other hand, Es84-24/seri//seri, Aytin98, Ca8055/krc66, Es84-24//ks82w409, and Gerek79 genotypes recorded the longest delay in heading date (4 days). The delay in heading date may be associated with internal plant water status. **Sarvestani**, ***et al***., (**2008**) reported that genotypes with a longer delay had tried to extract more water during the drought period, and consequently had higher water deficits. The results were in parallel with those achieved by **Bayoumi**, ***et al***., (**2008**) who reported that drought stress caused increases in days to 50% heading of all wheat genotypes.

5.3.3 Effect of irrigation systems on biomass (kg m^{-2})

Drought stress reduced total biomass of all genotypes except Lov/bll and Flamura85, which showed an increase in biomass under stress conditions; this may be a consequence of heading date, as Flamura85 genotype did not record any delay in heading date. The reduction in biomass might be due to the negative effects of drought on important metabolic processes, photosynthesis, and decreasing dry matter accumulation (**Nagarajan**, ***et al***., **1999**). In addition, the total biomass was reduced considerably due to reduction of plant height (**Mirakhori**, ***et al***., **2009**). The genotypes Altay2000, Kutluk94, Gun91, Ekg15//tast, Century, Ikizce96, Suzen97, Weston, Katia, Aytin98, Momtchill, Vorona/kauz, Ks82w422, Momtchill/gun, Krc/bez, Bayraktar, Mnch/5/ and Pastor, showed lesser reduction of biomass than Vona//no57, Hawk, Harmankaya99, F12.71/coc//kauz, and Soyer. This could suggest that the former genotypes have better adaptive responses such as the controlling stomatal closure and stability of organelles within the cell (**Setter** and **Flannigan**, **2001**). The results were in parallel with those achieved by **Sangtarash**, (**2010**).

5.3.4 Effect of irrigation systems on harvest index

Water stress had significant negative effects on harvest index of almost wheat genotypes. Harvest index is the proportion of the biological yield, which forms the economic yield. A mean reduction of 2.4% was recorded in HI for all genotypes. Zitnica, Pyn/bau, Izgi01, Gerek gm, Ikizce96, and Mufitbey recorded the minimum

reductions in HI, while Bolal2973, Ca8055/krc66, Century, Ekg15//tast, Gerek79, Hawk, Jagger, Kutluk94, Mnch/5/, and Suzen97 genotypes recorded equal HI values under both drought and well-watered conditions. The reduction in harvest index under drought may be due to effects of water shortage on seed weight (**Seghatoleslami**, ***et al***., **2008**). It may be also a result of reduction in yield and biomass (**Mirakhori**, ***et al***., **2009**). The drought conditions forces plant to complete its grain formation in a relatively short time (**Riaz** and **Chowdhrv**, **2003**). The genotypes which had the lowest reduction in plant height (i.e. Gerek gm, Izgi01, and Pyn/bau), and had a short or no delay in heading date (Izgi01, and Pastor) were associated with a small decrease or slight increase in harvest index. The reduction in harvest index in wheat genotypes under drought stress was reported by **Sangtarash**, (**2010**) and **Moayedi**, ***et al***., **(2010**). A maximum increase of 22.5% in HI was observed in Vona//no57 followed by Pastor, Es00-ke3, Tosunbey, Sonmez01, Soyer, Krc/bez, Kirac66, Dagdas, Ktk/ye2453, Karahan, Altay2000, Momtchill, Bezostaya1, and Seval. **Austin**, (**1994**) suggested that high harvest index might be due to improved resistance to drought, making the plants much shorter along with enhancing the supply of nutrient substances to young kernels.

5.3.5 Effect of irrigation systems on NDVI values

Normalized difference vegetation index (NDVI) is widely used for crop stress detection because of its high correlated with vegetation parameters such as biomass and green leaf area (**Curran**, **1980**). **Johnsen**, ***et al***., (**2009**) reported that the plant stress could be quantified with NDVI. In our study, values of NDVI were much lower under drought stress than under irrigated conditions except Momtchill (NDVI value was similar to control), and Es84-24//ks82w409, Es84-24/seri//seri, Gerek gm, Harmankaya99, Ikizce96, Izgi01, Jagger, Karahan, Katia, Ks82w422, Pyn/bau, Seval, Sonmez01, Suzen97, and Vorona/kauz genotypes (NDVI values were higher than controls). The reduction in NDVI values under drought stress may be due to the irrigated plants tending to have chlorophyll content higher than non-irrigated plants. Water stress produced changes in the chlorophyll contents in barley (**Anjum**, ***et al***., **2003**). **Lawlor** and **Cornic**, (**2002**) concluded that the photosynthetic rate decrease as the relative water content and leaf water potential decreases in higher plants. **Farooq**, ***et al***., (**2009**) reported that the drought stress mainly limits and reduced the photosynthesis

process through stomatal closure and non-stomatal limitation (metabolic impairment).The genotypes Bayraktar, Hawk, Mnch/5, Gun91, and Ktk/ye2453 recorded the lowest reduction in NDVI. **Shakya** and **Yamaguchi**, (**2007**) reported that the healthy vegetation gives high NDVI values, while the unhealthy plants give low values. Higher index values, being associated with greater green leaf area and biomass. **Baghzouz**, ***et al***., (**2006**) found that the NDVI decreased as the tissue water content decreases in in tall fescue and annual ryegrass. **Xiong**, ***et al***., (**2007**) concluded that NDVI was increased with increasing irrigation and nitrogen rate.

5.3.6 Effect of irrigation systems on SPAD values

Photosynthetic pigments are important to plants mainly for harvesting light (**Jaleel**, ***et al***., **2009**). In the present study, there was a genetic variation for SPAD values among wheat genotypes that were studied under drought stress in the field. The result show that drought decreased chlorophyll content (measured as SPAD value) of flag leaves of all wheat genotypes except Bayraktar, Bolal2973, Ca8055/krc66, Dagdas, Es00-ke3, F12.71/coc//kauz, F12.71/coc//prl"s"/ Gerek gm, Gerek79, Gun91, Harmankaya99, Jagger, Katia, Kutluk94, Sonmez01, Suzen97, and Zitnica genotypes, which showed high SPAD values under drought. **Rong-hua**, ***et al***., (**2006**) reported that the values of chlorophyll content in drought tolerance genotypes were significantly higher than in drought sensitive genotypes. The negative effects of drought stress on chlorophyll content may be consequences of inhibited photosynthesis (**Farooq**, ***et al***., **2009**), reduced synthesis of the main chlorophyll pigment complexes encoded by the cab gene family (**Allakhverdiev**, ***et al***., **2000**), and oxidative damage of chloroplast lipids (**Tambussi**, ***et al***., **2000**). Under drought stress, the lowest reduction in SPAD values were observed in Es84-24//ks82w409, Izgi01, Lov/bll/, Kirgiz95, Pastor, Pyn/bau, Kirkpinar79, Mufitbey, Krc/bez, Ks82w422, Ktk/ye2453, Hawk, Ekg15//tast, and Weston genotypes.

Water stress resulted in an accelerated chlorophyll breakdown starting in the wheat leaves. Meanwhile, the leaf senescence was delayed in Pyn/bau, Ca8055/krc66, Dagdas, Kirkpinar79, F12.71/coc//kauz, Es84-24//, Harmankaya99, and Suzen97 genotypes. The results suggest that the former genotypes are drought tolerant and are

able to retain green leaves longer than other genotypes under drought conditions. Wheat genotypes with green leaf retention may process drought-tolerance mechanism, which allow the plants to maintain metabolic activity, despite of low leaf water potential (**Fukai** and **Cooper**, **1995**). The ability to maintain the functionality of the photosynthetic machinery under drought stress is important in drought tolerance mechanisms. It may be possible to enhance drought tolerance by delaying senescence induced by drought (**Rivero**, ***et al***., **2007**). The obtained results are in agreement with those obtained by **Balouchi**, ***et al***., (**2009**).

5.3.7 Effect of irrigation systems on canopy temperature

Canopy temperature (CT) used in wheat breeding and selection for yield (**Saint Pierre**, ***et al***., **2010**). Generally, canopy temperature increased due to drought compared to well-watered conditions. Ca8055/krc66, Es00-ke3, Gerek gm, Gun91, Hawk, Ikizce96, Lov/bll, Pastor, Weston, Ktk/ye2453 genotypes recorded the opposite trend; with the temperature under stress was less than or equal to the temperature under well-watered conditions. The canopy temperature increased due to increased respiration and decreased transpiration rate resulting from stomatal closure (**Siddique**, ***et al***., **2000**). Under rain fed conditions Gun91, Dagdas, Ca8055/krc66, Es00-ke3, Karahan, Ktk/ye2453, Kutluk94, Lov/bll//, and Ekg15//tast were the coldest plants. For breeding and selection to drought resistance, it is very important to find genotypes that maintain lower canopy temperature as compared with other genotypes under drought stress conditions. These genotypes will use more of the available water in the soil, thus limiting the negative effect of water stress on plant functions (**Blum**, **1988**). These results were in accordance with those reported by **Olivares-Villegas**, ***et al***., (**2007**).

5.3.8 Effect of irrigation systems on yield and its components

Effect of irrigation systems on number of spikes m^{-2}

Number of spikes per m^2 is an important character in wheat breeding programmers (**Olgun**, ***et al***., **2006**). Most spikes were produced with well-watered conditions, while fewest spikes were produced when the drought stress was applied. Kirkpinar79, Tosunbey, Ca8055/krc66, Century, Es00-ke3, Lov/bll/, Bolal2973, Kirac66, Sonmez01, and Vorona/kauz genotypes recorded the lowest reduction in number of spikes m^{-2} under drought stress conditions. Similar results were demonstrated by **Moayedi**, ***et al***., **(2010**).

Effect of irrigation systems on number of grains $spike^{-1}$

Number of grains $spike^{-1}$ is an important trait of wheat contributing to yield. Generally, drought stress caused a significant reduction in NGS in most genotypes compared to the well-watered conditions except Vorona/kauz, Bezostaya1, Flamura85, Kirgiz95, Kutluk94, Momtchill, Ca8055/krc66, Pastor, Es84-24/seri//seri, Hawk, Mufitbey, Dagdas, Gerek gm, Bayraktar, Bolal2973, Vona//no57, Jagger, and Harmankaya99, which showed an increase in the grains number per spike under rain fed conditions. The genotypes Katia, F12.71/coc//prl"s", and Weston genotypes maintained the lowest reduction under stress conditions. This reduction could be a result from effect of drought on pollination (**Elhafid**, ***et al***., **1998**). Drought stress is associated with infertility and caused decrease in grains number $spike^{-1}$. **Sarvestani**, ***et al***., (**2008**) reported that stress might decrease translocation of assimilates to the grains, which increased the number of empty grains. Results were in harmony with those achieved by **Mirbahar**, ***et al***., (**2009**), and **Moayedi**, ***et al***., **(2010**).

Effect of irrigation systems on thousand grain weight

Sharp decrease in TGW was recorded under drought stress in all genotypes. The lowest of TGW was recorded in Stk52/trumbull (which flowered earlier). On the other hand, the highest TGW was observed in Momtchill at control as well as at drought. Under rain fed conditions, the minimum decreases in TGW recorded in Kirkpinar79, Aytin98, Izgi01, Dagdas, Bezostaya1, Es84-24//ks82w409, Sonmez01, Ktk/ye2453, Momtchill/gun, Katia, Suzen97, Century, Krc/bez, and Es00-ke3 genotypes. This reduction in TGW may be a result of disturbed nutrient uptake efficiency and photosynthetic translocation within the plant (**Iqbal**, ***et al***., **1999**). The drought conditions forces plant to complete its grain formation in a relatively short time (**Riaz** and **Chowdhrv**, **2003**). Amount and quality of storage material in wheat kernels depends on the accessibility of nutrients in soil (**Konopka**, ***et al***., **2007**). **Sarvestani**, ***et al***., (**2008**) reported that stress might decrease translocation of assimilates to the grains, which lowered grain weight and increased the number of empty grains. These results are in agreement with those of **Johari-Pireivatlou**, ***et al***., (**2010**) who observed that TGW of wheat was reduced due to drought stress.

Effect of irrigation systems on grain yield

Drought is one of the major abiotic factors that reduces grain yield of wheat (**Bhutta**, ***et al***., **2006**). In the present study, the GY was greater in well-watered plants than in the drought plants, a consequence of more spikes per m^2, and heavier grains (**Kilic** and **Yagbasanlar**, **2010**). Pastor and Flamura85 showed a reverse trend as the grain yield under drought conditions were more than under well-watered conditions. The yield of the forty-nine selected wheat genotypes varied in the range of 5.01 - 7.36 ton ha^{-1} and 3.11 - 6 ton ha^{-1} in irrigated and non-irrigated conditions, respectively. Under rain fed conditions, the highest yield reduction was observed in F12.71/coc//kauz, Izgi01, Stk52/trumbull, Hawk, and Gerek gm genotypes and the lowest yield reduction was in Suzen97, Ekg15//tast, Vorona/kauz, Bolal2973, Weston, Mnch/5/, Lov/bll/, Katia, Altay2000, Ktk/ye2453, Momtchill/gun, Kirgiz95, Ks82w422, Ikizce96, Es00-ke3, Gun91, Momtchill, and Krc/bez genotypes. **Sarvestani**, ***et al***., (**2008**) reported reduction in yield, which resulted from reduction in fertile panicle

number and filled grain percentage. GY depends on number of effective tillers and 1000-grain weight. **Chowdhry**, ***et al***., (**2000**) concluded that yield components like, grains per spike and TGW are main contributors to grain yield of wheat. Bolal2973, Sonmez01, Pastor, Zitnica, Harmankaya99, Krc/bez, Vorona/kauz, Pyn/bau, Tosunbey, Katia, Momtchill/gun, Flamura85, Es00-ke3, and Momtchill genotypes showed the best yielding in rain fed conditions, mainly due to higher grain filling period-no delay in heading date (**Kilic** and **Yagbasanlar**, **2010**). The results were in parallel with those achieved by **Sangtarash**, (**2010**).

Drought susceptibility index

DSI is independent of yield potential and drought intensity, and is potentially useful for comparisons of drought susceptibility of genotypes, since larger values of DSI indicate greater drought susceptibility. The results indicated that the wheat genotypes Pastor, Flamura85, Krc/bez, Momtchill, Gun91, Es00-ke3, Ikizce96, Ks82w422, Kirgiz95, Momtchill/gun, Ktk/ye2453, Altay2000, Katia, Lov/bll/, Mnch/5/, Weston, Bolal2973, Ekg15//tast, Vorona/kauz, Suzen97, Kutluk94, Sonmez01, and Bayraktar expressed lowest DSI. Moreover, the genotypes Gerek gm, Mufitbey, Hawk, Stk52/trumbull, Izgi01 and F12.71/coc//kauz had highest DSI values. The large values of DSI indicate greater drought susceptibility, while, the genotypes with low DSI values can be considered drought resistant, because they exhibited smaller yield reductions under drought stress compared with well-watered conditions than the mean of all genotypes. The results are in agreement with those obtained by **Bayoumi**, ***et al***., (**2008**).

Relative grain yield

Selection for drought tolerance typically involves evaluating genotypes for either high yield potential or stable performance under stress conditions (**Ahmad**, ***et al***., **2003**). High yield potential under drought conditions is an important target of wheat breeders (**Jaleel**, ***et al***., **2009**). The results showed that, Altay2000, Bayraktar, Ikizce96, Century, Karahan, Mnch/5, Gun91, Bolal2973, Pastor, Sonmez01, Harmankaya99, Zitnica, Krc/bez, Pyn/bau, Tosunbey, Vorona/kauz, Katia, Flamura85, Momtchill/gun,

Es00-ke3, and Momtchill genotypes, were relatively high yielding under water stress (**RY > mean RY**).This could suggest that these genotypes have better adaptive mechanisms such as controlling stomatal closure and stability of organelles within the cell (**Setter** and **Flannigan**, **2001**).

5.3.9 Correlation coefficient analysis under drought stress

The correlation coefficient analysis indicated that, there was significant positive correlations between grain yield and (biomass, 1000-grain, harvest index).These correlations suggested that increase in biomass would result in increasing of grain yield. Plant height had highly significant negative correlations with (canopy temperature, number of grains spike^{-1}). Negative correlation of plant height with number of grains was also reported by **Patil** and **Jain**, (**2002**). This suggested that increase in plant height would result in reduction of canopy temperature and number of grains. Heading date was negatively associated with, (number of grains spike^{-1}, number of spikes m^{-2}, and grain yield).These results were in parallel with those achieved by **Kilic** and **Yagbasanlar**, (**2010**) who found a negative correlation between grain yield and number of days to heading. **Sarvestani**, ***et al***., (**2008**) reported that the genotypes with longer delay in heading time had higher water deficits and were consistently associated with a larger yield reduction under drought.

There was a negative correlation between NDVI and canopy temperature. This suggested that increase in chlorophyll content would result in reduction of CT. The present findings are similar to those of **Fenstemaker-Shaulis**, ***et al***., (**1997**) who found a negative correlation between NDVI and canopy temperature. **Saint Pierre *et al***., (**2010**) reported that CT showed a strong negative phenotypic correlation with grain yield under drought conditions. On the other hand, **Olivares-Villegas *et al***., (**2007**) found that CT negatively associated with yield and it was highly associated with biomass at booting, and plant height. The potential of CT as screening tool for wheat genotypes under drought-stress based on its significant association with grain yield (**Reynolds**, ***et al***., **2001**).

In our studies, we found positive correlations between NDVI and plant height, heading date, SPAD, 1000-grain weight. NDVI is widely used as standard vegetation index for estimating biomass (**Barbosa**, ***et al***., **1999**). Because of the close relationship between vegetation vigor and soil moisture, NDVI be used in assessment of vegetation drought stress in arid and semi-arid regions (**Ji** and **Peters**, **2003**), also there is a positive correlation between NDVI and plant moisture content (**Fenstemaker-Shaulis**, ***et al***., **1997**). The NDVI was a better estimator of chlorophyll content in turfgrass (**Bell**, ***et al***., **2004**). **Kruse**, ***et al***., (**2005**) found that there is no correlation between biomass and NDVI. The healthy vegetation gives high NDVI values, while the unhealthy plant gives low values (**Shakya** and **Yamaguchi**, **2007**). SPAD observed positive significant correlations with heading date, NDVI, number of grains spike^{-1}, and 1000-grain weight, whereas canopy temperature and number of spikes m^{-2} were negatively associated with SPAD. These correlations suggested that increase in chlorophyll content would result in increasing of 1000-grain weight and Number of grains spike^{-1}. **Kilic** and **Yagbasanlar**, (**2010**) reported a positive significant correlation between the grain yield and chlorophyll content.

There was a significant positive correlation between TGW and Grain yield. **Nouri-Ganbalani**, ***et al***., (**2009**) reported a positive significant correlation between the grain yield and TGW. The high significant correlation of 1000-grain weight with grain yield implies that 1000-grain weight plays important role in the possible increase of the grain yield of the wheat genotypes. It was evident from the results that TGW had pronounced influence upon wheat grain yield in all genotypes. The present findings are similar to those of **Inamullah**, ***et al***., (**2006**) who observed positive association of TGW with grain yield. **Aycecik** and **Yildirim**, (**2006**) also reported positive correlations between grain yield with number of grains per spike, plant height and TGW. Number of grains spike^{-1} had positive and non-significant association with grain yield. **Kilic** and **Yagbasanlar**, (**2010**) reported a positive significant correlation between the grain yield and Number of grains spike^{-1}. The perusal of these correlation coefficient results suggested that number of grains per spike should given prime importance regarding its contribution to yield (**Akram**, ***et al***., **2008**).

6 CONCLUSION

Wheat is an attractive study system because of it is wide natural genetic variation in traits related to drought resistance. For a successful development of drought resistant genotypes, it is necessary to study the influence of drought on growth and the physiological characteristics of different wheat genotypes and compare between tolerant and susceptible genotypes under stress and non-stress conditions. In the present study, the effects of drought stress on morphological, physiological and molecular characteristics of some wheat genotypes were investigated. The differential response of wheat genotypes to imposed water stress condition indicates the drought tolerance ability. Since, all parameters like plant height, biomass, harvest index, 1000-grain weight, yield and yield components, were found to be influenced by drought stress.

Four important findings were obtained from this research. Firstly, the genotypes Altay2000, Bayraktar, Bolal2973, Es00-ke3, Flamura85, Gun91, Ikizce96, Katia, Krc/bez, Mnch/5/, Momtchill, Momtchill/gun, Pastor, Sonmez01, and Vorona/kauz showed high yield potential (RY) and high yield stability (DSI) under stress conditions, so these genotypes could be further tested for their other drought conferring characteristics. Secondly, based on the results of this research, the decreases in nutrient uptake were small in tolerant (BVD-22 and Sahal-1), but huge in susceptible genotypes (Giza-163 and Ozcan), which suggest that the nutrients uptake in wheat grown under drought conditions may have a role in drought tolerance. Calcium (participated in signaling mechanisms of drought induced proline accumulation), Potassium (had a key role in osmotic adjustments), Phosphorus (increased the water use efficiency and cell membrane stability), Zinc (protected plant cells from damage effects caused by ROS and inhibited NADPH oxidase), Cupper (had a key role in hormone signaling, oxidative stress response, co-factor for Cu-SOD enzyme), Manganese (amino acids biosynthesis, activated Mn-SOD enzyme which had a crucial role in antioxidant defense).

Thirdly, although drought tolerance mechanisms were seen in Sahal-1 and BVD-22, but its extent varies from genotype to other genotype and even within genotype from growth stage to other stage. For example, both genotypes used osmotic adjustment as a drought tolerance mechanism, but they used different organic solutes. Sahal-1 used soluble carbohydrate, however BVD-22 depend on proline accumulation. Furthermore, the plant response to drought may vary and depending on growth stage and genotype in terms of the types of enzyme that used in the antioxidant defense mechanism, which suggest that the developmental stages of wheat leaves might contribute to the differential prevention of oxidative damage in plants exposed to drought stress. Fourthly, the result of mRNA differential display clearly indicated that, mRNA-DD is an efficient approach to identify drought responsive genes and obtain gene expression profiles of Sahal-1 and BVD-22 genotypes which exposed to drought stress.

Finally, it is necessary to develop new wheat genotypes, which characterized by 1) maintaining lower canopy temperature as compared to other genotypes, 2) healthy vegetation and high NDVI values, 3) able to retain green leaves longer than other, and 4) characterized by high yield potential under drought conditions. The combination of molecular and morpho-physiological approaches is the key of a better understanding of drought resistance mechanisms in wheat. Thus, further work is required to identify and manipulated the genes controlling the physiological and molecular traits.

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Appendix: A

Supplies

Disposable Labware:

Centrifuge Tubes 2ml:	Axygen Scientific, USA (MCT-200-C).
Clickfit Cap Microtubes:	Trefflab, Switzerland (96.8185.9.03, 96.7811.9.03).
Clickfit Cap Microtubes:	Trefflab, Switzerland (96.9329.9.01).
Diamond® Tips:	Gilson, USA (D10, D200, D1000).
PCR-Tubes:	Trefflab, Switzerland (96.9852.9.01).
Petri Dishes:	ISOLAB Laborgeräte Gmbh, Germany (113.02.002).
Screw Cap Tubes 15ml:	Axygen Scientific, USA (SCT-15ML-25-S).
Screw Cap Tubes 50ml:	Axygen Scientific, USA (SCT-15ML-25-S).
Tips For Pipettes:	Axygen Scientific, USA (T-200-Y, T-1000-B, T-300).

Chemical Supplies and Kits:

2-Propanol Extra Pure:	Merck Kgaa, Germany (1.00995)
2-Propanol Puriss., ≥99.5% (GC):	Riedel-De Haën®, Germany (24137)
6x Dna Loading Dye :	Fermentas, Canada (R0611)
Acetic Acid:	Riedel-De Haen, Germany (27225)
Acetone:	Merckkgaa, Germany (100013)
Acrylamide 30%-0.8% Bi-Acrylamide:	Sigma, Germany (A3699)
Agar Type A, Plant Cell Culture Tested:	Sigma-Aldrich Co., USA (A4550)
Agarose:	Prona Basica LE Agarose, E.U.
Ampicillin Sodium Salt:	Applichem Gmbh, Germany (A0839)
Taq DNA Polymerase (Recombinant):	Fermentas, Canada (EP0402)
Boric Acid:	Sigma-Aldrich Co., USA (B6768)
Chloroform Biotechnology Grade:	Amresco® Inc., USA (0757)
Coomassie Brilliant Blue:	Fluka, Switzerland (27816)
D-(+)-Glucose Monohydrate:	Fluka, Switzerland (49158)
Datp, Molecular Biology Grade:	Fermentas, Canada (R0141)
Deoxyribonuclease I, Rnase-Free:	Fermentas, Canada (EN0521)
Diethyl Pyrocarbonate, ≥97% (NMR):	Sigma-Aldrich Co., USA (D5758)

Dnasei:	Fermentas, Canada (EN0521)
Dntp Mix 10mm:	Fermentas., Canada (#R0193)
Dntp Mix 25mm:	Fermentas., Canada (#R1121)
Ethanol Absolute Puriss:	Riedel-De Haen, Germany (32221)
Ethidium Bromide Solution:	Merck Kgaa, Germany (1.11608)
Generulertm 100bp DNA Ladder Plus:	Fermentas, Canada (SM0321)
Hydrochloric Acid 37%:	Merckkgaa, Germany (100314)
IPTG, Dioxane-Free:	Fermentas, Canada (R0393)
Lb Broth:	Sigma-Aldrich Co., USA (L3022)
Magnesium Chloride:	Riedel-De Haen, Germany (13152)
Magnesium Sulphate:	Riedel-De Haen, Germany (13246)
Methanol:	Riedel-De Haen, Germany (24229)
MS Medium (Salt Mixture):	Sigma, Germany (M5524)
Oligo(dT) Primer:	Invitrogen, USA (L3147)
Omniscript Rt Kit:	Qiagen Inc., USA(205111)
Pgem®-T Vector System II:	Promega, USA (A3610)
Qiaprep Spin Miniprep Kit:	Qiagen Inc., USA (27106)
Qiaquick Gel Extraction Kit:	Qiagen Inc., USA (28706)
Qiaquick PCR Purification Kit:	Qiagen Inc., USA (28104)
Sodium Chloride:	Riedel-De Haen, Germany (13423)
Sodium Hydroxide Pellets Pure:	Merck Kgaa, Germany (1.06462)
Sucrose Grade I, Plant Cell Culture Tested:	Sigma-Aldrich Co., USA (S5390)
Sodium Chloride EMPROVE®:	Merck Kgaa, Germany (1.06400)
Tris:	Fluka, Switzerland (93349)
Trizol® Reagent:	Invitrogen, USA (15596-018)
Tryptone:	Applichem Gmbh, Germany (A1553)
X-Gal:	Promega, USA (V3941)

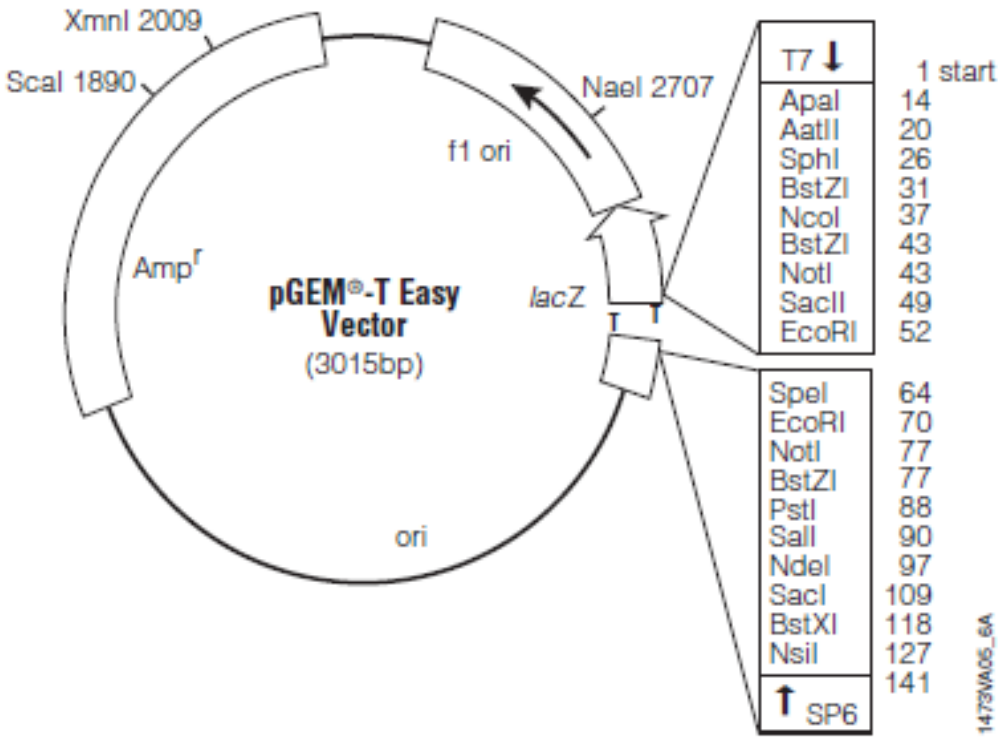
Appendix B:

Equipment

Autoclaves:	Hirayama, Japan (Hiclave HV-110)
	CERTOCCLAV® A-4050 TRAUN/AUSTRIA
Automatic Pipette:	Pipettus® -Akku, Hirshmann Laborgerate
Centrifuges:	Eppendorf, (Centrifuge 5415C, 5415D, 5415R), Germany.
Cold Room:	Alarko Carrier, Turkey.
Deep Freezer:	HERAEUS ® HERA FREEZE
Electronic Balances:	Sartorius, BP610, BP221S, BP221D, Germany.
Gel Documentation System:	Bio-Rad Laboratories, (Universal Hood II), USA.
Heating Block:	Fisher,(Bioblock Scientifictm), France .
Heating Magnetic Stirrer:	VELP Scientifica, Italy.
Ice Machine:	Scotsman Inc., Af20, USA
Incubator Shaker:	New Brunswick Scientific, (Innova 4330), USA.
Incubator:	Memmert, (D06059 Modell 300), Germany.
Laminar Flow Cabinets:	HERAEUS® HERA SAFE, Germany.
Magnetic Stirrer:	Velp Scientifica, Are Heating Magnetic Stirrer, Italy.
Micropipettes:	Gilson, Pipetman, France.
Microwave:	Vestel, Turkey.
Ph Meters:	WTW, Ph540glp Multical, Germany.
	Windaus Labortechnik,(Titroline Alpha), Germany.
Power Supply:	Biorad, Powerpac 300, Usa; Wealtec, Elite 300, USA.
Refrigerators:	Bosch, -20±C, +4±C, Turkey.
Spectrophotometer:	Schimadzu, UV3150, Japan; Nanodrop Tech, ND-1000, USA.
Thermal Cycler:	GMI, (MJ Research PTC-100), USA.
Thermo-mixer:	Eppendorf, Thermomixer Comfort, Germany.
Vortex Mixer:	VELP Scientifica, EU.
Water Baths:	Techne, (Refrigerated Bath RB-5A), UK.
	Huber Polystat Cc1
Water Purification System:	Millipore, (Milli-Q Academic), USA.
Liquid Nitrogen Tank:	DEWAR, Flask And Container, England.

Appendix C:

pGEM®-T Easy Vector Map and Sequence Reference Points



pGEM®-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
lacZ start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3