T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

DROUGHT AND SALT STRESS EFFECTS ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL CHANGES AND GENE EXPRESSION OF PHOTOSYSTEM II AND CATALASE GENES IN *ALLIUM CEPA* L.

USMAN KHALID CHAUDHRY

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USMAN KHALID CHAUDHRY

Doctor of Philosophy Thesis

Supervisor

Assist. Prof. Dr. Ali Fuat GÖKÇE

December 2020

Usman Khalid CHAUDHRY tarafından **Dr. Öğr. Üyesi Ali Fuat GÖKÇE** danışmanlığında hazırlanan "**Drought and Salt Stress Effects on Morpho-Physiological, Biochemical Changes and Gene Expression of Photosystem II and Catalase Genes in *Allium Cepa* L.**" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Tarımsal Genetik Mühendisliği Anabilim Dalı**'nda Doktora (İngilizce) tezi olarak kabul edilmiştir.

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Prof. Dr. Murat BARUT

MÜDÜR

THESIS CERTIFICATION

I certify that the thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Usman Khalid CHAUDHRY

SUMMARY

DROUGHT AND SALT STRESS EFFECTS ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL CHANGES AND GENE EXPRESSION OF PHOTOSYSTEM II AND CATALASE GENES IN *ALLIUM CEPA* L.

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Onion has tremendous importance with its health benefit, and it is used as the main condiment vegetable crop globally. Salinity and drought appear to be the major issues to be addressed to ensure food security. Although there are many works in literature summarizing responses of agricultural crops to these two most important abiotic stresses, data for onion appears to be quite limited. Therefore, a greenhouse study was conducted to observe the effect of drought and salinity on selected onion cultivars. Seven onion cultivars were compared for their morphological, physiological, biochemical and gene expression levels in responses to salt and drought stresses. Various morphological traits such as leaf length, leaf diameter, number of leaves and bulb characteristics (bulb diameter, bulb length and total bulb weight) were collected. Physiological data including relative water content (RWC), chlorophyll (a, b, total) and carotenoids contents, SPAD index, leaf temperature and gaseous exchange traits were observed. The activity of SOD, CAT and APX enzymes was also measured along with proline. Lipid peroxidation in onion plants was quantified by MDA contents. Results showed that among all the cultivars Inci, Perama and Seyhan performed well under both stress conditions. Contrarily, the response of Elit and Hazar cultivars was poor.

Keywords: Abiotic stress, ascorbate peroxidase, catalase, onion genotypes, photosystem II, superoxide dismutase, gene expression, short-day onion cultivars, photosynthesis, root morphology, yield

ÖZET

ALLIUM CEPA L.'DA FARKLI GÖSTERİM YÖNTEMİ İLE FİZYOLOJİK DEĞİŞİKLİKLER VE KURAKLIK STRESI İLE İLGİLİ ADAY GENLERİNİN TANIMLANMASI

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Tarımsal Genetik Mühendisliği Anabilim Dalı

Danışman :Dr. Öğr. Üyesi Ali Fuat GÖKÇE

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Soğan sağlığa yararları ile birlikte büyük öneme sahiptir ve dünya çapında yemeklerde lezzet için temel sebze olarak kullanılmaktadır. Tuzluluk ve kuraklık, gıda güvenliğini sağlamak için ele alınması gereken başlıca sorunlar olarak görünmektedir. Literatürde, tarımsal mahsullerin bu en önemli iki abiyotik strese tepkilerini özetleyen birçok çalışma olmasına rağmen, soğan için veriler oldukça sınırlı görünmektedir. Bu nedenle, kuraklık ve tuzluluğun seçilmiş soğan çeşitleri üzerindeki etkisini gözlemlemek için sera ortamında bir çalışma yapılmıştır. Yedi soğan çeşidi, tuz ve kuraklık stresine verdikleri tepkilerde morfolojik, fizyolojik, biyokimyasal ve gen ekspresyon seviyeleri açısından karşılaştırılmıştır. Yaprak uzunluğu, yaprak çapı, yaprak sayısı ve soğan baş özellikleri (çapı, uzunluğu ve ağırlığı) gibi çeşitli morfolojik özellikler incelenmiştir. Bağıl su içeriği (RWC), klorofil (a, b, toplam) ve karotenoid içerikleri, SPAD indeksi, yaprak sıcaklığı ve gaz değişim özelliklerini içeren fizyolojik veriler gözlenmiştir. SOD, CAT ve APX enzimlerinin aktivitesi de prolin ile birlikte ölçülmüştür. Soğan bitkilerindeki lipid peroksidasyon ve MDA içerikleri ile ölçülmüştür. Sonuçlar, İnci, Perama ve Seyhan çeşitlerinin her iki stres koşulu altında da iyi performans gösterdiğini göstermiştir. Elit ve Hazar çeşitlerinin tepkisi ise tam tersine zayıf bulunmuştur.

Anahtar Sözcükler: Abiyotik stres, askorbat peroksidaz, katalaz, soğan genotipleri, fotosistem II, süperoksit dismutaz, gen ekspresyonu, kısa soğan çeşitleri, fotosentez, kök morfolojisi, verim

PREFACE

Onion is the bulbous crop cultivated commercially in most parts of the world. It is the principal vegetable crop of Turkey holding 7th position in the world with a production of 2.0 million tons. It is consumed second in number after tomato, mostly both as a raw or in salad. The uncooked bulbs are the chief source of flavoring. They vary in taste from sweet to mild flavored and pungent for making onion aroma as a condiment in almost every cuisine. It is utilized as a condiment vegetable crop due to its unique aroma and taste. Onion consumption has numerous benefits including cardiovascular stability because of their anti-diabetic, anti-hypertensive, hyper-cholesterol, and antithrombotic effects. Onion is major vegetable crop with its uses and benefits, but abiotic stress causes significant yield losses. With climate change the severity of abiotic stress has been increased globally which is the main threat of future food security. Thereby it is essential to screen the local Turkish onion cultivars in response to the drought and salt stress. In this study seven onion cultivars were screened that can be used for future abiotic stress breeding.

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SYMBOLS AND ABBREVIATIONS

Symbols	Descriptions
%	Percentage
℃	Celsius degree
µg	Microgram
µL	Microliter
µmol	Micromole
mmol	Milimole
cm	Centimeter
g	Gram
L	Liter
M	Molar
mg	Miligram
min	Minute
mL	Milliliter
mM	Millimolar
nm	Nanometer
rpm	Revolutions per minute
sec	Second
h	Hour
T	Ton
m^2	Square meter
m^3	Cubic meter
cm^3	Cubic centimeter
cm^2	Square centimeter
mm	milimeter

Abbreviations	Descriptions
ANOVA	Analysis of Variance
LB	Luria-bertani Medium Diode
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid

RNA	Ribonucleic Acid
ddH2O	Double-distilled Water
DEPC	Diethyl Pyrocarbonate
dNTPs	Dinucleotide Triphosphate
DW	Dry Weight
FW	Fresh Weight
NaCl	Sodium Chloride
U	Units
SDS	Sodium Dodecyl Sulphate
ng	Nano Gram
OD	Optical Density
UV	Ultraviolet
TE	Tris Ethylene Diamine Tetra Acetic Acid
bp	Base Pair
FAOSTAT	Food and Agriculture Organization Statistical
LSD	Least Significant Difference
H_2O_2	Hydrogen Peroxide
EDTA	Ethylenediaminetetraacetic Acid
Tris-Cl	Tris-chloride
CRD	Completely Randomized Design
KCl	Potassium Chloride
TBE	Tris-borate-EDTA
MDA	Malondialdehyde
CAT	Catalase
SOD	Superoxide Dismutase
APX	Ascorbate Peroxidase
PSII	Photosystem II
pH	Potential for Hydrogen
ROS	Reactive Oxygen Species
SAS	Statistical Analysis Software
PCA	Principal Component Analysis

CHAPTER I

INTRODUCTION

Allium genus covers approximately 700 species distributed around the globe and its species are the oldest cultivated vegetables. Onion (*Allium cepa* L.) was formerly in *Alliaceae* family. Recently *Alliaceae* family divided into three subfamilies: *Amaryllidoideae*, *Agapanthoideae* and *Allioideae*. Onion is included in *Allioideae* family (Costa et al., 2020). It is the bulbous crop cultivated commercially in most parts of the world (Havey and Ghavami, 2018). It is originated from the mountainous region of Afghanistan, Iran, Pakistan, and central Asian regions of former USSR (Havey, 1997). China ranks first in number with average annum production of 17.8 million tons; India produces 11.4 million tons, while Turkey produces 2 million tons of onion annually (FAOSTAT, 2020) (Figure 1.1).

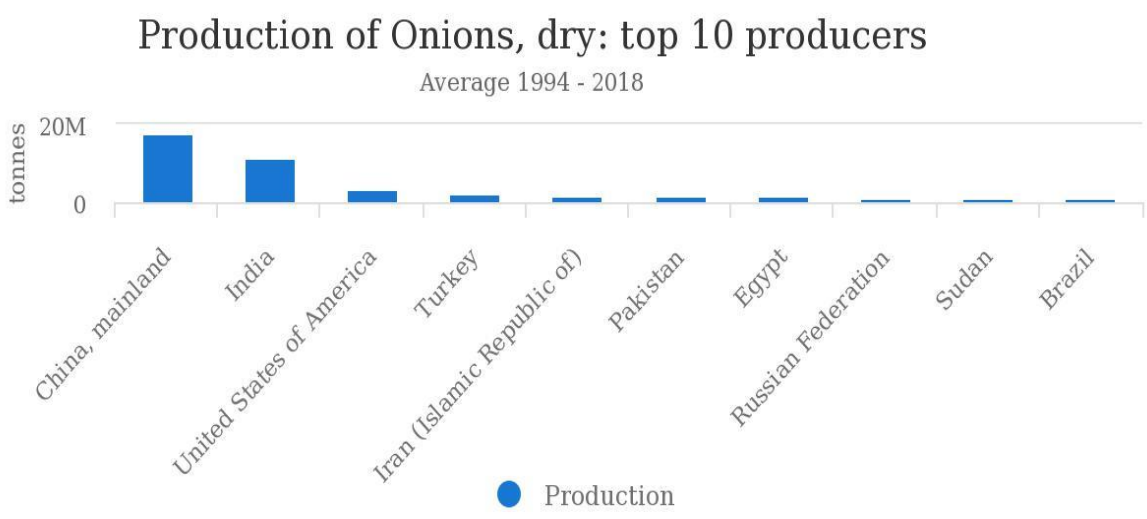

Figure 1.1. Top 10 onion producing countries in the world (FAOSTAT, 2020)

Worldwide annual production of dry onions along with green onions and shallots is exceeding 100 million tons from 7.6 million ha and they are distributed globally in wide range of geographic regions (FAOSTAT, 2020). Onion is widely distributed in world and within last 10 years its production has been increased significantly. Annual onion trade constitutes about 20 million USD (FAO, 2018). Global dry onion production over the past 20 years has been illustrated in Figure 1.2. Gross production value of onion over the entire

world is $42,743 million (FAO, 2018). Average yield of onion is 16 tons' ha^{-1} globally, but some farmers achieve up to 100 tons' ha^{-1} by adopting good agricultural practices and control measures (Soto, 2018).

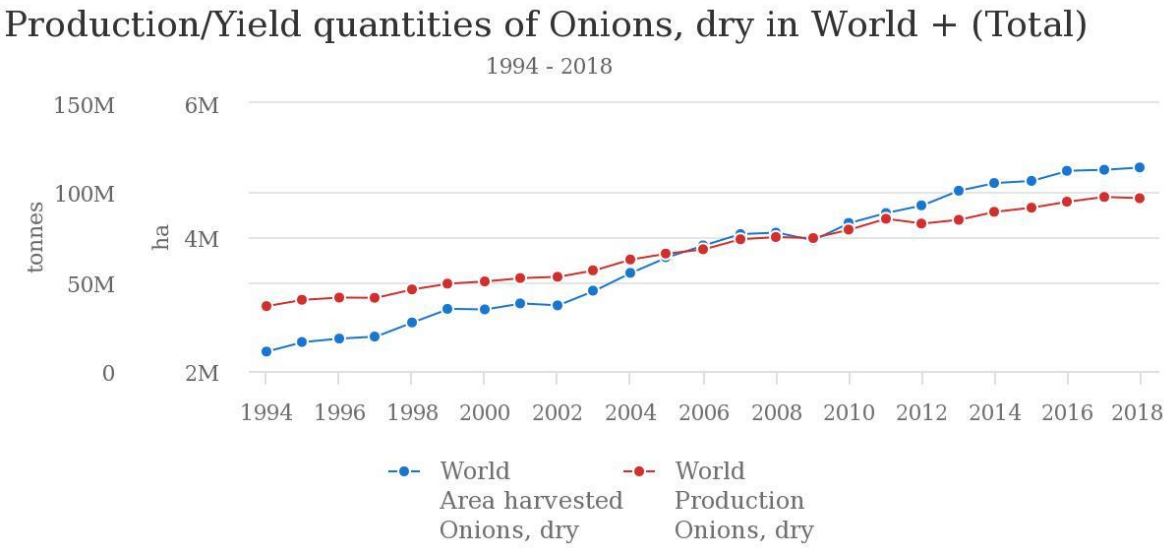

Figure 1.2. Onion production data for the last 20 years worldwide along with its production area (FAOSTAT, 2020)

Onion is the principal vegetable crop of Turkey holding 7th position in the world with a production of 2.0 million tons and covering an area of 105,000 ha (FAOSTAT, 2020). It is consumed second in number after tomato, mostly both as a raw or in salad. The uncooked bulbs are the chief source of flavoring. They vary in taste from sweet to mild flavored and pungent for making onion aroma as a condiment in almost every cuisine (Crowther et al., 2005). It is utilized as a condiment vegetable crop due to its unique aroma and taste. Onion consumption has numerous benefits including cardiovascular stability because of their anti-diabetic, anti-hypertensive, hyper-cholesterol, and antithrombotic effects. They also possess biological activities like anti-microbial, anti-cancerous, anti-asthmatic, antimutagenic and prebiotic activity (Corzo-Martinez et al., 2007; Gökçe et al., 2010).

Onions are classified as long-day, short-day and day-neutral. Long-day onions initiate bulb formation with a day length of more than 14 h and night length of less than 10 h, while short-day onion starts bulb formation starts with a day length of less than 12 h and night length of more than 12 h (Garner and Allard,1920). Bulb formation is not only

associated with day length but also with temperature, and the adequate bulb formation also differs with latitude and environment. Onion is adapted to wide range of climatic conditions, but it grows best at mild climatic conditions which lacks extreme climate (Mubarak and Hamdan, 2018). It also requires sufficient moisture content in soil with a soil pH of 6.0-7.0 for good production. The suitable areas for onion growth are temperate and subtropical regions (Brewster, 2008).

Abiotic stress is considered as an external environmental factor that negatively effects plant growth and economic yield (Farooq et al., 2009). It includes fluctuations in temperature, drought, salt, and heavy metals stress conditions (Mittler, 2006). Out of these stresses, drought and salt stress are the most common ones with moderate to severe threats in various countries of the world (Pitman and Läuchli, 2002; Hirayama and Shinozaki, 2010). Climate change is affecting rainfall patterns especially over arid regions which are under severe threat of drought stress (Lobell et al., 2011). Drought and salt stress are main problems which results in 70% yield reduction of staple food crops (Vorasoot et al., 2003; Ahmad et al., 2012). Drought is classified as agricultural, metrological, and hydrological, Agricultural drought includes the lower availability of moisture for plant growth with a least humidity mainly due to higher temperature (Khattak et al., 2019). Plants suffer from drought stress when soil moisture availability decreases as soil factors are also responsible because of lower water holding capacity (Anjum et al., 2011). It is obvious that salt stress problem has been existed long ago before humans and agriculture; however, poor quality irrigation water has worsened this situation (Zhu, 2001). Poor quality irrigation water deposits salt contents in soil which continues to increase gradually and cause salt stress. The higher evaporation rate with an increase in temperature causes these salt contents to accumulate at the upper surface of soil (Murtaza et al., 2017). High salt concentration interferes with normal functioning of plant which absorbs lesser amount of water and higher salt from roots (Horneck et al., 2007), thereby drought and salt stresses are interlinked with each other. Onion is sensitive to EC value of even lower as 1.2 dSm^{-1} (Koriem et al., 1994). It is believed that if they will leave uncontrolled, they may cause severe salinization in more than 50% of the arable lands by the year 2050 (Wang et al., 2001). Furthermore, it is predicted that abiotic stress conditions will become even worse with passage of time in the coming future due to global climate change (IPCC, 2014).

The mounting population pressure and increasing intensity of salt and drought stresses due to climate change is decreasing cultivable lands and available water sources. These stresses need to be addressed to ensure food security. Researchers are struggling to circumvent problems of abiotic stress for plant and unravel defense mechanisms for tolerance (Chaves et al., 2003; Maggio et al., 2006). Although there are many works in literature summarizing responses of agricultural crops to these two most important abiotic stresses, data for onion appears to be quite limited (Wakchaure et al., 2018; Rady et al., 2018). That is, there is a lack of knowledge that needs to be completed by observing the response of onion to salt and drought stress in different aspects.

Onion suffers from yield losses due to inadequate availability of moisture resulting in moderate to severe drought stress conditions across the Turkey (Sönmez et al., 2005), Central Anatolia is suffering from drought problem because it is a semiarid and driest region (Bagci et al., 2007). Salt stress also arises due to low rainfall in Anatolia region (Kaya et al., 2003) and other regions of Turkey (Ardahanlioglu et al., 2003, Kendirli et al., 2005, Cemek et al., 2007; Bilgili et al., 2011).

Salt and drought stress adversely affect the growth of the plant with a several modifications at physiological and biochemical level (Aprile et al., 2013). Numerous studies have been reported revealing the stress response of other important vegetables (Yang et al., 2020; Huang et al., 2019). As under natural environmental conditions plants are generally under pressure of combined stress. Recently studies are even focusing on the combination of stress response to physiological changes in potato and tomato (Demirel et al., 2020; Raja et al., 2020). Although in onion the least importance was given to observe its behavior towards physiological and biochemical changes even with the imposition of single stress as literature shows. For example, a search done on 1st August 2020, in the database with the keywords "physiological or biochemical response of onion to abiotic stresses" yielded couple of studies reported by Hanci and Cebeci (2015), Hanci et al., (2015), Semida, (2016), Ghodke et al., (2018) Rady et al., (2018), Semida et al., (2017). It is extremely necessary to understand the vital mechanism for physiological and biochemical changes in onion that take place during stress.

Roots provide anchorage, access to soil water, and essential nutrients required throughout the growth stages. Its morphological characteristics play an important role in growth and

production of plants (Ghosh and Xu, 2014). During stress, the plant roots are the first organ that sense adverse conditions such as moisture deficit or excessive salt in the soil. The study of root system is of great interest as the structure and distribution of roots assist in higher water uptake. It also maintains productivity of plant under salt and drought stress conditions (Comas et al., 2013). By considering the importance of roots, studies have been reported about the root response under stress conditions in garlic, potato, tomato, eggplant, and pea (Akinci et al., 2004; Al-Safadi and Faoury 2004; Karni et al., 2010, Khenifi et al., 2011; Pereira et al., 2020), but not even a single study has been reported on onion's root response to abiotic stress conditions. Moreover, onion has a shallow root system, its roots can only penetrate up to 0.76 m. Generally, its roots lie at a depth of 0.18 m in soil and fewer reports of its root penetration at a depth of 0.31 m was found. The irrigation water below 0.76 m is not available for onion root. These root characteristics of onion make it more prone to drought and salt stresses (Drinkwater and Janes, 1955).

In ancient times, classical plant breeding approaches were commonly used methods for crop improvement, but it requires a long period to make the plant tolerant against abiotic stress. With the introduction of modern genetic tools, crops can be improved, screened to better adapt to stress conditions. Thereby a term "speed breeding" is used. However, in terms of onion genomics, the information about onion genome is scarce as compared to other vegetables. For instance, tomato genome is fully sequenced and numerous studies have been reported about tomato response to salt and drought stress (Zhou et al., 2019). Contrarily limited transcriptomic studies have been reported in onion especially in response to abiotic stresses. It is probably because onion has complicated and extensively large genome size of 15.9 Gb, it is characterized by high heterozygosity, outcrossing nature, high level of repetitive DNA. These factors are major hurdles for identification of genes responsible for abiotic stress tolerance (Finkers et al., 2015). Onion sequence information is valuable to identify genes responsible for important traits such as salt and drought tolerance. Additionally, molecular mechanisms related to drought and salt tolerance have been extensively reported in monocot crops especially such as Brachypodium, rice, maize, wheat, and barley (Rossatto et al., 2017; Verelst et al., 2013; Ozturk et al., 2001; Jiang et al., 2012). However, a limited knowledge is available about onion behavior to endure stress conditions and no study has been reported about transcriptional response of onion. The unfloding of onion knowledge at molecular level is essential in studying the underlying mechanism for stress tolerance. The availability of

such information also leads to novel innovations which will be helping hand to speed up abiotic stress breeding of onion.

1.1 Aims and Objectives

The main aim of this thesis is to compare the response of onion cultivars against drought and salt stresses in physiological, biochemical and gene expression levels.
To achieve this objective, the following approaches were used.

1. Morphological changes in onion with the imposition of salt and drought stress.
2. Evaluation of physiological changes in onion cultivars against stress conditions.
3. Investigation of the effects of drought and salt stress on biochemical changes in onion leaves.
4. Determination of effects of salt and drought stress on response of onion roots.
5. Evaluation of the antioxidant defense machinery (SOD, CAT, APX) in selected onion cultivars.
6. Amplification of PSII and CAT gene from onion by exploiting degenerate PCR.
7. Measurement of gene expression levels of PSII and CAT gene against salt and drought stress.
8. Generating knowledge for the screening of onion cultivars to stress conditions.
9. Grouping of the selected onion cultivars as a tolerant and susceptible to salt and drought stress providing potent base for future abiotic stress breeding of onion.

CHAPTER II

LITERATURE REVIEW

2.1 Overview and Significance of Onion (*Allium cepa* L.)

Onion is a monocotyledonous vegetable belonging to genus Allium and family Amaryillidaceae. Onion has a vast history of its cultivation dating back to 3000 BC. It was originated from central Asia. Allium genus (x=8) has more than 700 species. *Allium cepa* L., is common bulb-type onion, which is commercially important member of its family. Several edible species, closely related to *A. cepa*, include *A. sativum* L. (garlic), *A. fistulosum* L. (Welsh onion), *A. ampeloprasum* L. (Leek), *A. tuberosum* L. (Chinese chives) and *A. schoenoprasum* L. (chives). Onion is either biannual or perennial depending on the region and cultivation conditions. Almost all the species of Allium are native to the northern hemisphere of world and first domesticated in the mountainous areas of Pakistan, Afghanistan, Tajikistan, and Iran (Brewster, 1994). Onion bulbs have been categorized depending on their bulb size (small, medium, and large), shape (globular, flattened and round), skin color (white, yellowish, silvery, buff, bronze, purple, violet or red) and fleshy scale color (white to bluish red) (Rabinowitch and Currah, 2002). Cultivated onions are grown and eaten in many regions of the world. The tangy onions seem to receive much acclaim worldwide as it is the second most cultivated vegetable in the world with a total production of 97.8 million tons in 2017. China is leading onion producer with 17.8 million tons followed by India 11.4 million tons and United States of America 3.3 million tons. Turkey ranks fourth among top onion producing countries in the world. The production of onion in Turkey was reported 2.0 million tons. Pakistan stands sixth in the world with total production of 1.57 million tons. According to FAOSTAT, (2020), the continent Asia contributes higher production of onion in the world (Figure 2.1).

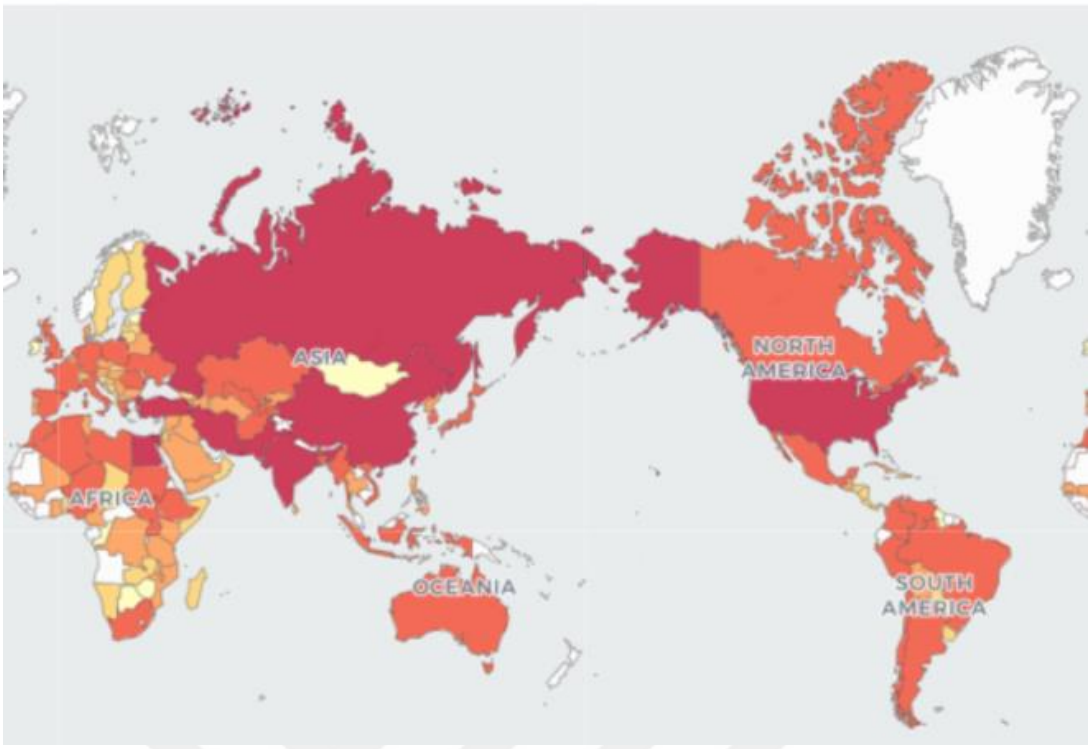

Figure 2.1. Production of onion in different continents of the world

Onion is consumed both as green leafy state and dry bulbs. Onion has distinctive flavor to be used in salad, sandwiches, soup, and dishes. Moreover, it is also cooked as a vegetable in some parts of the world. Onion bulbs pungency is praised globally thereby it is used in almost every dish as a condiment globally. Its pungency is due to the presence of volatile oil (allypropyl disulfide) (Ahmed, 1994). There is no limit to its use in diet as an essential condiment and vegetable by any nationality. Thereby its demands remain high all the year.

The word “onion” is derived from Latin which means “large pearl”. It is not because of its similarity of shape with pearl but due its highly nutritious value and medicinal quality (El-Samad et al., 2011). Onion is an indispensable item of human’s diet due to its nutrition and flavoring qualities. Fresh onion bulb contains approximately 86.6% moisture, soluble sugar 6-9%, carbohydrates 11.6%, protein 1.2%, fat 0.1%, Ca 0.2-0.5%, P 0.05%, traces of Mn, Zn, Fe, Cu, Al and vitamins A, B, C (Jilani et al., 2010). Onion’s nutritional value helps in alkaline reaction of human body and vital element for neutralizing acid by products formed during digestion of cheese, meat, and other related foods (Yousuf et al., 2013).

2.2 Introduction to Major Abiotic Stresses

Plants as being sessile grow under natural environmental conditions where so many factors are involved for their nurturing. So, any deviation from their required growth conditions and at different growth stages exerts pressure on them. Plants as being immutable suffer from multifarious environmental adversities in field (Zlatev and Lidon, 2012). Various abiotic stress factors are involved for curtailing growth of plants. Abiotic stresses are environmental adversities that negatively influence the plant growth and cellular functioning's. Abiotic stresses are the major hurdles in sustainable agriculture development. The prime climatic pressure that are under thorough investigation in context of its effect on plants are drought (deficient moisture with dry wind conditions), salinity (excessive salt concentrations), soil contamination (heavy metals stress) and extreme temperature (heat and cold stress). Intensity of these adversities has been triggered mainly due to anthropogenic activities and poor management practices. Severity of these stresses is lethal for plant. Currently it is the main challenge for maintaining plant growth and crop productivity under such stress scenario for sustainable agriculture (Mittler, 2006). All these environmental factors alone or in combination disrupt plants functioning. Abiotic stresses are chief cause of deprived yield and crop failure in some regions around the globe. Abiotic stresses reduce yield of many major crops up to 50% (Bray et al., 2000). To cope with these environmental stresses, the insights into plant acclimation to such stresses has been observed for considering morphological, physiological, biochemical, and molecular approaches (Anjum et al, 2011; Gürel et al, 2016). Some plants could survive during short period of abiotic stresses. Plants most often encounter harsh climatic changes. Plant varies in their response to harsh climatic conditions, as one environment is harmful for one species while it is not harmful for other plant species (Munns and Tester, 2008). This was elaborated that every plant species has multitude of mechanism against stress response. Plants have inherent buffering capacity to alleviate period of abiotic stresses. The two main strategies adopted by plants to cope the negative influence of stress are stress avoidance and tolerance. Avoidance is the plants shifts themselves to protective strategy by delaying the influence of stress incurred by plants. Contrarily stress tolerance is the inherited plant potential to acclimate to stressed conditions (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). General effect of major abiotic stresses on plant growth is illustrated in Figure 2.2. The abiotic stress is increasing rapidly; likely mounting

population pressure is a great challenge to agricultural production to ensure 70% more food for 2.3 billion people by the year 2050 globally.

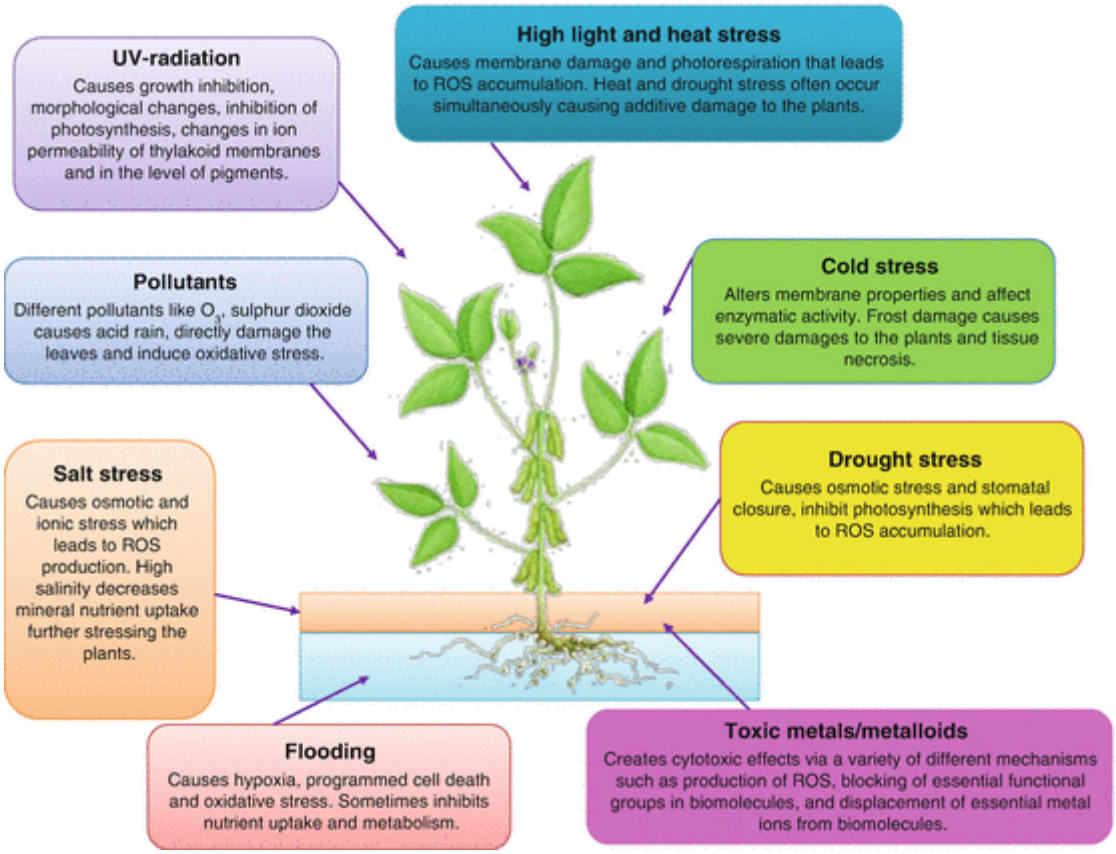

Figure 2.2. General effect of all the prevailing major abiotic stresses to plant (Hasanuzzaman et al., 2017)

2.2.1 Impact of drought stress on crops

Water is an essential component of life. It is required by humans, animals, and plants. Its consumption is increasing due to the increasing population globally, and its resources are becoming limited. Increased usage of water resources is depleting year by year and many agricultural lands are becoming vulnerable to drought stress (Mahmood et al., 2020). Limited availability or non-availability of moisture for plants during a short period of time or prolonged time span is coined as drought stress. As we know that water is the integral part for plants life from seedling emergence to final harvesting of crop. However, the requirement of water varies among plant species. Drought can be characterized into three categories (agricultural, metrological, and hydrological drought). Agricultural drought is a condition of low soil moisture and air humidity with the raise in high

temperature (Shahzad et al., 2016). Drought problem is increasing at rapid rate due to several issues. Drought problem is increasing at a rapid rate globally, drought affected areas have been doubled in last 3 decades (Liu et al., 2019).

Arid areas are more prone to drought stress as they are totally dependent on rainfall for crops growth. Drought arises due to insufficient rainfall events or least moisture availability in soil mostly in arid regions (Khattak et al., 2019). Drought is the most devastating stress for plants among other abiotic stresses. Currently it covers an area of 1-3 % and it is expected to increase up to 30 % by 2090 (Kogan et al., 2016). Global food production is severely influenced by drought stress. Among all the stresses that affect crop yield and hinder its growth, most alarming is drought, it is also most resistant to breeders' efforts (Tuberosa and Salvi, 2006). Plant faces drought stress when moisture availability to the root becomes restricted, transpiration rate decreased at a rapid rate. It limits plant growth and resultantly lower yield of crops (Aksoy et al., 2015). Drought is a versatile stress influencing plants at every growth stage. There are three major strategies that plant uses against water deficit conditions: "escape", it allows plants to complete its growth cycle before intense deficiency of water, this process mainly occurs in short cycle crops. Secondly, "avoidance" is a strategy in which a plant decreases transpiration and enhance water absorption. Lastly, "tolerance" is a mechanism which is the ability of a plant to tolerate a stress without altering its growth stage (Tardieu, 2005). To find out adequate process and to adapt against drought, we must know about the plant behavior under drought and this is not easy as plant responses are complex when it faces drought (Jones, 2004). Drought tolerance can be described as the crops ability to produce constant yield, under environmental adversity (Cattivelli et al., 2002). Drought is a serious menace to crop productivity. However, at molecular level these conditions are more complex under drought stress; it is a polygenic quantitative trait (McWilliam, 1989).

2.2.2 Salt stress: Saline soils and threat to crops

Agricultural soils are affected with salt stress due to climate change and various anthropogenic factors such as excessive application of fertilizers, improper application or poor quality of irrigation and drainage. All these factors contribute to excessive accumulation of salts in soil root zones. Saline soil has following characteristics having

electrical conductivity of 4 dS m^{-1} and 15% exchangeable sodium percentage that causes salt stress for crop (Murtaza et al., 2006; Shahzad et al., 2017). The soils having such characteristics reported to reduce the growth of crops up to 20% (Munns and Tester, 2008). Farmers are unaware of long-time losses of soil in the form of salinity that is increasing gradually and difficult to reclaim (Shahbaz and Ashraf, 2013). Soil salinity is major pressure for decreased productivity of crops (Haque, 2006). Natural resources are becoming limited day by day to population pressure and higher demand of food. Globally almost all the countries are suffering from this issue and it covers the 3.1 % (800 million hectares) of the total lands of the world. Soil salinity damages crop production with a severe loss from million hectares and billions of dollars yearly (Munns and Tester, 2008; Shabala and Cuin, 2008). Moreover, it is predicted that it will increase in number by the year 2050 (Ashraf and Akram, 2009).

Natural salinity is caused by weathering of rocks which releases various kinds of soluble salts i.e., chlorides of carbonates, sulfates, magnesium, calcium, and sodium. Among all sodium chloride is released in an abundant quantity. Ocean water is also rich in salt which carried by winds and rainwater to soil and becomes part of it for an infinite period. Rainwater alone contributes to deposit sodium chloride (6-50 mg kg^{-1}) in the coastal regions. Secondary salinity includes clearance of agricultural lands, rise in water table that increases the concentration of salt around the roots of plant (Kaushal et al., 2017). Agricultural lands are under the pressure of salinity due to brackish water irrigation. The other main cause in coastal regions is encroachment of sea water and its percolation deep into the soil layers. Higher concentration of salts resulted in declined growth; however, magnitude of yield losses is difficult to predict as salinity is non-uniformly dispersed in given area and at a depth. Salinity stress is one of the most predominant abiotic stresses resulting in heavy losses of crop production. It is an alarming situation for the future crop production under salinity stress scenario. Therefore, it is essential to understand the influence of salinity stress on onion plant growth to select best cultivars tolerance for future harsh conditions.

The water used for irrigation contains sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}). During hot weather water evaporation increases which often precipitates Ca^{2+} and Mg^{2+} into carbonates and Na^+ dominate the soil. Resultantly Na^+ concentration increases as compared with other essential macronutrients. Enhanced Na^+ concentration in soil

inhibits the activity of nutrient ions with an extreme production of Na^+/K^+ or Na^+/Ca^{2+} ratios (Tang et al., 2019).

Excessive accumulation of soluble salts in soil pockets interrupts the water potential with a severe reduction of water availability for roots to uptake by plants. In this way water availability becomes restricted and creates moisture stress as well. Salinity stress is more detrimental for plants as it triggers drought stress conditions with the least absorption by the roots. Sodium ions absorbed by the roots is transported to the transpiration stream of shoots and ultimately ends up its journey by depositing in leaf tissues (Hasegawa et al., 2000; Munns and Tester, 2008).

Plants are categorized in to two major groups based on their adaptation against salinity conditions. First one is halophytes that grow normally under saline conditions second group includes glycophyte that lacks the ability to prevail saline environment leading to death of plant under severe stress conditions. The major cultivable crops fall in the second group "glycophytes". The glycophytes plants include onion, maize, rice, beans, and lettuces which are sensitive to salinity stress. Wheat is moderately salt tolerant, while barley is the most salt tolerant monocot plant. Although rice which is the model plant for monocot crops is highly sensitive to salinity stress, even at the EC of 4.0 dSm^{-1} (Munns and Tester, 2008). Thereby salinity is the major hurdle for plants growth which is a serious threat to salt sensitive crop productivity and eventually global food security. Soil salinity disrupts the functioning of plant by two ways. High salt concentration in soil makes it difficult for roots to absorb water, likely high Na^+ accumulation in plant tissues creates toxic environment (Horie et al., 2012).

Salt stress interrupts the processes of plant. Salinity stress causes to increased respiration rate of plant and disturbed mineral ion distribution i.e displacement of calcium and potassium with Na^+ ion, ultimately ion toxicity. Salt tolerance of plant is termed as the plants able to survive during stress period, although slower development confers harvestable yield. Some crop species are susceptible to salinity, so the effect on its final crop yield depends on plant species, its duration to exposure and crop developmental stages (Kinraide, 1999).

2.3 Drought Stress Effect on Plant Morphology

Drought stress influences seed germination and seedling emergence. Poor seedlings stand and non-uniformity of seedlings are the obvious symptoms of drought stress (Farooq et al., 2009). Drought stress imparted negative influence on seedlings emergence of major monocot crops such as rice (Pirdashti et al., 2003), maize (Queiroz et al, 2019), wheat (Abid et al, 2018) and barley (Abdel-Ghani et al, 2015). Poor seedling establishment leads to weakened morphological growth of plant. Under normal conditions plant growth occurs by cell differentiation, division, and cell enlargement. Drought damages mitotic division likewise elongation of cells and eventually growth retardation. Loss of turgor pressure is mainly due to water deficit conditions which restricts cell growth (Taiz and Zeiger, 2006). Least water availability/uptake by plants limits flow of water from xylem to its surrounding cells with a reduction in cellular growth (Nonami, 1998). Water stress significantly represses the expansion and growth of cell with a disturbed turgor pressure of plant. Decreased cellular growth declines the size and number of leaves with the onset of drought stress conditions. The obvious symptom of water deficiency on plant is the reduction plant leaf area. As the water contents decline, cell volume decreases due to shrinkage of cell. Drought stress not merely effect the leaf area, but it also causes reduction in number of leaves, branches, and results in stunted growth (Taiz and Zeiger, 1998).

Maize growth was reported to be significantly reduced with a stunted plant height, decreased leaf size, and reduced stem girth under limited water growth conditions (Anjum et al., 2016). Onion plant height and stem girth showed a drastic decrease under drought stress conditions (Wakchaure et al., 2018). In another study, similar results of growth reduction were reported in onion with moisture stress (Hanci and Cebeci, 2015; Zheng et al., 2013). Among the monocot crops, rice is the most sensitive to drought as it is a submerged crop with an influence on its morphological growth (Ullah et al., 2018). Wheat and maize are also sensitive to drought stress conditions regarding their growth. Studies conducted on dicots to explore their morphological response also revealed a negative influence on soybean with a higher decrease in stem girth (Specht et al., 2001). The stem length of potato was also observed to be reduced due to drought stress. The main reason behind the reduction in morphological characteristics is a disruption of cell enlargement and greater leaf senescence with drought stress (Chang et al., 2018).

2.4 Salt Stress Effect on Plant Morphology

Salinity is the excessive accumulation of soluble salts around the vicinity of plant roots. The higher salt concentration favors its higher uptake by plant roots to the aerial parts of the plant. It also diminishes the uptake of other essential nutrients vital for plant growth. In this way, it disturbs the metabolic activities, ion toxicity, and decreased cell division (Zörb et al., 2019). The plant vegetative growth becomes restricted with the uptake of salt. It has a great effect on almost every stage of plant development leading to the death of crops. The sensitivity of plants varies with the growth stage among different genotypes. As some of the crop species are sensitive to salinity during germination but tolerate salinity stress with the later growth stages (Safdar et al., 2019).

Globally, salt is considered the most toxic substance for rice growth. The morphological symptom of Na^+ accumulation results in leaf rolling and molting. Cl^- is also harmful as it causes burning symptoms on the edge of the leaf in rice. Onion is also sensitive to salinity especially, at the bulb formation stage. The typical response of a plant to mitigate salinity stress is the reduction of leaf area. A decreased number, of leaves and growth is the earliest plant response against salinity. Moreover, reduction in canopy area might be the inherent avoidance mechanism adopted by glycophytes. As it restricts the loss of water by transpiration and limits the movement of toxic ions from roots to shoots and eventually leaves. Saline conditions also change the properties of cell wall and turgor pressure. Salts accumulate in higher concentration in older leaves as compared to younger leaves, so less damage to young growing leaves occurs during salinity stress (Munns et al., 2005). Salinity stress resulted in growth reduction of important species belonging to the Allium genus i.e., onion, garlic, leek, chive (Rady et al., 2018; Astaneh et al., 2019; Kiremit et al., 2016; Arslan et al., 2018).

2.5 Abiotic Stress Influence on Root Morphology

Plant responses to salt and drought stress on the above-ground plant parts (i.e., number and diameter of leaves, plant height, leaf area, shoot length, etc) have been studied extensively over the decades (Franco et al., 2006; Chaves et al., 2011). However, few studies have explored the role of roots in response to stress conditions. The general response of a plant to abiotic stresses results in a reduction in plant growth and yield

which is due to the limited ability of plant roots to absorb water and failure to transport it to the shoots (Navarro et al., 2008). Despite the importance of roots to mitigate stressed conditions and favor sturdy growth, little work has been reported with no single study on onion regarding salt and drought stress response. Roots are the base of the plant that supports the plant and provides essential nutrients required throughout the growth stages. Roots might be vulnerable/tolerant to salt and drought stress depending upon the genotypic differences. Genotypes that are sensitive to such stresses show poor root growth leading to reduced plant growth ending up with yield losses (Vamerali et al., 2003). Decreased root length with the exposure to salt stress in peanut was due to toxic effects imparted on peanut roots growth, moreover, it also resulted in decreased water uptake by the plant (Aydinşakir et al., 2015). In potato root, morphological changes in response to drought stress exhibited drought hardening of the tolerant genotypes (Zhang et al., 2018). Numerous studies have shown that the study of root traits is an important trait for the screening of tolerant and sensitive genotypes (Zarzyńska et al., 2017; Zhang et al., 2019; Bian et al., 2017).

2.6 Abiotic Stress Influence on Physiological Changes

Excessive sodium toxicity around the roots makes it difficult to extract water and disturb its uptake by roots to shoots and leaves. Decreased absorption of water increases osmotic stress in the plant. It affects the water potential, solute potential, and turgor pressure in a plant cell. Salinity negatively influences water and osmotic potential in a plant while turgor pressure continues to increase. A Linear relationship has been observed with the increase in salt concentration to decrease in water potential (Khan et al., 2013). Salinity aggravates further two stresses in plants such as osmotic stress and ionic stress. Osmotic stress impedes plant water uptake, whereas ionic stress is the enormous accumulation Na^+ in the plants. Severe Na^+ toxicity resulted in necrosis and chlorosis of plant leaves (Horie et al., 2012).

Due to salinity stress, higher uptake of Na^+, and Cl^- interrupts physiological functioning. As Na^+ reduces the uptake of K^+ ions which disrupts stomatal activity resulting in detrimental water losses, whereas Cl^- ions damage the production of chlorophyll contents ending with chlorotic toxicity. It was reported that Cl^- is more deleterious to plants compared with Na^+ (Tavakkoli et al., 2011).

High sodium concentration replaces potassium and calcium in the cell likewise it causes dehydration which eventually inactivates enzyme activities and deleterious to metabolic processes of the plant. Osmotic pressure initiates plasmolysis above the cell causing shrinkage of protoplast and plasma membrane detaches from the cell wall. In this, it creates large gaps between the cell wall and plasma membrane and favors the movement of salts by the artificial formation of apoplast pathway. Saline water enters the transpiration stream and causes damages to the cells (Volkov and Beilby, 2017).

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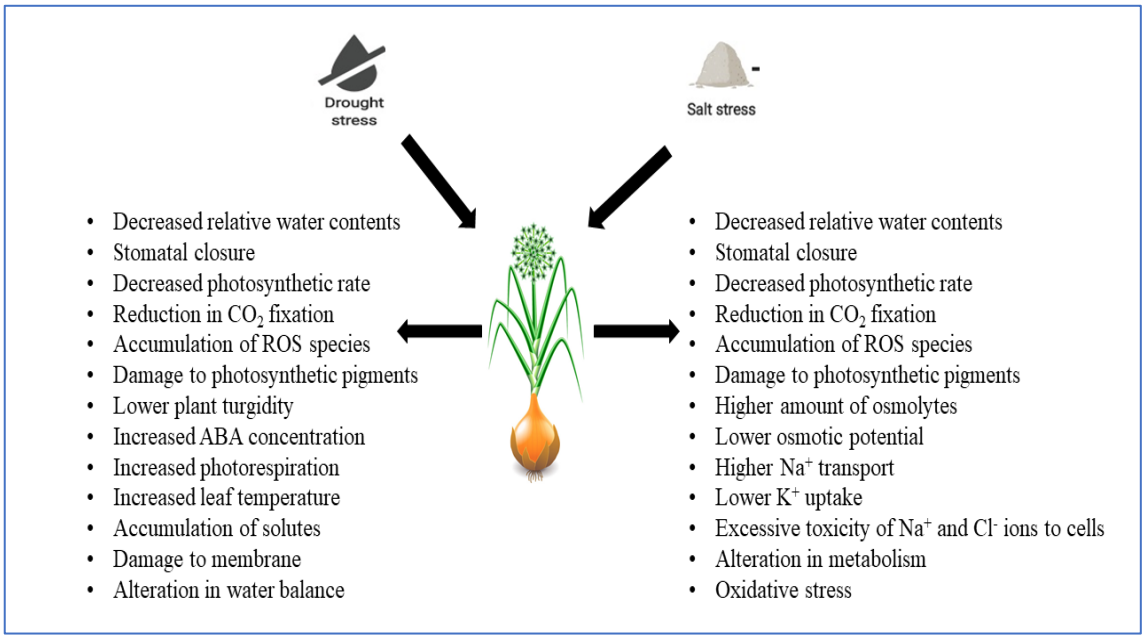


Figure 2.3. Drought and Salt stress effect on physiological changes in onion

2.7 Photosynthesis under Stress Conditions

Plants being autotrophic must produce their own food. For this purpose, photosynthesis is an essential to process to provide food for plants to function. It is the process in which the light energy is absorbed from the sun rays by the plants, converted into chemical energy, and stored in the bonds of organic compounds and sugars. Another important process that occurs during this process is the intake of carbon dioxide, synthesis of carbohydrates from water, and release of oxygen as a by-product to the environment. Photosynthesis takes place in chloroplast, a specialized organelle containing photosynthetic pigments. It is the chief metabolic process highly sensitive to salinity stress. It is extremely essential to normalize the flow of energy for optimized carbon fixation to avoid light-dependent injury. Photosynthesis comprised of light-dependent

and light independent fixation of carbon. A light-dependent photosynthetic electron transport regulates by converting photon energy to chemical energy. Photosynthetic electron transport is in the thylakoid membrane which is embedded in the chloroplast to conduct the light reaction. Photosynthesis is essential for plant growth which becomes restricted with the salinity stress. It decreases the intake of CO_2 via stomata and mesophyll ultimately disrupts the photosynthetic machinery. Excessive salts in the photosynthetic tissues cause shrinkage of thylakoid membrane. Sunlight is absorbed by different pigments present in the reaction centers and light-harvesting complexes. The main pigments in the light harvesting complexes are chlorophyll to capture light energy. Chlorophyll contents are the important components of photosynthesis as they absorb light energy and convert into chemical energy to be used for photosynthesis (Chaves et al., 2009; Ashraf and Harris, 2013).

2.7.1 Drought and salt stress effect on photosynthesis

Photosynthesis is the intricate physiological process that is affected by the short phase and long phase of drought and salinity stress to plants. As photosynthesis involves multifarious components which include photosystems, photosynthetic pigments, electron transports systems and CO_2 assimilation. Thereby abiotic stress at any stage causes a reduction in photosynthetic capacity. It is mainly affected due to limited stomatal activity with decreased CO_2 assimilation. It leads to prompt growth reduction even with the exposure of a few hours to drought and salt stresses (Ashraf and Harris, 2013).

Decreased stomatal conductance is beneficial for plants to minimize water loss and restrict toxic ions delivery to the roots of a plant. It has been highlighted that stomatal closure during the beginning of salt stress results in reduced in-flow of toxic ions to the transpiration stream. Water status of leaf interacts with stomatal conductance which deciphered a potent correlation for water potential of a leaf with stomatal activity under drought stress. Stomatal closing instantaneously limits the rate of photosynthesis. Numerous studies reported that short term exposure resulted in inhibited photosynthesis rate with stomatal closure of the plants while non-stomatal restrictions under long term exposure of salt (Bolandnazar et al., 2007; Shahbaz et al., 2013; Rahnama et al., 2010; Vysotskaya et al., 2010). The photosynthesis rate is also reduced due to the synthesis of rubisco. As it controls carbon assimilation during photosynthesis. Further, it was

supported with a decreased synthesis of rubisco enzyme under stressful environments (Salehi-Lisar and Bakhshayeshan-Agdam, 2016).

2.7.2 Role of photosystem II in photosynthesis

Photosystem II (PSII) is the first complex protein that works in the catalyzation of light-induced water oxidation in oxygenic photosynthesis. It is in the thylakoid membrane, a site that captures light energy for photosynthesis along photosynthetic pigments embedded in it. The light energy is absorbed by the PSII resulting in the formation of higher energy electrons that are transferred to photosystem I in the electron transport chain. It obtains substitute electrons from H_{2O} which split into H^+ and O_2 atoms. Later, H^+ atoms continues to move in the lumen, and O_2 is converted into molecular oxygen released into the atmosphere. The higher flow of H^+ in the photosynthetic membrane enables the plant to produce adenosine triphosphate (ATP). The remaining high-energy electrons are released by the absorption of light energy by photosystem I assist in the synthesis of nicotine adenine dinucleotide phosphate (NADPH). It takes substitute electrons from the electron transport chain. Finally, ATP gives energy and NADPH gives H^+ essential to drive photosynthetic reaction as Figure 2.3 shows. In the case of abiotic stress conditions, plant absorbs more light than required for photosynthetic carbon fixation. It leads to the impairment of photosynthetic machinery particularly PSII, resulting in photoinhibition (Takahashi and Murata, 2008). Photosystem II is relatively sensitive to salt and drought stress (Lu and Zhang 1999; Khan et al., 2017). Several studies have reported the alleviation of PSII photoinhibition improved the antioxidant activity in chloroplast with better photosynthesis under stress conditions (Sun et al., 2010; Hou et al., 2018; Huang et al., 2019).

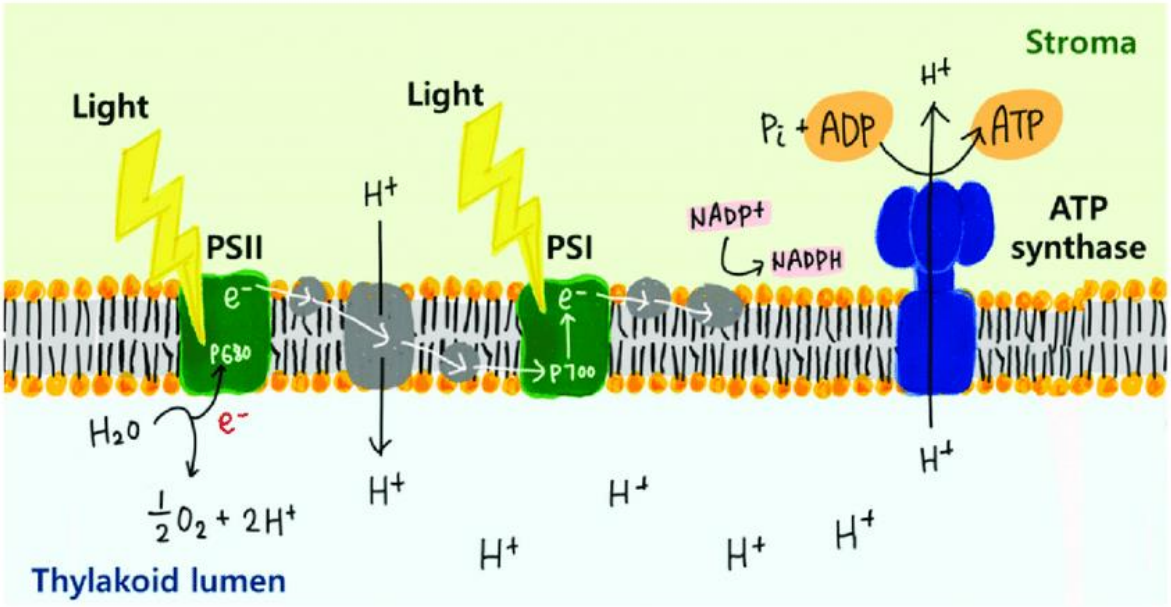


Figure 2.4. Schematic mechanism of the role of PSII in photosynthesis in plants (Ryu et al., 2019)

2.8 Synthesis of ABA under Salt and Drought stress

Abscisic acid is the fundamental plant tool for development and potent stress-responsive phytohormone to attenuate stress. Abscisic acid induces closure of stomata under water deficit conditions. It works as a signaling molecule after perceiving the initial stress phase to shift plants to water conservation strategy. Guard cells configure stomatal pores in the epidermis of the leaf. It allows the plant to maintain a balance of CO_2 intake for photosynthesis and restricts water loss by transpiration (Vishwakarma et al., 2017).

Synthesis of ABA starts in dry roots and it moves to the xylem. It has been observed that its concentration increased by 30-fold in drought-stressed plants. ABA reaches a higher concentration for causing an efflux of ions via guard cells to hinder stomatal opening. ABA initiates a high influx of Ca^{2+} ions and conversely lowers K^+ ions that favor stomatal closure. Moreover, malate and chloride anions are also synthesized by guard cells for closing the stomata (Outlaw, 2003).

Salinity aggravates the osmotic effect with a higher accumulation of abscisic acid (ABA) which also decreases stomatal conductance rate, the concentration of CO_2, chlorophyll

contents, and accumulation of sucrose with changes in electron transport. Stomatal signals are perceived by plants via roots with the accumulation/generation of ABA which is a chief chemical compound in this regard. Reduced stomatal conductance is the adaptive strategy by plants to cope with saline conditions as well as decreasing salt load to leaves (Koyro et al., 2006).

An increased amount of ABA has been observed in photosynthetic tissues of barley immediately after exposure to salinity conditions. It was also predicted that a rapid increase in ABA concentration is correlated to the in-situ ABA synthesis in photosynthetic tissues. Limited intake of water from roots perceives drought conditions and send signals for stomatal closure. The photosynthetic rate of salinity stressed plants was unchanged even with a reduction of stomatal conductance (Davies et al., 2005; James et al., 2002; Fricke et al., 2004).

2.9 Production of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are derivatives of oxygen which is a highly reactive compound. Independent oxygen radical elements comprised of singlet oxygen ($^1O2$), superoxide anions ($O2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$) (Mhamdi and Van Breusegem, 2018). A nonradical elements consisted of H_2O_2 and O_3. ROS are produced in different cell compartments of plants, generally the site of its production are chloroplast and mitochondria (Choudhury et al., 2017). ROS species are always generated due to the leaking of electrons of O_2 from electron transport regulating activities of chloroplast and mitochondria. They also formed because of numerous metabolic pathways located in mitochondria chloroplast and peroxisomes (Mittler, 2002; Blokhina and Fagerstedt, 2010).

In chloroplast thylakoid membrane harbors photosystem PSI and PSII which are the prime site of ROS production. Photoproduction of ROS is hugely influenced by environmental factors stimulating physiological changes. Moreover, the rate of its production elevates due to intense photon energy more than required in CO_2 fixation by plants (Asada, 2006). In drought stress conditions limited water and CO_2 availability likely severe light intensity excites electron transfer to molecular oxygen, initiating the generation of superoxide anion in PSI by Mehler's reaction. Singlet oxygen ($^1O2$) is

formed at the PSII by excitation of the triplet state of chlorophyll at the reaction center of P680 when electron transport chain is over-reduced (Asada, 2006).

Superoxide anion undergoes univalent reduction likewise protonation resulting in the generation of moderately reactive H_2O_2 ROS species. It is mainly produced in the electron transport chain in the chloroplast, endoplasmic reticulum, mitochondria, cell membrane, fatty acid oxidation, and photorespiration. H_2O_2 works as a double-edged sword because of its dual role. It is favorable at a lower concentration. Conversely, it imparts a devastating effect to plant cells at higher concentrations. It regulates signals for vital physiological processes such as senescence, photosynthesis, photorespiration, cell cycle, stomatal conductance, development, and growth (Hung et al., 2005; Khan et al., 2018).
It is a general perspective that ROS always plays a destructive role in plant tissues. Besides this, it is also beneficial but at a low concentration. Reactive oxygen species take part in the signaling cascade to regulate the activity of ionic channels and gene expression. It is observed that at least 1% O_2 is being converted to ROS from out of its total consumption of O_2 by a plant (Mano et al., 1987). Stomatal closure occurs with salinity stress which leads to a reduction in CO_2 availability for the plant. Consequently, a decline in carbon fixation makes chloroplast subjected to excitation energy and favors attenuation of photosynthetic electron transport resulting in excessive ROS production.

It is understood that high salinity triggers oxidative stress, whereas plants activate antioxidant defense mechanisms to mitigate oxidative stress. Damage to the plant caused by salinity varies with the severity of exposure and plant's genetic nature (sensitive). ROS damage the integrity of membranes, affects cellular homeostasis, disrupts numerous enzymatic activities, protein oxidation, lipid peroxidation. Moreover, damage to nucleic acid has been reported in pea plants (Bhattacharjee, 2019). Production of surplus ROS resulted in damages to nucleic acids, lipids, cell structures, and protein. Abiotic stress damage to DNA is called genotoxic stress. It might be due to deletion of bases, dimmers of pyrimidine, breaking of strands, cross-links, and modification of bases by oxidation or alkylation (Shahzad et al., 2019). $^{\cdot}OH$ ion is most harmful among other ROS species as it damages both pyrimidine and purine bases likewise the backbone of deoxyribose. DNA damages caused by ROS resulted in decreased protein synthesis. It also damages the proteins of photosynthetic machinery leading to weak development and growth of plants (Yang et al., 2017).

Excessive production of ROS is deleterious for plant tissues. Generally, under abiotic stress, its production increases and exerts intensifying pressure on plant cells. Thereby the role of ROS in their production and harmful effects is fundamental to decipher in response to salinity (Chan et al., 2016).

2.10 Role of Antioxidant Enzymes to Mitigate Drought and Salt Stress

The Plant faces oxidative damage with the exposure to salinity and drought that are generally relieved by the inherent ability of plant by producing excessive reactive oxygen species (Hussain et al., 2018). Antioxidant enzymes play a vital role in this regard to alleviate ROS. The main ROS enzymes are ascorbate peroxidase (APX), Superoxide dismutase (SOD), and catalase (CAT) (Hossain and Dietz, 2016). These enzymes also serve as an indicator of tolerance and sensitivity of cultivars (Ashraf and Harris, 2004).
Superoxide dismutase works as a first in the antioxidant system by catalyzing the dismutation of superoxide radical ($O2•−$) to H_2O_2 and O_2 in the cytosol. SODs are classified into three classes depending upon their prosthetic metals i.e., copper-zinc, iron, and manganese-containing SODs. In plants, the localization of SOD differs among plant species with stress conditions. Therefore, CuZn-SOD and FeSOD are present in chloroplasts (Bowler et al., 1994; Ogawa et al., 1995), while MnSOD is in mitochondria and different types of peroxisomes (Bowler et al., 1994; Corpas et al., 2006). So, in each plant genome, there is a high probability that the minimum of one copy of MnSOD may be found, and this enzyme aids in the protection of mitochondria against ROS injury in plants (Møller, 2001).

Ascorbate peroxidase is the main player of detoxifying hydrogen peroxide in plant chloroplast, mitochondria, cytosol, and peroxisomes during stress conditions. It utilizes ascorbic acid as an electron donor for the reduction of H_2O_2 to H_2O (Mittler et al, 2004; Shigeoka et al., 2002). Catalase also alleviates oxidative damages by converting H_2O_2 to H_2O (Bor et al., 2003; Demiral and Türkan, 2005). Plants can tolerate drought and salt stresses by maintaining antioxidant enzyme activity or increase in their levels to cope with oxidative stress caused due to environmental adversities in various plant species (Fu and Huang, 2001). Numerous studies have illustrated the correlation of antioxidant system against abiotic stress in monocots species (Caverzan et al., 2016; Zahedi et al., 2016; AbdElgawad et al., 2016; Kibria et al., 2017).

In oxidatively stressed plants, all the ROS scavengers work together to overcome salt stress for improved growth and development of a plant. Numerous organs of maize seedlings were observed for the activity of antioxidant enzymes under salinity stress. Catalase activity was elevated in roots and both mature and young leaves of maize. However, SOD and APX increased solely in root parts (AbdElgawad et al., 2016). Wheat salt-tolerant and susceptible cultivar's response to salinity for antioxidant enzyme revealed that tolerant cultivar had higher APX, CAT, and POD activity, whereas sensitive one had reduced antioxidant enzyme activity (Siddiqui et al., 2017). So increased antioxidant activity during salinity stress conquers oxidative stress and confers tolerance. Several plants have been studied for the activity of ROS scavenging enzymes in drought-stressed plants. Rice plants were studied to observe the response of antioxidant enzyme activity. Imposed drought stress stimulated enhanced enzymatic activity in rice. Rice seedlings exhibited better growth response by alleviating ROS species in tolerant rice cultivars (Guo et al., 2006). In another study on rice elucidated the defensive role of SOD in decreasing the intracellular level of ROS (Yang et al., 2014). For instance, above-mentioned studies suggested that enhanced antioxidant enzyme activity is beneficial to mitigate salinity toxicity in a plant. On the other, some studies highlighted the cause of the difference in enzymatic activities. It might be due to the differential expression of enzymatic genes and the extent of salinity (Guan et al., 2015; Cunha et al., 2016). In a previous report, it was noticed that enzymatic activity elevated after exposure to salt stress for the mitigation of ROS species. It also aided in increasing the tolerance level against abiotic stress in the dicot plant as well (Koca et al., 2007). It was reported from a study in cotton that tolerant cultivar exhibited a higher level of antioxidant enzymes (Ibrahim et al., 2017). Potato stressed plants produced a higher amount of H_2O_2 which generated oxidative stress. Antioxidant enzymes illustrated to alleviative the role of ROS to confer tolerance against stress (Fidalgo et al., 2004).

2.11 Lipid Peroxidation in Response to Stress Conditions

Decomposition of polyunsaturated fatty acids results in the formation of malondialdehyde. Lipid peroxidation triggers excessive ROS accumulation where malondialdehyde is the product formed. It depicts the damage caused to the plant due to abiotic stress. It also serves as a marker of oxidative damage caused to plants (Davey et al., 2005). Osmoregulation is the prime characteristic of stress resistance which increases

MDA contents. Lipid peroxidation reflects the damage to the cell membrane under stress (Wang et al., 2019). MDA also helps in a screening of the tolerant and susceptible cultivars. Rice tolerant cultivars showed a lower formation of MDA which means lower membrane damage under drought stress (Yang et al., 2014). However sensitive rice genotypes showed a marked increase in MDA contents (Farooq et al., 2009). Further studies on maize cultivars confirmed that higher MDA formation is correlated with a sensitivity of the plant while tolerant genotypes exhibited lower MDA accumulation (Shafiq et al., 2019). Salt stress also aggravated membrane damage as it was reported in wheat (Chen et al., 2019).

2.12 Role of Proline against Abiotic Stress Conditions

Proline synthesis in plants occurs by the glutamate and ornithine pathway. The glutamate pathway is mainly involved in an accumulation of proline under osmotic stress with the reduction of glutamate to glutamate-semialdehyde by the enzyme pyrroline-5-carboxylate synthetase (P5CS). This enzyme spontaneously converts P5CS to pyrroline-5-carboxylate (P5C). Later the reductase of P5C (P5CR) reduces it to proline. Generally, in plant species, two genes encode for P5CS while only one gene encodes for P5CR. Catabolism of proline occurs in mitochondria by consecutive action of proline oxygenase or proline dehydrogenase (POX or PDH) (Hu et al., 1992; Savouré et al., 1995). Proline is also synthesized by an alternative ornithine pathway transmitted firstly by ornithine-delta-aminotransferase producing glutamate-semialdehyde and pyrroline-5-carboxylate which is ultimately converted to proline (Delauney et al., 1993).

Proline is known to be proteinogenic amino acid necessary for metabolism. The first report of proline accumulation was in the wilting of rye perennial grass (Kemble and Macpherson, 1954). Proline works in osmoregulation of plants which assists plant cells to maintain higher water contents. In this way, it is helpful for a plant in alleviating oxidative stress and is known to be a powerful ROS scavenger (Szabados and Savouré, 2010). Moreover, it also regulates the redox status of a cell. Proline accumulation under stress is a positive signal of plant stress tolerance (Verslues et al., 2014). Proline is static osmolytes for the protection of cellular, subcellular, and macromolecules against osmotic stress. Its accumulation influences stress tolerance in numerous ways. Its imperative functions are to work as a molecular chaperone capable of protecting the integrity of the

protein. Likewise, it improves enzymatic activities such as avoidance of aggregation of protein and maintenance of M4 lactate dehydrogenase (Rajendrakumar et al., 1994). Various studies have documented the higher accumulation of proline in response to different environmental adversities in plants. It has been reported to accumulate with drought and salinity stress as well (Zegaoui et al., 2017; Kibria et al., 2017). Proline also has a characteristic like an antioxidant enzyme as it quenches singlet oxygen and diminishes ROS activity (Matysik et al., 2002). Moreover, it aids in the removal of hydroxyl radicals in photosystem II of the thylakoid membrane (Saradhi and Mohanty, 1997). It serves as an alternative to ROS scavengers; it protects antioxidant enzymes and helps in the activation of substitutive pathways for detoxification. Proline enhanced the activity of detoxification enzyme (methylglyoxal) which increased the activity of superoxide dismutase, peroxidase, and catalase under salt stress in tobacco plant cells (Hoque et al., 2008, Islam et al., 2009). Proline not only have scavenging and protective feature, but it also further stabilizes ionic homeostasis under salinity stress. It protects chloroplast and photosynthetic pigments from the damage of salinity by an increased buildup of P5CS1 in the chloroplast (Székely et al., 2008; Rayapati et al., 1989). The protective role of proline for mitochondria was also deciphered. It protected the complex II of the mitochondrial electron transport chain under salt stress and stabilized the respiration of mitochondria (Hamilton and Heckathorn, 2001). High proline concentration rendered salt tolerance to halophyte. It stabilized enzymatic detoxification, improved protein machinery, and stimulated an increased amount of protective protein against stress conditions (Khedr et al., 2003). Higher proline accumulation helps plant in osmotic adjustment, assist in the alleviation of free radicals and maintenance of cellular redox potential (Ashraf and Foolad, 2007). A higher concentration of proline is correlated with a tolerance of cultivar, as tolerant cultivar depicts higher accumulation of proline in response to stress (Hayat et al., 2012). Wheat genotypes were categorized as tolerant and susceptible based on proline accumulation in wheat. Tolerant cultivars showed a higher level of proline under drought stress (Johari-Pireivatlou, 2010). Moreover, exogenously applied proline also results in drought tolerance in wheat (Farooq et al., 2017). Maize plants also showed the same response towards proline accumulation under drought stress (Moussa and Abdel-Aziz, 2008). Onion plants documented for the higher synthesis of proline except for the sensitive onion cultivars under both drought and salt stress conditions (Hanci and Cebeci, 2015). Proline sinks in every potato organ in response to drought stress. It served as the pool of energy for regulating redox potential in potato,

simultaneously it helped in osmotic adjustment to overcome osmotic stress (Farhad et al., 2011).

2.13 Stress Inducible Genes under Drought and Salt Stress

Plant response to abiotic stress is complex which involves a various set of genes with changes in the mechanism of biochemical and molecular responses. Analysis of functions of the stress-inducible genes provides a potent tool to unravel the molecular mechanism of plants for stress tolerance. It also provides insight into genes involved to manipulate them to render stress tolerance. Generally, the perception is that hundreds of genes are involved in abiotic stress tolerance (Seki et al., 2003). These genes regulate gene expression in response to abiotic stress conditions. Initially, the transcriptional response is comprised of a set of multiple genes later as the stress progress these stress-responsive genes become specific. Some of the genes that are induced by drought and salt stress are induced by cold and heat stress, so it suggested a similarity of the existence of genes mechanism to respond against stress (Ma and Bohnert, 2007).

These genes are categorized into three different groups; the first one is genes involved for direct protection of plant cell i.e., LEA proteins, chaperones, osmoprotectants, free radical scavengers, and detoxification enzyme (Wang et al., 2003). The second group includes transcriptional control and signaling cascades i.e., transcriptional factors, calcium-dependent protein kinase (CDPK), mitogen-activated protein kinase (MAPK), SOS kinase, and phospholipases (Ludwig et al., 2004; Zhu et al., 2001; Frank et al., 2000; Shinozaki et al., 2000). The third group includes ion transporters and aquaporins responsible for ion, water uptake and transport in plants (Blumwald et al., 2000).

2.14 Stress Signaling and Transcription Factors

Abiotic stresses negatively influence plant functioning. In response to adverse conditions, plants regulate a network of signals cascade to initiate nuclear gene. Plant exhibit specific gene expression to combat stress conditions as shown in Figure 2.5. It was assumed previously that plant cells can sense numerous stress signals. Despite several efforts, only a few reports of putative sensors have been documented. Activated nuclear genes triggered the expression of various genes. The first stress signal is perceived by the

receptors at the membrane level. The main receptors include G-protein-coupled, histidine kinase, and ionic channels. It results in the initiation of secondary signal molecules i.e., inositol phosphate, Ca^{2+} channels, ROS formation, and synthesis of abscisic acid. Abiotic stress signal cascade is well established whereas it is extremely complex to understand all the cross-talks. The simple reason explained for all this is that abiotic stress signaling is a complex trait controlled by various genes. However, in the last two decades, studies elaborated the role of single gene expression or multiple gene expression, enabled genome-wide expression strategy to understand complex traits (Zhu, 2016).

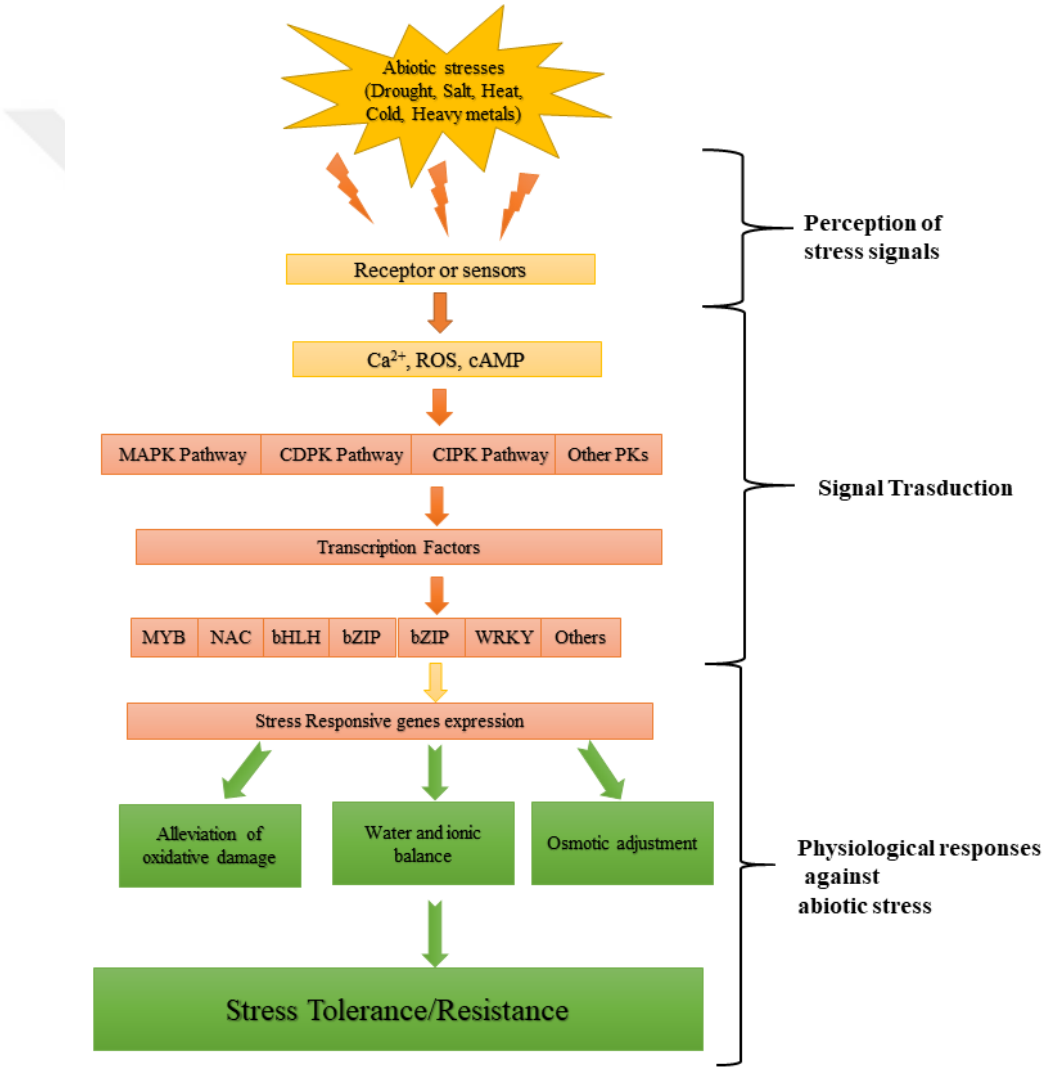


Figure 2.5. Transcription factors modulating stress signals and regulating physiological changes to mitigate the harmful effects of abiotic stresses

Despite various signaling pathways in response to abiotic stress, the mitogen-activated protein kinase (MAPK) signaling cascade is a major pathway. It links external stimulus

with endogenous cellular responses. MAPK are a conserved module for signaling in eukaryotes. It plays a core role in the transduction of various type of signal. Arabidopsis genome showed approximately 10 MAPKKs, 20 MAPKs, and 80 MAPKKs (de Zelicourt et al., 2016, Saijo and Loo, 2020). In nucleus, the stress signal transduces stress-responsive genes for stress adaptation. There are two types of stress-responsive genes. One group of a gene is known as early responsive genes that activate immediately within minutes after perceiving transient stress. Other group includes delayed genes that activate in the long run and with the severity of the stress. Multifarious abiotic stress-associated genes, regulatory sequences, and transcription factors in plant promoter regions have been identified and studied. Transcription factors are proteins known to be prime regulators for controlling the expression of a gene. Transcription factors responsive to abiotic stresses include WRKY, bZIP, NAC, AP2, MYB, bHLH, and DREB. These transcription factor genes can control the expression of target genes by binding to cis-acting elements in the promoter region. In this way, they are upregulating the gene expression of the candidate genes. Transcription factors follow an ABA-independent and ABA-dependent signal pathway. Both these pathways play an extensive role in controlling gene expression related to abiotic stress (Xiong et al., 1999). The expression level of the bZIP gene was observed under long salinity stress duration. The expression of the gene was upregulated in salt susceptible wheat cultivars; conversely, its expression decreased in salt-tolerant cultivar (Agarwal et al., 2019). The NAC transcription factor genes were overexpressed in wheat and rice. It mitigated the harmful effect of salinity and conferred salinity tolerance (Alshareef et al., 2019). Transcriptional regulators have been elucidated in rice to mitigate abiotic stress. The transcription factor ZFP179 and NAC5 showed upregulation in response to salinity. It might regulate production and accumulation of proline, LEA protein, and sugar which rendered stress tolerance (Song et al., 2011). Rice as being considered as a model plant has been explored to decipher the knowledge of transcription factors in response to abiotic stress conditions. Recently in rice SALT-RESPONSIVE ERF1 (SERF1), a transcription factor gene has been reported that revealed a root-specific induction upon salt and H_2O_2 treatment. Moreover, it was showed that SERF1 genes are responsive to H_2O_2 (Schmidt et al., 2013). The role of the NAC transcription factor depicted that guard cells induced under dehydration (Alshareef et al., 2019).

2.15 Role of Transporters in Salinity Stress

Salinity stress imposes ionic stress as well as water deficit conditions to plant cells. Due to higher sodium toxicity and a relatively higher concentration of solute in soil, it makes plants unable to absorb/uptake water as required for normal growth. Moreover, it disturbs ionic homeostasis of the plant resulting in ionic stress. Alteration in K+/Na+ ratio and elevated Na+ and Cl- ions creates hostile environment for plant cells. It is due to the higher influx of Na+ ions via same pathways that accumulate potassium. Another assumption is that it is hard for plant transporter protein to discriminate between K+ and Na+ because of the similarity of hydrated ionic radii. It is believed to be chiefly responsible for Na+ toxicity in plant. Furthermore, both Na+ and K+ are competitors for binding sites of K+. Plant cytosol is not capable of thriving at ahigh salt levels likely cytosolic enzymes are inefficient to cope with the influx of Na+ (Bernstein, 2019).

Glycophytes adopted a strategy against salinity with a low Na+ concentration in cytosol and higher K+ concentration. It maintains a higher K+ ion in the cytosol is sodium compartmentation and extrusion which is an active process. Na+/H+ antiporters facilitate Na+ compartmentation in vacuole and extrude from cell. These two are the important processes of Na+ detoxification from the cytosol. It also helps in osmotic adjustment essential for salinity tolerance. Though the mechanism of Na^+ entry in the plasma membrane is not evident it can be transported via K^+ carriers. Moreover, numerous selective pumps favor the uptake of K^+ compared to Na^+ Plant cells employ high and low-affinity K^+ transporters to uptake K^+ extracellular medium. Low-affinity K^+ transporters AKT1 which is inward channels responsible for the activation of an influx of K^+ at hyperpolarization of the plasma membrane. It is highly selective for external Na^+ and K^+ concentrations. Nonetheless, it could also facilitate higher Na^+ uptake due to excessive Na^+ concentration in soil with salinity stress. High-affinity K^+ transporter is HKT1 which activates at a minute range of external K^+. It is originally categorized as H^+/ K^+ and Na^+/ K^+ symporter. Under normal conditions when Na^+ is a normal range in the soil it stimulates K^+ transport (Schachtman et al., 1994; Nieves-Cordones et al., 2019; Ali et al., 2019).

High-affinity potassium transporter (HKT) protein was the first potassium selective transporter that play a selective role in K^+ uptake against Na^+ in numerous plants

(Schachtman and Schroeder, 1994). Its main role is the exclusion of Na^+ from plant leaves and assists in maintaining K^+ homeostasis. In wheat, $HKT_{2:1}$ was reported to confer salinity tolerance. The Na^+/H^+ transporter is found in the tonoplast that play role in the outward movement of Na+ from cytosol to apoplast or vacuole. However, it is energy-consuming process for cells and proton pumps give force for transporting Na^+ contrary to the electrochemical gradient (Blumwald et al., 2000). Its beneficial role was reported in cotton to confer tolerance in cotton by excluding higher Na^+ concentration and favors a higher influx of H^+ (Kong et al., 2012). Salt overly sensitive pathway (SOS) also works as an exchanger in the plasma membrane. Its activation under salinity stress also excludes higher Na^+ concentration to render salinity tolerance in plants (Xu et al., 2018; Yin et al., 2019).

2.16 Degenerate PCR Approach

Degenerate PCR is a technique used to identify a gene using the gene or amino acid sequence of a species in which there is no earlier genomic information reported or available (Telenius et al., 1992). The degenerate approach paved the path to solve this issue to create knowledge or identify new genes. Generally, polymerase chain reaction with a set of primers is used to amplify the region in the genome, but these primers is specific. Contrarily, degenerate PCR is coined due to the degenerate primers used in PCR rather than specific primers. The degenerate primers differ in having wobble with possible options for binding to the base as compared to specific primers. As, for instance, the 3rd nucleotide of the primer sequence harbors three possible nucleotide bases to anneal with template sequence. In this way, it provides flexibility for amplification. Moreover, it can also amplify the target sequence of all possible variants. Numerous successful studies with degenerate PCR have been reported. In grapevine calcium-dependent protein kinase gene expression was studied with the help of degenerate PCR in response to salinity stress (Dubrovina et al., 2013). In another study degenerate, PCR technique was used by Fernández-Ocaña et al. (2011) to study the function of superoxide dismutases (SODs) gene in sunflower. Salinity tolerance can be achieved with a higher influx of K^+ as mentioned earlier, so in one study NHX gene expression was observed by employing a degenerate PCR approach (Jha et al., 2011). Transcription factor WRKY is known to play a vital role in mitigating abiotic stress, thereby its gene expression was measured in tobacco in response to salinity stress by using degenerate PCR (Liu et al., 2013).

2.17 Expression Analysis of Genes in Response to Drought and Salt Stress

Knowledge about gene expression provides imperative information of gene functions in plant's life at every growth stage. Especially during stress conditions, it regulates a different set of genes which upregulates and downregulates. Various methods can be employed to measuring gene expression such as RNA sequencing, microarray, Northern blotting, semi-quantitative PCR, quantitative real-time PCR (qRT-PCR), and RNase protection analysis (VanGuilder et al., 2008). Microarray paved a path to study several gene expression simultaneously, however, contrarily it has several major limitations of this technique is quite costly. The other major challenge for this approach is the quantity and quality of RNA. Moreover, its other weaknesses include low precision, accuracy, specificity, and high sensitivity to setup experiments for variations in hybridization temperature. The gene expression by exploiting this technique is also affected by the purity of genetic material, its degradation, and amplification process (Blair et al., 2009; Draghici et al., 2006; Opitz et al., 2010). Alternatively, in the past three decades, qRT-PCR approach has been widely used for observing gene expression (Higuchi et al., 1992). It is utilized extensively even for low expression level studies because of its efficiency and precise quantification of target genes (Holland, 2002). It is a convenient and quick PCR approach that employs conventional RT-PCR with fluorescence resonance energy (Hu et al., 2014). Genes encoding enzymes required for antioxidative defense have been widely studied in several types of plants. Measurement of antioxidant enzyme gene expression of tomato under a combination of abiotic stresses was investigated with qRT-PCR (Martinez et al., 2018). Salt stress was imposed to investigate the gene expression of SOD, APX, and CAT in ryegrass by using qRT-PCR. Expression of levels of SOD, CAT, and APX enzymes were also observed under drought and salt stress in rice, wheat, maize, barley, Arabidopsis, tomato, potato, sweet potato (Shafi et al., 2017; Wang et al., 2005; Feng et al., 2016; Ueda et al., 2013; Jiang et al., 2019; Shiriga et al., 2014; Odat, 2018; Yan et al., 2016).

CHAPTER III

MATERIALS AND METHODS

The present research work focused on the response of selected onion cultivars against salt and drought stress. To observe the behaviour of onion under stress salt and drought conditions following materials and methods were used.

3.1 Experimental Materials

3.1.1 Plant material, growth conditions and sampling

Seven short-day onion cultivars (Table 3.1) were used in this greenhouse study collected from MTN Seed Co. Ltd, Bandirma. Plastic 10 L pots were filled with torf and perlite (3:1), twelve onion seeds were sown, and necessary management practices were taken to ensure proper growth (Photograph 3.1).

Table 3.1. List of short-day onion cultivars used in this study

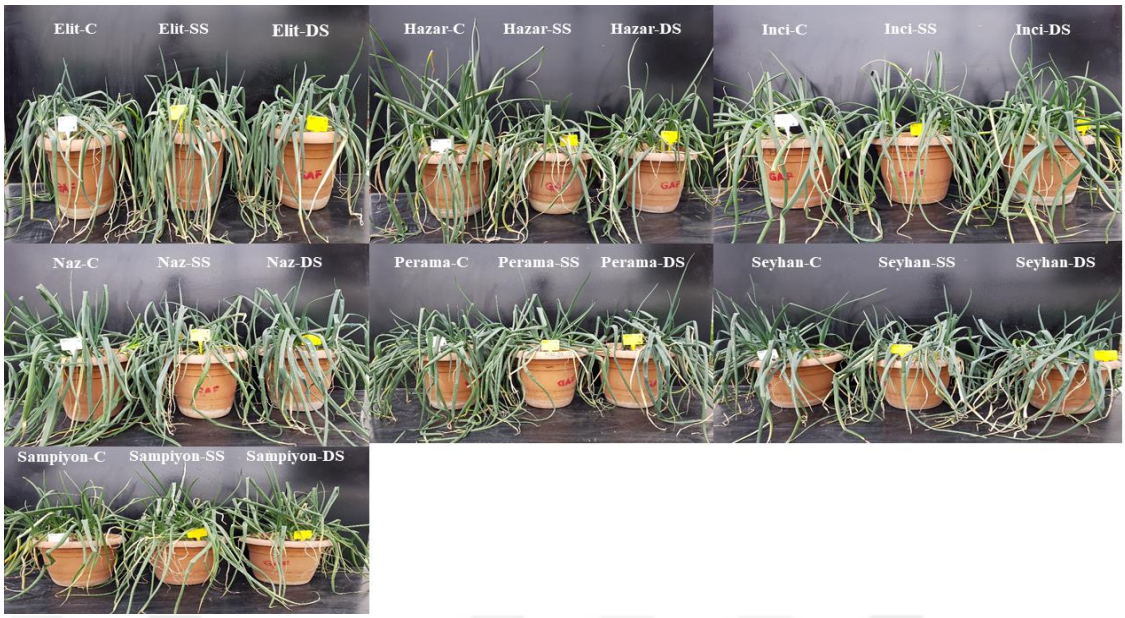
Cultivars Name	Source	Color	Shape
Elit	CommercialDomestic	Yellow	Round
Hazar	CommercialDomestic	Brownish-Yellow	Top
Inci	CommercialDomestic	White	Top
Naz	CommercialDomestic	Yellow	Round
Perama	CommercialDomestic	Red	Round
Seyhan	CommercialDomestic	White	Globe
Sampiyon	CommercialDomestic	Yellow	Round

Photograph 3.1. Sowing of onion seeds in pots under greenhouse conditions

After germination, 10 plants of similar physiological age per pot were maintained, and onion plants were divided into three groups. The first group included control plants (C) without any stress application, while the second group of plants was under salt stress (SS), and the third group was subjected to drought stress (DS) (Photograph 3.2).

Photograph 3.2. Seed germination and seedling establishment of onion cultivars under greenhouse conditions

The treatments were arranged according to a completely randomized design (CRD) under factorial arrangements with three replications. Stress treatment initiated at the onion bulbification stage as shown in Photograph 3.3.



Photograph 3.3. Onion cultivars before salt and drought stress application under greenhouse conditions

Plants were watered daily to the control group, whereas water was suspended for 20 days for DS onion plants. Salt stress application was started with irrigating with a salty water of 100 mM sodium chloride (NaCl). After every 3 days, salt stress was applied once with an increasing salt concentration as 125 mM, 150 mM, 175 mM, and 200 mM NaCl to the same pots designated for salt stress. In total 750 mM, NaCl was used for salt stress application as given in Table 3.2. Plants after stress application are shown in Photograph 3.4. At the end of stress treatment onion leaf samples were collected, directly frozen in liquid nitrogen, and stored at -80˚C for biochemical analyses and gene expression studies.

Table 3.2. Concentration of salt applied for salt stress application during the study

Days at which salt stress applied	Salt Application (mM)	Salty water applied to each pot	NaCl required for 1 L (g)	NaCl required for 1.5 L (g)
1st day	100	1.5 L	5.84	8.76
4th day	125	1.5 L	7.30	10.95
8th day	150	1.5 L	8.76	13.14
12th day	175	1.5 L	10.22	15.33
16th day	200	1.5 L	11.68	17.52
20th day	Leaf samples collected and pots were washed			

Photograph 3.4. Onion cultivars at the end of salt and drought stress application under greenhouse conditions

3.2 Physiological Measurements

Physiological parameters were measured on the 0^{th} before stress application, on the 10^{th} and 20^{th} day after stress application from the salt stress, drought stress, and control group plants between approximately 09:00 h-13:00 h.

3.2.1 Chlorophyll index

Leaf chlorophyll index was measured from control as well as from salt and drought-stressed plants with SPAD 502 Chlorophyll–Meter (Soil Plant Analysis Development; Minolta, Japan). Onion leaf (3^{rd} or 4^{th} fresh leaf) was selected from each pot and measurements were carried out as the average of three replications.

3.2.2 Stomatal conductance

Stomatal conductance measurements from control and stress application groups (drought and salt stress) were conducted with a constant light intensity of photosynthesis device (1500 μmol m^{-2} sec^{-1}), CO_2 amount (400 μmol) and airflow (500 μmol s^{-1}) and when photosynthesis machine gained steady-state measurement was recorded with a portable gas exchange system LiCor 6400 XT (Li-COR Biosciences, USA).

3.2.3 Photosynthetic rate

The Photosynthetic rate was measured the same as for stomatal conductance from control, salt stress, and drought stress group onion plants with a portable gas exchange system LiCor 6400 XT (Li-COR Biosciences, USA).

3.2.4 Transpiration rate

Transpiration rate was also measured from control, salt stress, and drought stress group onion plants stress group and control group onion plants with a portable gas exchange system LiCor 6400 XT (Li-COR Biosciences, USA).

3.2.5 Relative water content (RWC)

Onion leaf (3rd or 4th) from each pot was collected and fresh weight of onion leaves from both control and stressed plants were measured and turgid weight of the leaves was determined after keeping in distilled water overnight (Photograph 3.5). The leaves were then dried in a microwave at 500 W for 10 minutes followed by oven drying 95 ℃ for 2–3 hours to ensure complete drying of the leaves before determining dry weight. RWC values of plants were calculated according to the following equation.

$$\text{RWC}\ (\%) = [(\text{Fresh weight} - \text{Dry weight})/(\text{Turgor weight} - \text{Dry weight})] \times 100$$

Photograph 3.5. Relative water contents measurements from onion cultivars under control, salt, and drought stress application under greenhouse conditions

3.2.6 Leaf temperature (℃)

Leaf temperature was measured every mid-day using Infrared Thermometer (IRT) device (MASTECH BM380). The measurement was taken from the top of the onion leaves from the control, salt stress, and drought stress group. Measurement was taken in three replicates and the mean value was given for the leaf temperature.

3.2.7 Chlorophyll and carotenoids contents

Leaf chlorophyll and carotenoids contents were determined by collecting 0.5 g of fresh onion leaves (3rd or 4th fresh leaf) homogenized in 80% of 10 mL acetone. The Homogenized solution was kept at 4℃ overnight for complete extraction. The mixture was centrifuged at 10,000 rpm for 5 minutes at 4℃. Absorption of the supernatant was measured by using a UV-Vis spectrophotometer (UV-1800, Shimadzu) at 470 nm, 646

nm, and 663 nm. Calculation of chlorophyll contents was determined by formulas given below.

$$\text{Chl } a \text{ (µg/ml)} = 12.7(\text{A663}) - 2.69(A645)$$
$$\text{Chl } b \text{ (µg/ml)} = 22.9(\text{A645}) - 4.68(A663)$$
$$\text{Total chlorophyll (µg/ml)} = 20.2(\text{A645}) + 8.02(A663)$$

3.3 Biochemical Measurements

3.3.1 Lipid peroxidation (MDA)

Lipid peroxidation of onion leaves was assessed by measuring malondialdehyde (MDA) contents by following the procedure described by Heath and Packer (1968) using thiobarbituric acid (TBA) reaction. Onion leaf samples (0.2-0.25 g) were grounded and mixed with 2 ml of 0.1% trichloroacetic acid (TCA). The homogenates were centrifuged at 10,000 rpm for 20 min. 2 ml of 20% TCA having 0.5% TBA was mixed with the collected supernatants and incubated in a hot water bath for 30 minutes. The boiling reaction was ended by putting on ice for 10 minutes. Samples were separated in two separate 2 ml tubes and final centrifugation was done at 10,000 rpm for 5 min. The supernatant was taken, and absorbance was observed at 532 nm with the blank of a solution of 0.5% TBA and 20% TCA. To determine MDA contents, the second absorption was taken for nonspecific absorption at 600 nm which was subtracted from 532 nm. MDA values are expressed as nmol g^{-1} FW^{-1}.

3.3.2 Proline measurement

Leaf proline content was determined by the procedure described by Bates et al. (1973). Fresh young onion leaves were collected from each pot in three replicates for each treatment group. Onion leaf samples (200-300 mg) were ground in 3% sulfosalicyclic acid (2 ml) and centrifuged. The supernatant (1 ml) was collected and mixed with 1 ml of acid ninhydrin solution (ninhydrin in glacial acetic acid mixed with 6 M orthophosphoric acid). The mixture was shaken and incubated in a hot water bath (100 °C) for 1 hour. The reaction was stopped by keeping on ice for 10 minutes. After that 2 ml of toluene was added to the reaction mixture. The tubes were kept at room temperature under dark for 1

hour to allow separation of the aqueous and solvent phases. Finally, the toluene phase was collected carefully to measure the absorbance at 520 nm via UV-Vis spectrophotometer (UV-1800 Shimadzu). Toluene was used as a blank. Proline content was expressed as μmol g^{-1} FW**.**

3.3.3 Antioxidant enzyme analysis

3.3.3.1 Enzyme extraction

Fresh young leaves were taken at the end of stress application. Leaf samples of 200 mg were put in a 2 ml tube and 50 mM K phosphate buffer (including 2mM Na-EDTA and 1% PVP) was added and mixed. Homogenate was taken and centrifuged at 4℃ and 10,000 rpm for 10 minutes. The upper phase was collected and stored at -80℃ for enzymatic measurements.

3.3.3.2 Superoxide dismutase (SOD)

SOD was measured by a method provided by Giannopolitis and Ries et al. (1977). The decline in nitroblue tetrazolium (NBT) at 560 nm was observed. For the preparation of the reaction mixture Na-EDTA (0.1 mM) was taken with riboflavin (75 μM) and potassium phosphate buffer (50 mM, pH 7.0), methionine (13 mM), and enzyme extract (50 μl) in a test tube and kept them under fluorescent light for 5 minutes to initiate the reaction. The reaction was stopped by the removal from light to dark conditions. The blank sample contained all the reaction mixture apart from enzyme extract. The content of each of the samples and blank was immediately mixed by inversion and the increase in absorbance (ΔA) at 560 nm was recorded.

3.3.3.3 Catalase (CAT)

The activity of CAT antioxidant enzyme in leaves was defined as μmol of H_2O_2 per min per milligram H_2O_2 breakdown and monitored as a change in absorbance up to 0.01 following the method given by Chance and Maehly et al. (1955). The reaction mixture was prepared with the addition of 2670 μL of 50 mM potassium phosphate buffer (pH 7.0), 30 μL of enzyme extract, and 300 μL of 100 mM H_2O_2 and which was added at the

end for starting the reaction. The absorbance was measured at 240 nm via UV-Vis spectrophotometer (UV-1800 Shimadzu). The blank was prepared as the reaction mixture except for enzyme extract.

3.3.3.4 Ascorbate peroxidase (APX)

The activity of APX was measured by taking enzyme extract of 70 µl and homogenized in 2900 µL of APX solution containing 0.5 mM ascorbic acid, 1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.0) (Nakano and Asada, 1981). The reaction was initiated with the addition of 30 µL of 10 mM hydrogen peroxide in a 3 ml cuvette and the change in absorbance was measured at 290 nm using by observing the change in the rate of absorbance after 2 min by using UV-Vis spectrophotometer (UV-1800 Shimadzu).

3.4 Morphological Parameters

All the morphological parameters were measured on the 20^{th} day of stress application before termination of stress application.

3.4.1 Length of leaf

Leaf length (cm) was measured with measuring tape from the control and stress group plants in three replicates.

3.4.2 Leaf numbers

A number of leaves per plant was counted by selecting three plants from each pot in three replicates from control, salt stress, and drought stress group.

3.4.3 Leaf diameter

The diameter of leaf per plant was measured with measuring tape by selecting three plants from each pot in three replicates from control, salt stress, and drought stress group.

3.5 Yield Parameters

Yield parameters were measured after rescuing the onion plants that were allowed to grow until harvesting. When onion plants showed bending of the neck and started falling they were harvested through uprooting for measuring bulb yield parameters.

3.5.1 Length of bulb

Onion bulb length (cm) per plant was measured after stress application and harvesting bulbs. It was measured with the help of measuring tape by selecting three plants from each pot in three replicates from control, salt stress, and drought stress group.

3.5.2 Diameter of bulb

Onion bulb diameter (cm) per plant was measured after stress application and harvesting bulbs. It was measured with the help of measuring tape by selecting three plants from each pot in three replicates from the control, salt stress, and drought stress group.

3.5.3 Bulb weight

Onion bulb weight (g) was measured with weigh balance from each pot in three replicates and weight was measured at the end of stress applications by selecting three plants from each pot in three replicates from control, salt stress, and drought stress group.

3.6 Root Morphological Characteristics

The roots were collected from each pot in three replicates for each group. The root samples were washed with distilled water to remove dirt. The roots were placed in a 20 cm wide and 30 cm long acrylic tank with one-inch distilled water and placed on an EPSON scanner. The scanned images of roots were analyzed by WinRHIZOTM 2013 (Régent Instruments Inc, 2013) for root morphological traits including total root length (cm), the total surface area of the root (cm^2), average root diameter (mm), and root volume (cm^3).

3.7 Amplification of PSII and CAT Gene by Degenerate PCR Approach

3.7.1 RNA extraction

Onion leaves sample (100 mg) was collected from stressed and control group for RNA extraction by using the PureZOL™ (Bio-Rad, Hercules, CA). Samples were ground with liquid nitrogen and PureZOL™ reagent (1 ml) was added and transferred to a microcentrifuge tube with vortexing. The resulting supernatant was shifted to a new 2 Ml microcentrifuge tube, chloroform (0.5 volumes) and acid phenol (0.5 volumes) was added and vortexed for 2 min. Centrifugation of the mixture was performed at 10,000 rpm for 15 min. After centrifugation, the upper aqueous phase from the mixture was carefully taken and transferred to a new Rnase-free 1.5 Ml microcentrifuge tube. Ninety µL of 3M sodium acetate and 600 µL isopropanol was added with thorough mixing. Incubation of the extract was done for 10 min and centrifuged for 10 min at 10,000 rpm. After centrifugation, aqueous phase (upper) was collected and the washing of the pellet was done by 1 Ml of 75% ethanol. Pellet was dissolved in 50 µL DEPC-treated, sterilized ddH_2O, and stored at -80 °C. Evaluation of purity and intactness of RNA concentrations was measured by nanodrop spectrophotometer (Thermo Fisher) and by electrophoresis on a standard Rnase-free agarose gel.

3.7.2 cDNA synthesis

Cdna was synthesized by using iScript™ Cdna Synthesis Kit (BIO-RAD). The reaction mixture for Cdna synthesis consisted of Dnase treated RNA, 5x iScript reaction mix,

iScript reverse transcriptase, and nuclease-free water Table 3.3. Incubation was done in thermocycler by following protocol (Bio-Rad Laboratories Inc., Hercules, California, USA) given in Table 3.4.

Table 3.3. Components of the reaction mixture for the synthesis of Cdna

Components	**Volume per reaction (µl)**
5x iScript reaction mix	4µl
iScript reverse transcriptase	1µl
Dnase treated RNA template	2µg
Nuclease-free H_2O	to 20µL

Table 3.4. Reaction protocol for the synthesis of Cdna

Priming	5 min at 25℃
Reverse transcription	20 min at 46℃
RT inactivation	1 min at 95℃
Hold step	4℃

3.7.3 Designing of degenerate PCR primers

Currently, there is no data available regarding antioxidant enzyme gene CAT and PSII gene in onion. In this context degenerate PCR approach was the best option for amplification of these genes. Catalase degenerate primers were designed from the conserved regions of rice (XM_015769909.2), maize (NM_001254879.2), and Brachypodium (XM_003563195.2), while for PSII rice (NC_001320.1), maize (NC_001666.2), and wheat (NC_002762.1) conserved regions were used for designing degenerate primers (Figure 3.1). The degenerate primers for CAT and PSII are given in Table 3.5.

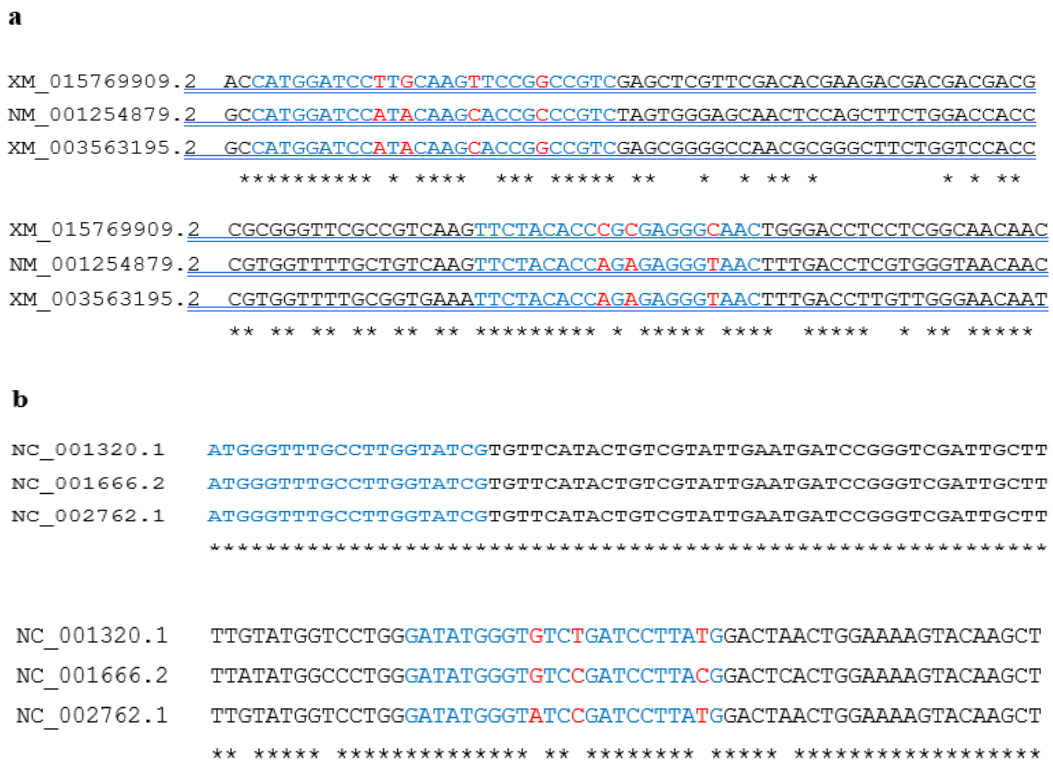


Figure 3.1. Sequence alignment of genes where blue regions shows regions of degenerate primers. (a) catalase gene, (b) photosystem II gene. Rice (XM_015769909.2), maize (NM_001254879.2) and Brachypodium (XM_003563195.2) for catalase gene, while for photosystem II, rice (NC_001320.1), maize (NC_001666.2) and wheat (NC_002762.1) were used.

Table 3.5. List of degenerate primers used in this study

Primer Names	Primer Sequence	Product Size (bp)	Degeneracy fold
CAT	F 5'-TTCTACACCMGMGAGGGYAAC-'3	396	2
	R 5'-TCDGGCCAMGTCTTGGTGWCATC-'3		2
PSII	F 5'-ATGGGTTTGCCTTGGTATCG'-3	527	0
	R 5'-CCGATMCTTATYGACTAACTYG-'3		2

where D=A/G/T, M=A/C, W=A/T and Y=C/T

3.7.4 Degenerate PCR approach

Degenerate PCR reaction consisted of 25 µl volume containing cDNA template and all the necessary ingredients for PCR reaction as shown in Table 3.6. The steps used for degenerate PCR is given in Table 3.7.

Table 3.6. Chemicals used for degenerate PCR

cDNA template	1µl
Forward primer (100 pM)	0.25µl
Reverse primer (100 pM)	0.25µl
dNTPs mix (10 mM)	0.2µl
$MgCl_2$ (25 mM)	2.5µl
Taq buffer with KCl (10 X)	2.5µl
Taq polymerase (U)	0.5µl
H_2O	17.8µl

Table 3.7. Steps for degenerate PCR

Steps	**Temperature**	**Time (h:m:s)**	
Initial denaturation	95°C	00:05:00	
Denaturation	95°C	00:02:00	35 cycles
Annealing	45°C	00:01:30	
Extension	72°C	00:01:00	
Final extension	72°C	00:10:00	

3.7.6 Purification of PCR amplified fragment from agarose gel

Amplified fragments of CAT and PSII were purified from agarose gel with GeneJET Gel extraction and DNA cleanup micro Kit (Thermo Scientific- Cat# k0832) in such a way that the required band gene was cut with the help of a sterilized blade from the gel, weighed and incubated with 200 µl of extraction buffer solution in a 1.5 ml tube at 55 °C till gel slice gets dissolved. After that incubation, 200 µl of ethanol (96-100 %) was added and mixed thoroughly. The mixture was then passed through DNA Purification Micro

Column and kept at room temperature for 10 minutes so that the column absorba the DNA. The column was then centrifuged for 60 seconds at 14,000 rpm. The supernatant was removed after centrifugation and the pellet was washed three times with Pre-wash and wash buffer was provided in the kit. Again, the supernatant was removed through centrifugation, the pellet was air-dried and warm elution buffer (65 ℃) was added onto the column to resuspend the pellet and kept at room temperature for 30 minutes. After 30 minutes the column was put for centrifugation and the supernatant was stored in a 1.5 ml tube at -20 ℃.

3.7.7 TA cloning of eluted fragment and bacterial transformation

Eluted PCR products were cloned by using Qiagen PCR Cloning Kit according to the protocol given in the manual. An eluted gene fragment was inserted into a TA vector solution Figure 3.2. Ligation protocol was followed as per instructions provided in the manual. The *E. coli* strain Top10 was used for the transformation of the plasmid and ligation mixture. The ligated product (2 µL) was transformed into Top10 chemical competent cells (50 µL) and mixed by tapping. The incubation of the cells was done on ice for 1 h and after that heat shock was given at 42 °C for 45 seconds. LB broth medium was added and incubated at 37 °C on a shaking incubator for 1 hour and spread on LB-kanamycin plates (50 µg mL^{-1}). Overnight incubation of the plates was done at 37 ºC.

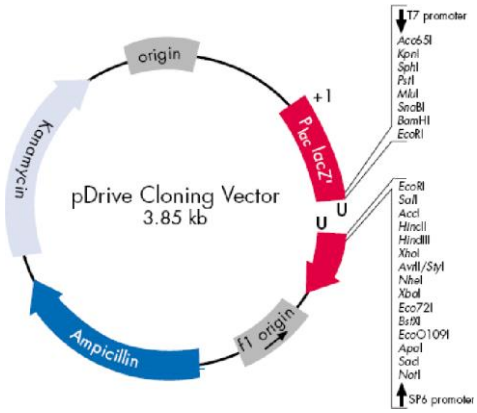


Figure 3.2. Map of the pDrive cloning vector

3.7.8 Screening and sequencing of positive clones

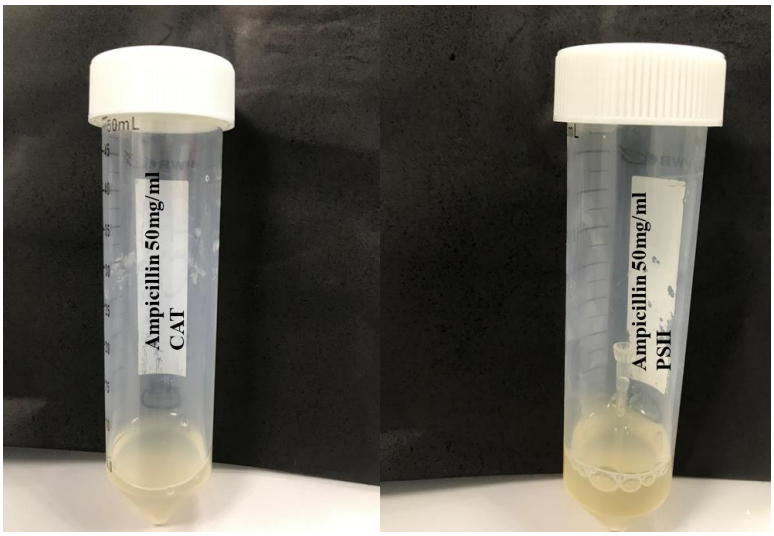
A colony PCR was conducted by selecting 10 colonies randomly. PCR reaction was done by using standard M13 primers (Table 3.8 and Table 3.9). PCR positive colonies were picked for inoculation in 10 mL of LB broth (Figure 3.3). The confirmation of the positive clones was further done with restriction analysis of DNA with *Eco*R1. The obtained results were evaluated on 1% agarose gel.

Table 3.8. Chemicals used for colony PCR

cDNA template	1µl
M13 F primer (100 pM)	0.25µl
M13 R primer (100 pM)	0.25µl
dNTPs mix (10 mM)	0.2µl
$MgCl_2$ (25 mM)	2.5µl
Taq buffer with KCl (10 X)	2.5µl
Taq polymerase (U)	0.5µl
H_2O	17.8µl

Table 3.9. Steps for degenerate PCR

Steps	Temperature (°C)	Time (h:m:s)	
Initial denaturation	95	00:05:00	
Denaturation	95	00:02:00	35 cycles
Annealing	55	00:01:30	
Extension	72	00:01:00	
Final extension	72	00:10:00	



Photograph 3.6. Overnight grown culture of CAT and PSII gene plasmids suspensions

3.7.9 Plasmid DNA isolation from positive clones

Cells were harvested at 5000 rpm for 1 minute. 150 µL of solution-1 (50 mM Glucose, 10 mM EDTA, 25 mM Tris-Cl) was added to resuspend the pellet, and samples were incubated on ice for 10 min; 200 µL of solution (0.2 M NaOH 1% SDS) was added and incubated at room temperature for 10 min until it became transparent. After that 200µL of 3M Potassium Acetate (pH 4.8) was added and kept on ice for 10 minutes. Centrifugation of the tubes was done for 10 minutes at 13000 rpm. The supernatant was recovered in new tubes and extraction with an equal volume of chloroform: phenol and isoamyl alcohol was done. The supernatant was taken and transferred carefully into new tubes. DNA was ethanol precipitated and washing of pellet was done with 70% ethanol after that air drying dissolved in sterilized water. Samples were given RNAase treatment with 1 µL of RNase A (Sigma-Aldrich).

3.7.10 Restriction analysis of cloned DNA

Positive clones were confirmed with restriction analysis of DNA with *Eco*R1 (Table 3.10). Clones were subjected to incubation at 37 °C for 1 hour. The obtained results were evaluated on 1 % agarose gel by running the digested samples.

Table 3.10. Restriction digestion reaction

DNA to be digested	1.5µl
10X Fast digest green buffer	1µl
*Eco*R1	0.5µl
H_2O	To make volume up to 10 ul

3.7.11 Sequencing of positive clones

The clones confirmed after PCR and restriction analysis were sent for sequencing purposes using an M13 primer. These primers are away from the site of PCR- Product where it is being inserted. Therefore the sequence reads with M13 primers contained extra sequences of a vector with a sequence of the desired PCR-Product. Targeted sequences were retrieved by the elimination of vector sequences.

3.7.12 Sequence data analysis

The obtained DNA Sequences were blasted once again to have a clear picture of similarities and differences with the already reported sequences from different monocot plants.

3.8 Gene Expression of PSII and CAT Gene by Real-Time Quantitative PCR (qRT-PCR)

Primers for qRT-PCR studies were designed using Primer 3 software. The CAT, PSII, and Actin primers were used given in Table 3.11. The reaction mixture for qRT-PCR given in Table 3.12 included Iq SYBR Green (BioRad) master mix (2X), 1 Ml of both forward as well as reverse primers (50 Pm each), sterile Rnase-free distilled water, and diluted Cdna (1:10) template 1 µg. The Reaction mixture was incubated by using Qiagen real-time PCR (Rotor-Gene Q) (Table 3.13). Melting curve analysis was performed with incubation at 99 °C to 70 °C with a change rate of 1.0 °C/min. The Ct values of samples for selected target gene expression were measured by Rotor-Gene Q Software. After getting the results of qRT-PCR, the expression levels of the selected genes were calculated by using the $2^{-\Delta\Delta Ct}$ proportional calculation method provided by Livak and

Schmittgen (2001). The fold change in transcript levels of genes under study was given relative to the control plants.

Table 3.11. List of qRT-PCR primers used in this study

Primer Names	Primer Sequence	Product Size (bp)
CAT	F 5'-CTCTCAAACCGAACCCAAAA-'3 R 5'-GCCAGAACCTTCCATGTGTC-'3	149
PSII	F 5'-GATTGGGGTTGTTGCTGAAC-'3 R 5'-GTCCGCCTCAACGTCTCTAC-'3	137
Actin (GU570135.2)	F 5'-CGATGAAGCACAATCCAAGA-3 R 5'-TGTTCTTCAGGAGCAACACG-'3	138

Table 3.12. Chemicals used for qRT-PCR

Chemicals	Amount (µl)
iQ SYBR Green (BioRad) master mix (2X)	5.0
F primer (2 µM)	0.4
R primer (2 µM)	0.4
dH2O	1.7
cDNA	2.5
Total volume	20

Table 3.13. PCR conditions for qRT-PCR

Steps	Temperature (℃)	Duration (h:m:s)	Cycle
Initial denaturation	94	00:02:00	No
Denaturation	94	00:01:00	30
Annealing	60	00:00:15	30
Extension	72	00:00:20	30
Final extension	4	∞	No

3.9 Statistical Analysis

The study was laid out according to CRD design under factorial arrangements. Analysis of variance (ANOVA) was done for all the data for confirmation of variability within the data and validation of results. The least significant difference test (LSD) was conducted to determine significant differences by using Statistical Package Statistix 8.1. Pearson correlation coefficient was applied to observe the association of variables with each other under investigation in this study with the use of SAS software. A P value of less than 0.05 was accepted to be statistically significant. Principal component analysis (PCA) was performed by using XLSTAT for visualizing differences between the treatments for different biochemical variables related to salt and drought stress response.

CHAPTER IV

RESULTS AND DISCUSSION

The current study was conducted to observe the morphological, physiological, biochemical, and molecular responses of seven different onion cultivars under salt and drought stress conditions in a pot experiment at the Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University. The results obtained during the whole course of the study are described in this chapter.

4.1 Physiological Parameters

To mimic the natural salt stress and drought stress conditions in the field for selected onion cultivars, a continuous SS and DS were imposed by applying NaCl and withholding water. Physiological traits of selected onion cultivars under salt and drought stress were observed as given below. Abiotic stresses are the leading obstacles resulting in retarded growth, development, and yield losses of the onion bulbs. The onion susceptibility to salt and drought stress varied on the intensity of both stresses. It was monitored that onion manifested the least disruptions in physiological changes after 10 days of under stress conditions. So, onion can tolerate a transient stress period. The onion is vulnerable to prolonged stress duration quantified on the 20^{th} day of continuous stress. Onion cultivars subjected to salt and drought stress demonstrated varying responses regarding physiological changes.

4.1.1 Relative water contents (RWC)

Relative water contents appreciably declined in all the onion cultivars exposed to drought and salt stress conditions (Figure 4.1). Control onion cultivars did not show any significant change. The RWC continued to decrease at a steady rate after every salt stress application and withholding water supply. A remarkable decrease in RWC was noticed on the 10^{th} day in all the SS and DS group plants compared with control plants. The RWC contents were measured on the 20^{th} day and the Inci cultivar had the highest RWC (83%) in the case of SS while the Perama cultivar had the highest RWC (81%) in the case of DS

even with no significant (P≤ 0.05) difference from the control. The lowest RWC was assessed from Naz cultivar (68%) in the case of SS, whereas the Sampiyon had lowest RWC (57%) under DS. Relative water content is an important physiological attribute to estimate the internal water status under stress conditions. Drought and salt stresses resulted in decreased RWC in the current study. Substantial reduction in RWC is also attributed to the susceptibility of plants to certain stress (Dien et al., 2019). The cultivars 'Elit' and 'Hazar' showed decreased RWC under both stresses whereas 'Sampiyon' under DS condition. As, RWC is considered as an indicator of stress tolerance (Dien et al., 2019), results of this study suggested that the cultivars 'Elit', 'Hazar' and 'Sampiyon' were susceptible to applied stress. Findings of this study and previous studies indicated that SS and DS affect negatively RWC which also leads to oxidative stress in plants (Egert and Tevini, 2002, Astaneh et al., 2018). The cultivar 'Inci', showed more RWC only under SS condition, whereas 'Perama' and 'Seyhan' were the best performers under DS. Interestingly, these cultivars could have exhibited higher osmotic regulation through an accumulation of osmolytes to alleviate osmotic stress (Moharramnejad et al., 2019). The lower RWC with the influence of salt stress was reported in onion (Semida, 2016). Water deficit conditions also have been reported to cause an enormous effect on reduced internal water contents of multifarious monocot crops which includes maize, wheat, garlic, and chive (Egert and Tevini, 2002; Çiçek and Çakirlar; 2002; Kaya et al., 2006; Astaneh et al., 2018). Thus the result of the study and already earlier reports strongly demonstrate the effect of stress on RWC which further aggravates oxidative stress in plant.

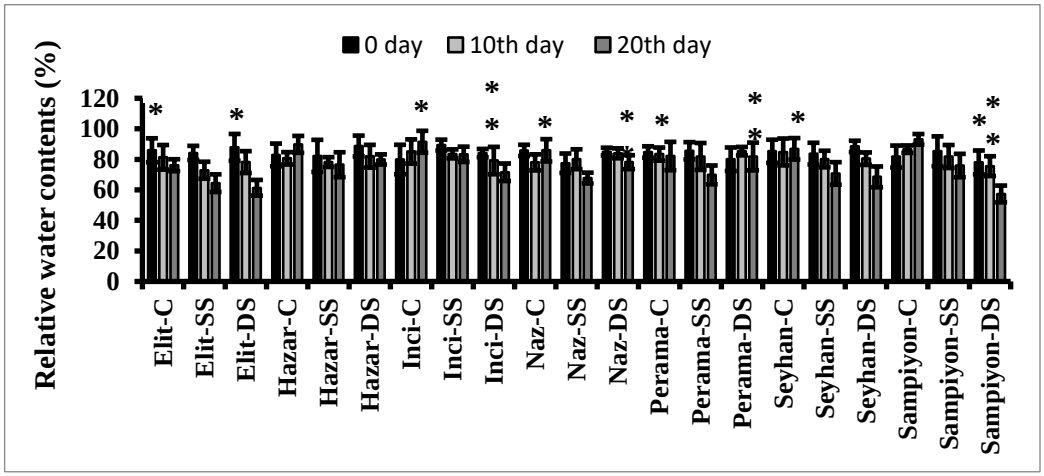


Figure 4.1. Relative water contents of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.1.2 Chlorophyll index

Leaf chlorophyll index was measured at three different intervals. Once it was measured before stress application (0^{th} day) and a second time after stress application on 10^{th} day finally on 20^{th} day before the termination of stress application. The chlorophyll index was the same for all the cultivars and all the groups categorized as control, salt stress, and drought stress before initiation of stress application. Minimal decreases in chlorophyll index were observed in all cultivars on the 10^{th} day with both stress groups. However, the higher reduction was in drought-stressed group as compared with salt-stressed group. Quantification of chlorophyll index indicated deterioration in all onion cultivars with a prolonged stress environment. The cultivar 'Hazar' showed a decrease of 17% in response to SS and a 23% reduction in cultivar 'Sampiyon' was recorded under DS after exposure to 20 days of stress, as compared to their respective control plants. However, the cultivars 'Perama' and 'Seyhan' showed a minimum decrease in chlorophyll index in response to both stresses (Figure 4.2). Decreased chlorophyll index is due to the damage caused by stress which is also in accordance with the earlier report by Shah et al., (2017). The result of this study with the decreased chlorophyll index is also in accordance with the previous reports in monocot crops (Talebi, 2011; Li et al., 2006).

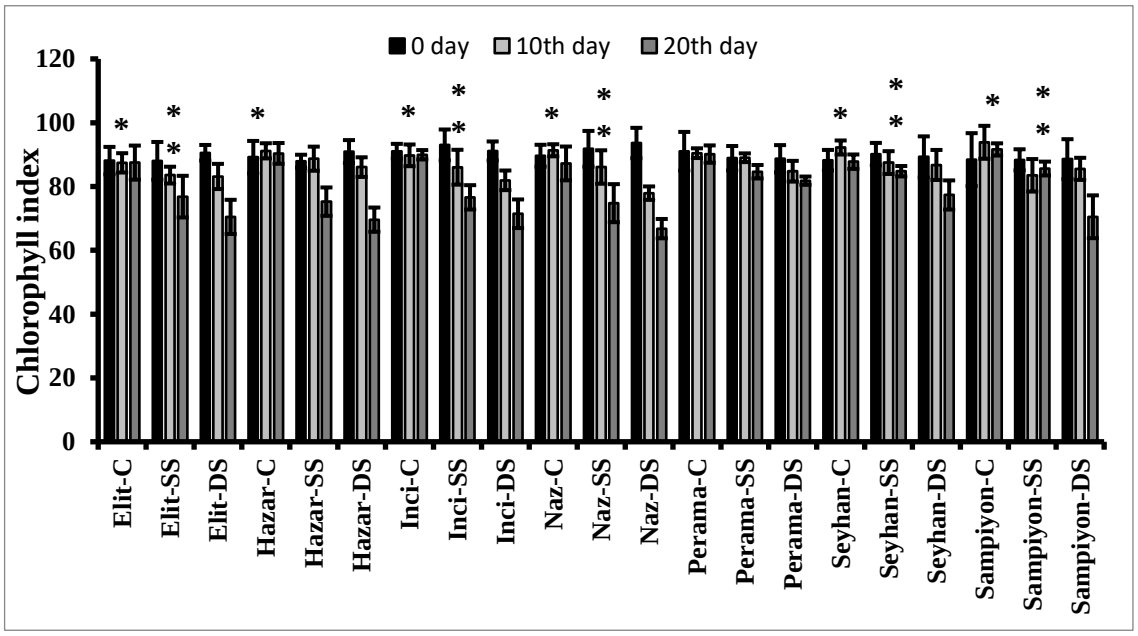


Figure 4.2. Chlorophyll index of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.1.3 Stomatal conductance

Onion cultivars that were subjected to stress conditions resulted in repression of stomatal conductance rate. Stomatal conductance was found to be consistently reduced irrespective of the onion cultivars with the course of both stresses. There was no significant difference at the 0^{th} day among all the cultivars. However, The cultivars acclimatized to stress conditions showed a significant reduction ($p \leq 0.05$) in stomatal conductance activity when compared with their respective controls. The cultivar 'Perama' excelled the other cultivars with the least decrease in stomatal conductance after 10 days of salt and drought stress. In contrast, cultivar 'Hazar' showed a decrease of 70% and 84% in stomatal conductance after 10 days of SS and DS, respectively. The response of onion cultivars was also noted after 20 days of stress imposition. The cultivars 'Hazar' and 'Sampiyon' showed a significant decrease up to 90% and 93% in stomatal conductance after 20 days of salt and drought stress, respectively (Figure 4.3). Stomata is the key site for the exchange of gases between leaf and environment, which is affected under the influence of salt and drought stress. Stomatal regulation is a key process to prevent the plant from desiccation as well as maintains an internal CO_2 level under abiotic stress conditions (Medici et al., 2007). The decreased stomatal activity was reported in important crops under stress conditions (Anjum et al., 2011; Pazzagli et al., 2016; Zhang et al., 2019).

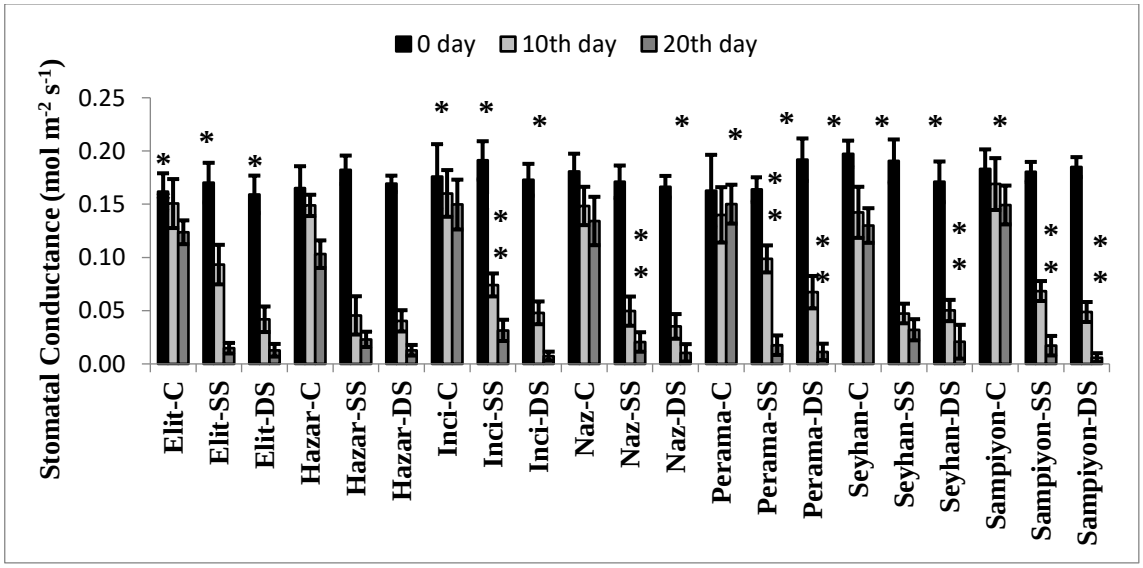


Figure 4.3. Stomatal conductance of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents significant difference. Two vertical asterisks (**) shows significant difference among stressed counterpart (salt or drought stress)

4.1.4 Transpiration rate

Transpiration rate also dropped ($P \leq 0.05$) under both stress regimes. Results revealed that SS and DS resulted in a reduction of all the cultivars at the 10^{th} and 20^{th} day of stress intervals. The Perama cultivar showed the least decline in transpiration rate at 10^{th} day under SS by 17%, and 20^{th} day by 56%, whereas under DS at 10^{th} day by 21% and 52% at 20^{th} day of stress. The highest reduction in transpiration rate was quantified in Elit and Sampiyon. It declined by 80% under SS in Elit and 93% under DS in Sampiyon after 20 days (Figure 4.4). Reduced transpiration rate is the symptom of the sensitivity of cultivars to stress conditions as reported by Jezdinský et al., (2013) in leek. It is also attributed to a decline in internal leaf water contents which disrupts the transpiration process of a plant. It might be the reason that Elit and Sampiyon cultivars showed maximum reduction transpiration rate as compared to other cultivars under study. The results of this study were also endorsed by the previous report in onion exhibiting the decreased transpiration rate of sensitive cultivars that failed to hold water contents resulting in damaged photosynthetic machinery (Kutty et al., 2014). Decreased transpiration was also noticed in potato in response to drought stress (Zhang et al., 2018). Higher Salt stress results in osmotic stress which ultimately causes ion toxicity in plants. Sensitive cultivars might failed to compartmentalize ions within tissues as reported in rice (Radanielson et al., 2018).

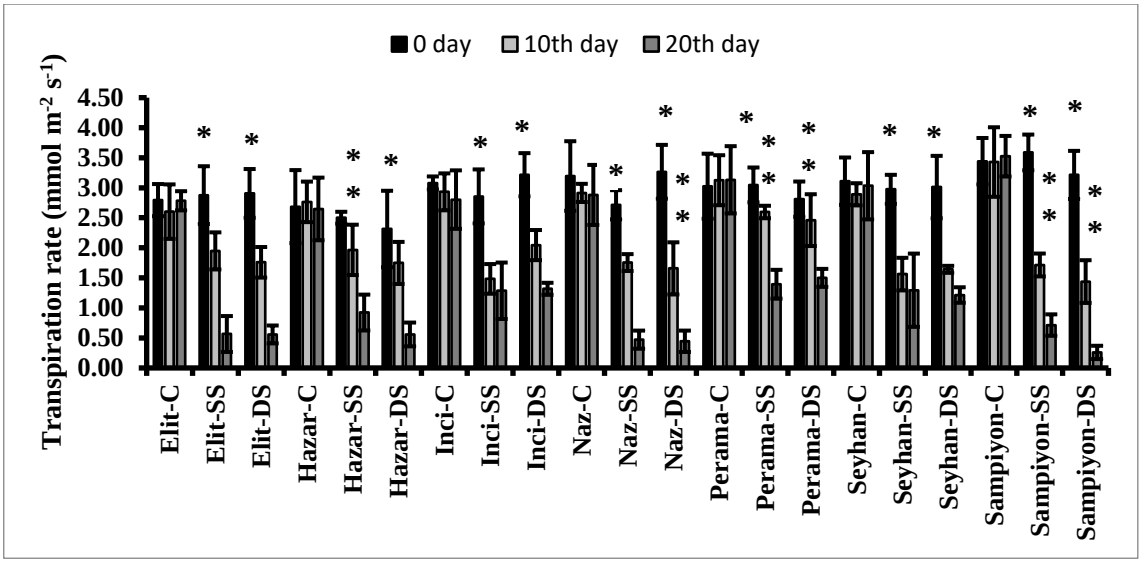


Figure 4.4. Transpiration rate of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.1.5 Photosynthetic rate

Data regarding photosynthetic activity was taken at the 0^{th} day among cultivars before the onset of any stress application. Photosynthetic rate significantly ($P \leq 0.05$) suppressed in all the cultivars after exposure to a total of 750 mM NaCl and 20 days of DS. The maximum photosynthetic rate was observed from Perama cultivar under salt (19.06 µmol m^{-2} s^{-1}) and drought stress (18.53 µmol m^{-2} s^{-1}) conditions at the 10^{th} day of stress applications. The minimum photosynthetic rate at the 10^{th} day was noted from Elit cultivar under both stress regimes with a reduction of 35% and 40%, respectively. Naz cultivar showed exceptional response regarding photosynthesis in response to both stress regimes as compared with all other cultivars under investigation. It showed maximum reduction in photosynthesis under SS (12.31 µmol m^{-2} s^{-1}) whereas its counterpart under DS (17.22 µmol m^{-2} s^{-1}) showed the least reduction in photosynthesis. The Perama and Seyhan cultivars showed the least decline in the photosynthetic rate at the 20^{th} day of stress applications while all other cultivars showed a lower photosynthetic rate under both stress conditions with a reduction of 39-50% (Figure 4.5). Photosynthesis along with cell growth is the primary process that are affected due to the closure of stomata (Fahad et al., 2017). Decreased photosynthesis in these cultivars might have resulted due to the decline in leaf internal CO_2 concentration which is a consequence of stomatal closure and is evident by the decreased transpiration rate (Vesela et al., 2017). The sensitivity of these

cultivars might also be attributed to the damaged photosynthetic apparatus caused by the generation of reactive oxygen species (ROS), which are accumulated under the circumstances of reduced CO_2 influx and excess/continued light exposure (Farooq et al. 2014). The reduction of cellular water caused by the contact of roots with the stressful environment subsequently reduces the transport of assimilates, which eventually affects the photosynthetic rate (Chaves et al. 2009). The least influence on gaseous exchange traits in the cultivars 'Perama' and 'Seyhan' was due to their high-water contents. It includes changes in the cellular osmotic behaviour evident by high relative water contents in the cell under stress conditions (Hussain et al., 2018). The synthesis of osmoprotectants combined with high chlorophyll pigments and enhanced photosynthesis could be a major decisive factor for the stress tolerance response of these cultivars (Farooq et al., 2012). The findings of this study grouped the cultivars 'Perama' and 'Seyhan' as 'tolerant' based on their better adaptive response to both the stresses. Demirel et al. (2020) investigated the effect of drought stress on gaseous exchange characteristics of potato cultivars and suggested that a higher photosynthetic rate was a key attribute exhibited by tolerant cultivars. A similar trend was observed in this study. Drought stress had been reported to suppress photosynthesis and the transpiration rate of leek (Jezdinský et al., 2013). Similar results were quantified in this study with a severely deleterious effect on gaseous exchange traits among the cultivars. Photosynthetic sensitivity of onion plants both to salt and drought stresses is obvious from Figure (4.3) which was primarily associated with the decrease in stomatal conductance. The least influence on gaseous exchange traits in Perama and Seyhan cultivars might be due to its characteristic of high water contents retention against a moisture deficit period and responsiveness against stress. On the other hand, the cultivars most affected might had previous stress imprints This finding suggested that genotypic variation exists in onion for photosynthesis against its response to stress conditions.

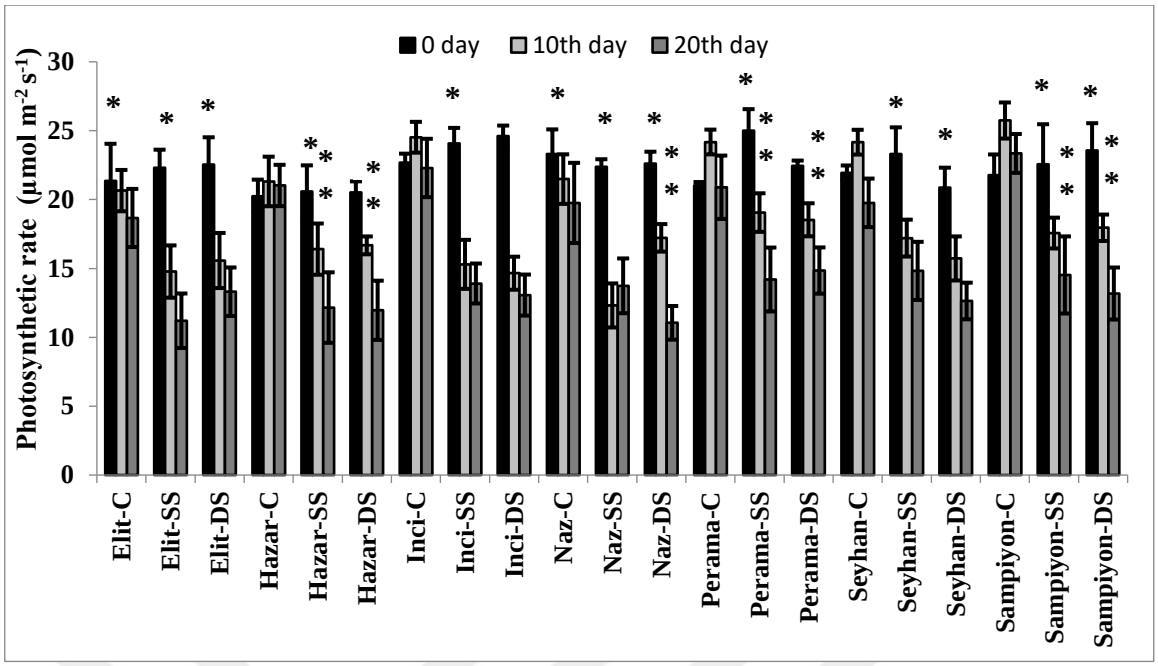


Figure 4.5. Photosynthetic rate of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.1.6 Leaf temperature (°C)

Change in leaf temperature was observed during stress application. The leaf temperature of Inci, Perama, and Seyhan cultivars was lowest under SS and DS on the 10th day respectively with no significant ($P \leq 0.05$) difference from control plants. The rise in leaf temperature was also reckoned on the 20th day before the termination of stress application. Leaf temperature of the cultivar 'Inci' was lowest under SS (23.6 °C) and DS (24.9 °C) on the 20th day, respectively, with no significant ($P \leq 0.05$) difference from control plants. However, the cultivar 'Elit' had the highest rise in leaf temperature under SS (28.6 °C) and the cultivar 'Sampiyon' under DS (29.1 °C) (Figure 4.6). Leaf temperature was elevated under both the stressed conditions with a higher increase under DS. The higher leaf temperature was noticed in the cultivars 'Elit', 'Hazar' and 'Sampiyon' under both the stresses, which is attributed to lower RWC and disruption in gaseous exchange traits. Isoda (2010) reported that water-stressed plants showed lower transpiration rates resulting in higher leaf temperature. The reason is that there is a close relationship between stomatal closure and increased leaf temperature (Liu et al., 2011). The cultivar 'Inci' exhibited comparatively lower leaf temperature under SS, due to its capability to preserve higher levels of RWC. The cultivar 'Seyhan' prevented the tissue damage by regulating

metabolic processes such as increased stomatal opening, transpiration rate, and enhanced photosynthesis that resulted in lowering of leaf temperature. Other factors that might have contributed to better adaptation response of this cultivar under both stresses is linked to the manipulation of the antioxidant system to scavenge oxidative stress caused by SS and DS (Farooq et al., 2019).

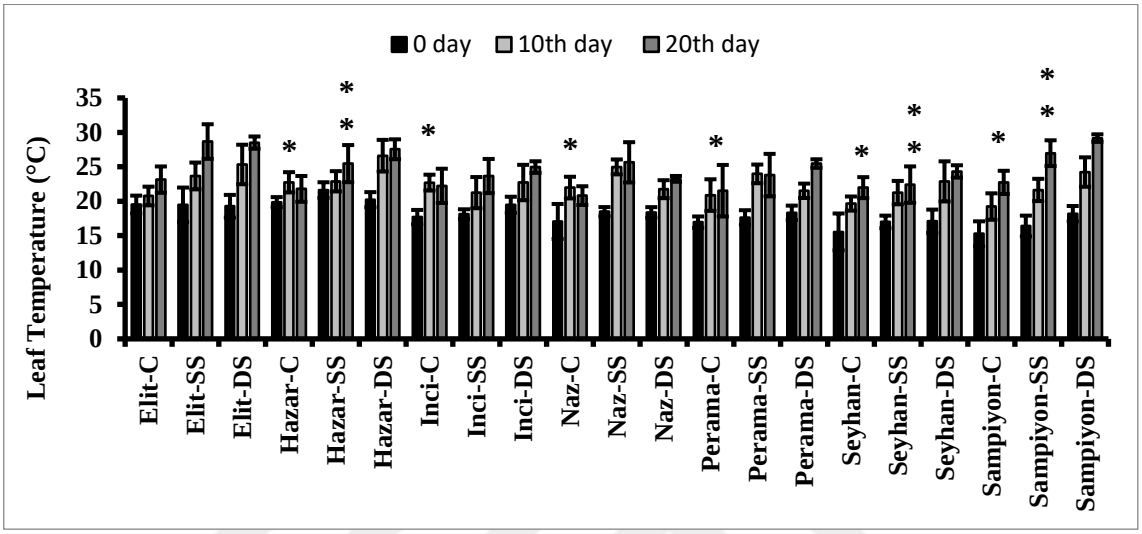


Figure 4.6. Leaf temperature of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2 Biochemical Parameters

Biochemical changes in onion leaves following salt and drought stress treatments showed a differential response for all the cultivars as compared to their control under investigation in this study.

4.2.1 Chlorophyll a content

The chlorophyll a contents, indicated that stress application of 750 mM NaCl and 20 days of drought stress damaged the photosynthetic pigment. Salt contents in onion leaf imparted deleterious effect to chlorophyll a content in Hazar cultivar with a reduction of 28% and 34%, respectively. However, Sampiyon had maximum damage to chlorophyll a content under drought stress with a reduction of 38% (Figure 4.7). The chlorophyll content is the main component of chloroplast involved in photosynthesis and, therefore,

has a positive association with the rate of photosynthesis. Both chlorophylls, chlorophyll a and b, are prone to abiotic stresses (Ghaffar et al., 2019; Alhoshan et al., 2019; Moharramnejad et al., 2019). The decrease in chlorophyll content under salt and drought stress is a general symptom of oxidative stress which ultimately causes degradation of photosynthetic pigments and arrest photosynthesis (Maghsoudi et al., 2015; Zaefyzadeh et al., 2009). Results obtained in this study indicated that photosynthetic pigments of the cultivars Hazar and Sampiyon were reported to decrease in response to SS and DS, respectively. This decrease was due to colossal damage caused by reactive oxygen species (ROS) in susceptible genotypes. Under stress conditions precipitation of Mg^{2+} occurs resulting in impairment of plant pigments, moreover oxidative stress triggers chlorophyllase enzyme activity which degrades chlorophyll contents (Abdel Lateef and Tran, 2016).

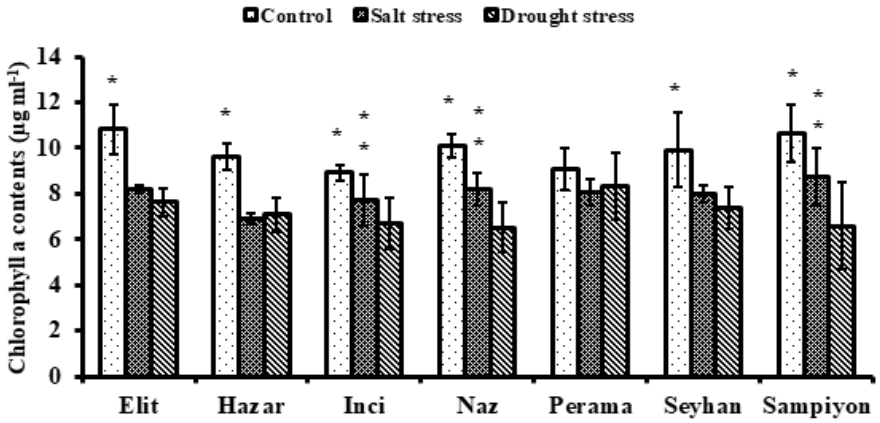


Figure 4.7. Effect on Chlorophyll a content (µg ml^{-1}) of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.2 Chlorophyll b content

The chlorophyll b content was also measured on the 20th day of stress application from all the groups. Drought and salt stress imparted a deleterious effect to chlorophyll b content of onion cultivars under investigation. The Hazar cultivar showed greater damage to chlorophyll b content with a reduction of 34% under salt stress conditions. However, Sampiyon showed maximum damage to chlorophyll b content under drought stress with a reduction of 42%. The cultivar Perama exhibited the least damage to chlorophyll b

contents in response to both stress conditions (Figure 4.8). The reduction in chlorophyll b content with the exposition to SS and DS is due to damage to the chlorophyllase enzyme. Moreover, it is also attributed to a weakening of protein pigments lipid complex (Rahdari et al., 2012). The cultivars Hazar and Sampiyon depicted higher damage to chlorophyll b content as they had higher oxidative stress. Similar results with the decreased chlorophyll b content were reported in onion in response to both SS and DS (Hussein and El-Faham, 2018; Hanci and Cebeci, 2014; Hanci and Cebeci, 2015).

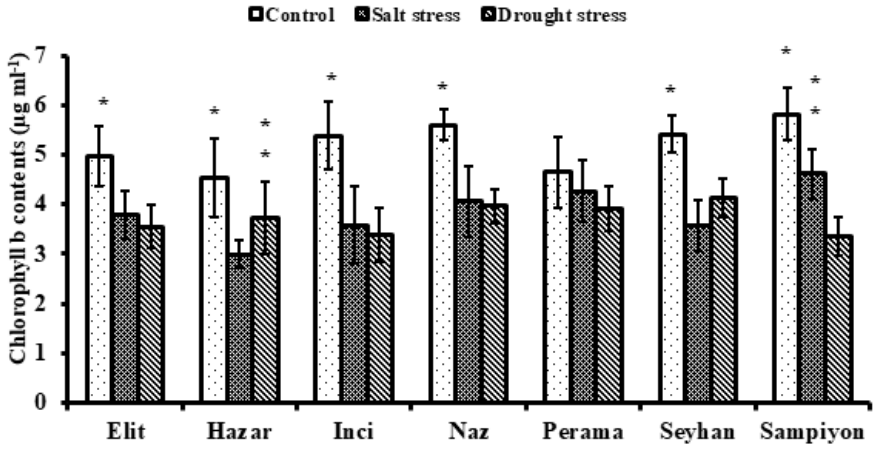


Figure 4.8. Effect on chlorophyll b content (µg ml^{-1}) of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress).

4.2.3 Total chlorophyll contents

Total chlorophyll content was also measured on the 20th day of stress application from all the groups. Drought and salt stress imparted a deleterious effect to the total chlorophyll content of onion cultivars under investigation. The Hazar cultivar showed greater damage to total chlorophyll contents. The maximum reduction in total chlorophyll contents was quantified from Hazar under SS with a decrease of 31% while a 40% decrease was observed in Sampiyon cultivar under DS condition. Contrarily least damage to total chlorophyll contents in response to both stress conditions was observed in Perama and Seyhan cultivars among all the cultivars (Figure 4.8). In this study, the exposition of onion plants to SS and DS damaged the photosynthetic pigments of all the cultivars. The maximum impairment in total chlorophyll content was depicted by the cultivars 'Elit', and 'Hazar' in response to SS and 'Sampiyon' under DS (Figure 4.9). This is due to the

damage caused by ROS in these cultivars. It resultantly destroyed the chloroplast structure of the cultivars. Moreover, a decrease in the chlorophyll of stressed plants is a general symptom of oxidative stress which is attributed to inhibition in the synthesis of chlorophyll (Santos, 2004). The findings of damaged chlorophyll contents are also supported by an earlier study of Romdhane et al. (2020). Total chlorophyll content was higher in the cultivar 'Perama' that was credited to the minimal effect on the light-harvesting complexes present on the thylakoid membrane, as evident from enhanced photosynthesis compared to others.

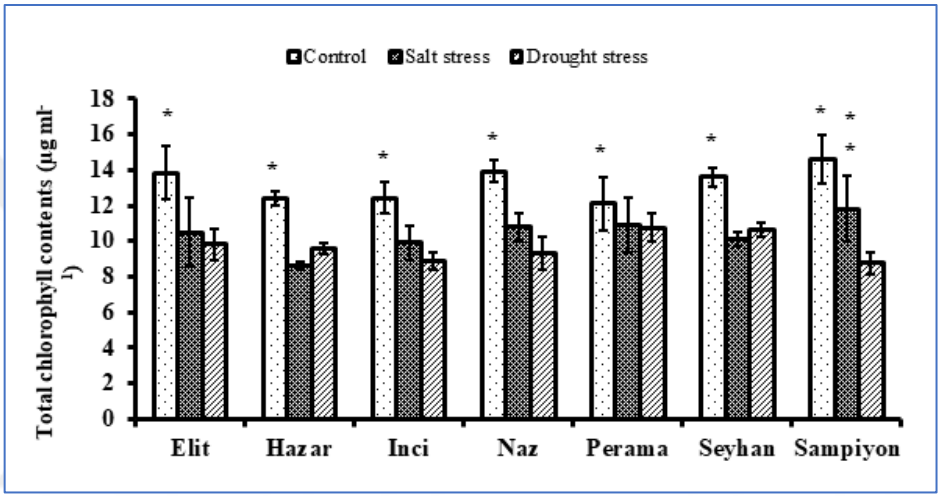


Figure 4.9. Effect on total chlorophyll contents (µg ml^{-1}) of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.4 Carotenoid contents

Carotenoid content was measured on the 20th day of stress application from all the groups. Drought and salt stress exhibited higher damage to the carotenoid content of all the onion cultivars used in this study. The Hazar cultivar showed greater damage with a reduction of 39% to carotenoid contents under salt stress conditions and Sampiyon under drought stress with a decrease of 52%. The least damage to carotenoid contents was observed in Inci, Perama, and Seyhan cultivars in response to both stress conditions (Figure 4.10). Carotenoid is an antioxidant with the potential for detoxifying the harmful effects of ROS in plants. The current study reported the negative impact of SS and DS with damaged carotenoid contents causing photoinhibition. In cultivars 'Elit' and 'Sampiyon', a

significant reduction of carotenoid contents indicated susceptibility to SS and DS, whereas the cultivar 'Hazar' performed poorly under SS. These cultivars showed a decline in RWC and total chlorophyll content. Additionally, carotenoid effectively scavenges provoked free radicals in tolerant genotypes. Thereby in these two aforementioned cultivars, Elit and Sampiyon significant disruption of carotenoid highlighted susceptibility against stress. Contrarily, the cultivars 'Inci' and 'Seyhan' were the richest in carotenoid content and this was possible by retaining higher RWC under SS. This finding was also supported by previous reports in onion (Hanci and Cebeci, 2014, Hanci and Cebeci, 2015, Hanci et al., 2015, Semida, 2016).

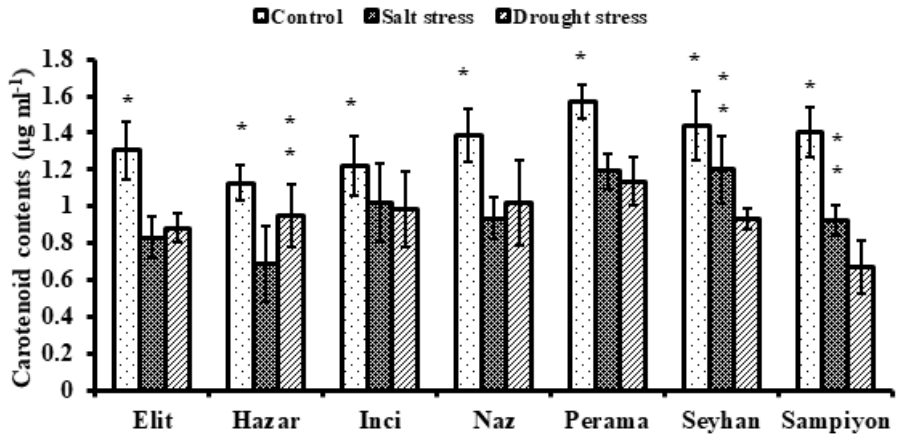


Figure 4.10. Effect on carotenoid content (µg ml^{-1}) of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.5 Malondialdehyde contents

Malondialdehyde contents were quantified on the 20th day of salt and drought stress application from all the groups. MDA significantly ($P \leq 0.05$) increased in the Elit and Hazar cultivars under both SS and DS conditions, whereas Sampiyon cultivars exhibited higher MDA contents under DS. In the case of the Naz cultivar, a slightly higher peroxidation rate of membrane lipid was noticed based on MDA contents compared with other cultivars. However minimal trend was observed in remaining cultivars under both stress conditions (Figure 4.11). Lipid peroxidation was observed by measuring MDA contents. It is the key indicator of the negative effect on cellular membranes of plants with the influence of stress (Hussain et al., 2018). In this regard, enhanced level of MDA

was noticed in Elit and Hazar under both stress regimes while Sampiyon in case of drought stress (Figure 4.10). It showed the sensitivity of these cultivars to stress conditions, contrarily other cultivars exhibited a nominal increase in MDA contents. It was reported that increase in MDA content is due to the result of oxidative damage in a plant with the overproduction of ROS species leading to higher lipid peroxidation (Hanif et al., 2020). Results found in this study are in accordance with the earlier studies showing the negative effect of MDA in response to stressed conditions in monocot crops (Yang et al., 2014; Hussain et al., 2019; Abid et al., 2018).

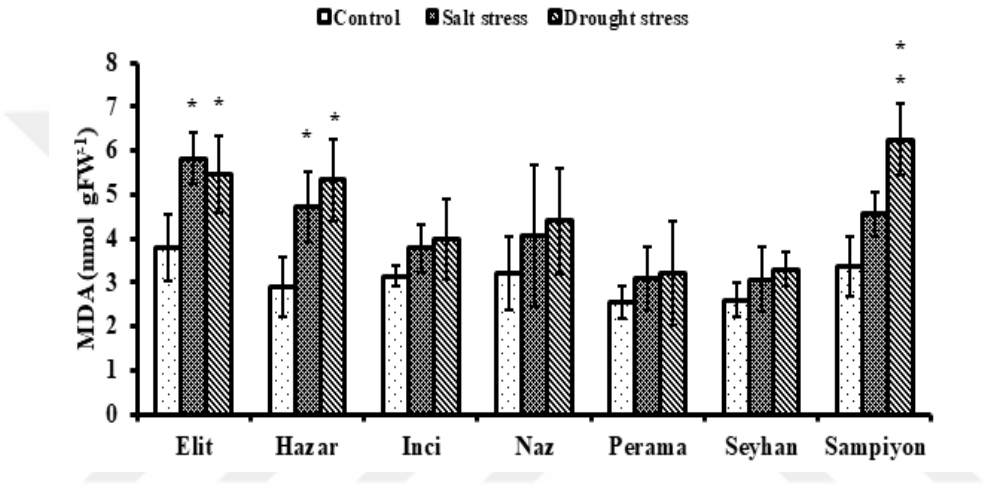


Figure 4.11. Malondialdehyde (MDA) contents of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.6 Proline contents

Proline content was measured on the 20th day of SS and DS application from all the groups. The onion cultivars differed in accumulation to proline in response to stress conditions. The proline contents increased in all the cultivars irrespective of stress conditions but a significant ($P \leq 0.05$) higher increase was observed in Perama and Seyhan cultivars under both stress conditions, while lower proline accumulation was quantified in Elit and Hazar cultivars under both stress conditions. Sampiyon exhibited higher accumulation under the SS environment while its counterpart showed the least proline accumulation (Figure 4.11). Proline is the chief osmoregulator in plants. It is the indicator of stress tolerance as it maintains the cellular functioning of the plant under a stressful

environment. In this study higher accumulation of proline in Inci, Perama, and Seyhan cultivars were observed (Figure 4.12). It suggested a favorable role of compatible solute that aided in stress tolerance in onion leaves with detoxification of ROS species, stabilization of protein, and protection of cellular membrane (Hayat et al., 2012). Decreased proline accumulation in the Elit and Hazar might be due to the sensitivity of these cultivars to stress conditions. It was reported in numerous studies that sensitive plants failed to synthesize higher proline due to massive degradation of proline contents against SS and DS conditions (Lutts et al., 1996; Kibria et al., 2017). Moreover, several studies found a positive correlation of proline contents to confer stress tolerance in monocots crops (Lum et al., 2014; Anjum et al., 2016; Mwadzingeni et al., 2016; Ghassemi-Golezani et al., 2018). Enhanced proline accumulation in this study is in accordance with study of Efeoğlu et al. (2009), who found the same response of increased proline in maize in response to drought stress. Results regarding proline accumulation are also in line with the findings of Hanci and Cebeci (2015), as they also reported a remarkable increase in proline contents in onion under SS and DS conditions.

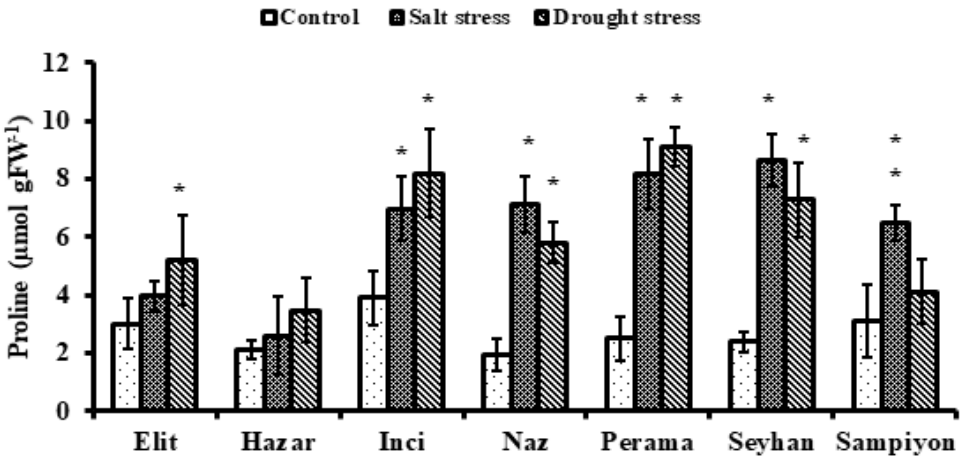


Figure 4.12. Proline contents of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.7 Antioxidant enzyme activity

All the onion cultivars differed in their antioxidant enzyme activities with the imposition of stress application. The Elit and Hazar cultivars showed relatively no change in response

to SS and DS conditions. Sampiyon also didn't show any change in antioxidant enzymatic activity under DS while under SS it showed significant ($P \leq 0.05$) change compared to control. Perama and Seyhan exhibited elevated enzyme activities in response to both stress regimes. Inci and Naz cultivars also showed higher antioxidant enzyme activities. Plants respond to stress conditions by activating ROS scavenging enzymes to mitigate oxidative stress and protect plant cells. However, plants differ in their capability to accumulate antioxidant enzymes. Therefore, to assess this hypothesis, the current study was conducted on onion with the imposition of SS and DS on different onion cultivars. Enzymatic activities increased in all the cultivars compared with their control. Although this increase was not as pronounced in Hazar and Elit under both stress regimes, Sampiyon's response was also the same under drought stress. Conversely, all the other cultivars showed significantly ($P \leq 0.05$) higher enzymatic activities.

4.2.7.1 Superoxide dismutase activity (SOD)

Superoxide dismutase activity was observed in response to salt and drought stress conditions in all the selected onion cultivars used in this study. Higher SOD activity was noticed in Inci, Perama, and Seyhan cultivars under SS and DS conditions, whereas a less increase in SOD activity was observed in Naz cultivar under DS as compared to its counterpart under SS conditions. Sampiyon cultivar accumulated higher SOD under SS condition whereas under DS conditions it showed minimal accumulation of SOD enzyme (Figure 4.13). Increased SOD activity in some of the cultivars suggested a positive role in stress tolerance. Its higher accumulation highlighted the defensive mechanism of onion cultivars to dismutase superoxide anion under SS and DS. The results of this study are in line with the previous study that exhibited the higher accumulation SOD in tolerant cultivars in response to salt stress in onion (Mohamed and Aly, 2008). Moreover, the Persian shallot also showed a similar response to drought stress with higher levels of SOD enzyme to alleviate oxidative stress (Ghassemi-Golezani et al., 2018). Increased SOD activity in response to drought stress was also reported in tolerant tomato genotypes as compared to the sensitive one which confirmed the positive role of SOD accumulation under stress conditions (Rahman et al., 2004).

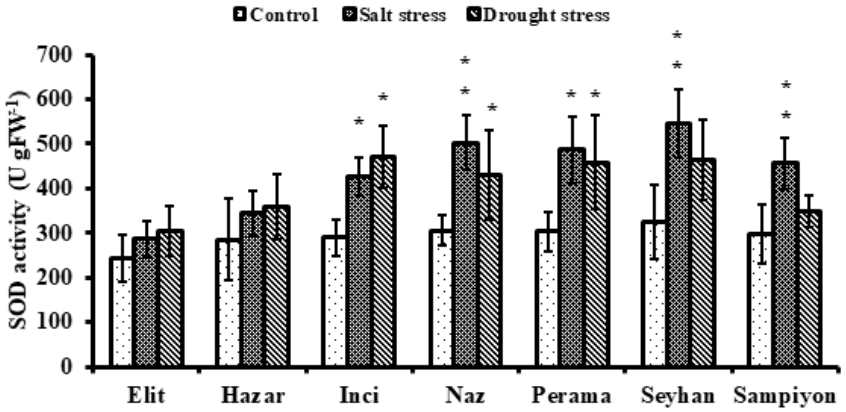


Figure 4.13. Superoxide dismutase activity of onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.7.2 Catalase activity (CAT)

Catalase activity was observed in response to SS and DS conditions in all the selected onion cultivars used in this study. Higher CAT activity was noticed in Inci, Perama, and Seyhan cultivars under SS and DS conditions, whereas a less increase in CAT activity was observed in Naz cultivar under DS as compared to its counterpart under SS conditions. Sampiyon cultivar accumulated higher CAT under SS condition whereas under DS conditions it showed minimal accumulation of CAT enzyme. The least change in CAT enzyme was noticed in the cultivars Elit and Hazar in response to both stresses (Figure 4.14). Catlase is involved to scavenge elevated level of H_2O_2 under stress conditions, therefore higher accumulation CAT enzyme in cultivars Inci, Perama and Seyhan was might be due to their resilient behaviour against the applied stress. It helped in alleviating the toxic ROS species to protect the onion from cellular and oxidative damage (Hasanuzzaman et al., 2017). The upregulation of CAT also assisted in reducing the H_2O_2 buildup, it is chiefly involved in alleviating its oxidative stress in the peroxisome (Mittler, 2002). In onion higher accumulation of catalase showed better tolerance to salt stress which supported the findings of this study (Rady et al., 2018). The results of this study were further corroborated with the previous reports in monocot crops (Ali et al., 2013; Kamal et al., 2019; Wang et al., 2019). Moreover, these cultivars (Inci, Perama, and Seyhan) showed better performance as compared to the cultivars Elit and Hazar under both stress conditions and Sampiyon under DS.

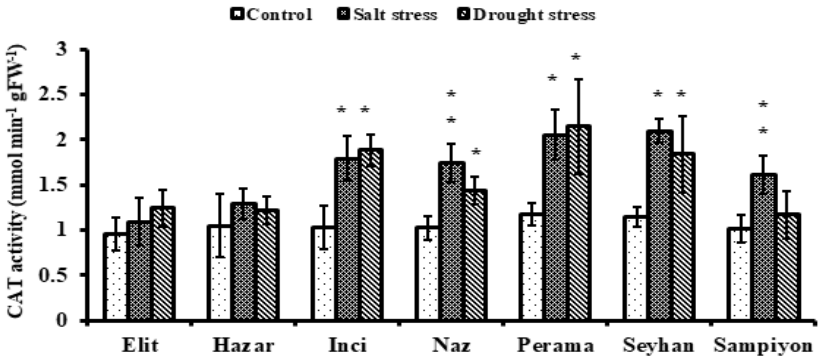


Figure 4.14. Catalase activity of onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.2.7.3 Ascorbate peroxidase activity (APX)

Ascorbate peroxidase activity was observed in response to SS and DS conditions in all the selected onion cultivars used in this study. Higher APX activity was observed in Inci, Perama, and Seyhan cultivars under SS and DS conditions, whereas a less increase in APX activity was observed in Elit, Hazar cultivars under DS. Sampiyon cultivar accumulated higher APX under SS condition whereas under DS conditions it showed minimal accumulation of APX enzyme (Figure 4.15). Seyhan and Perama peaked in response to stress for accumulating APX, which suggested its strong defense mechanism against oxidative stress. It was reported in an earlier study that APX is chiefly responsible for protecting plant cells from oxidative stress under SS and DS (Wang et al., 2010). It might be the case that Elit and Hazar cultivars had a weaker defense system and they were under high pressure of oxidative stress. APX did not accumulate to higher levels in these cultivars as they were measured from the other cultivars in this study. Results are also supported by studies conducted on rice and sorghum that exhibited higher APX activity in tolerant genotypes under SS and DS (Lum et al. 2014; Rossatto et al., 2017; Guo et al., 2018).

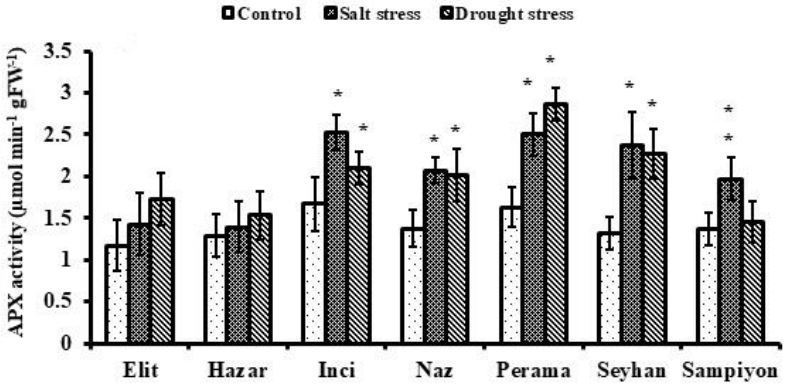


Figure 4.15. Ascorbate peroxidase activity of onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.3 Morphological Parameters of Onion

Morphological changes indicated diminished vegetative growth both in SS and DS conditions. Both stresses strongly influenced the above ground biomass of onion plants.

4.3.1 Number of leaves per plant

Decreased number of leaves per plant was observed in response to both SS and DS conditions. A maximum number of leaves per plant was calculated from the Perama cultivar under SS and DS conditions. The Minimum number of leaves were counted from Naz cultivar with a decrease of 53% under DS, whereas in Elit cultivar leaf growth halted up to 38% SS (Figure 4.16). The cultivar ‘Naz’ showed the minimum number of leaves under both the stresses whereas the cultivars ‘Elit’, ‘Inci’ and ‘Sampiyon’ demonstrated a decreased number of leaves under DS. The stressed condition inhibited the growth of the cultivars with alteration in cell size division and resulted in decreased production of leaf and promoted senescence (Ghodke et al., 2018). Moreover, the findings of this study were corroborated with the earlier report that salt stress negatively influenced the growth of onions genus member leek (Kiremit and Arslan, 2016). The differences in vegetative growth among the genotypes found in this study can be attributed to differences in their genetic traits. Results are also in accordance with Hanci and Cebeci (2015), which reported a reduction in the growth of onion in response to SS and DS.

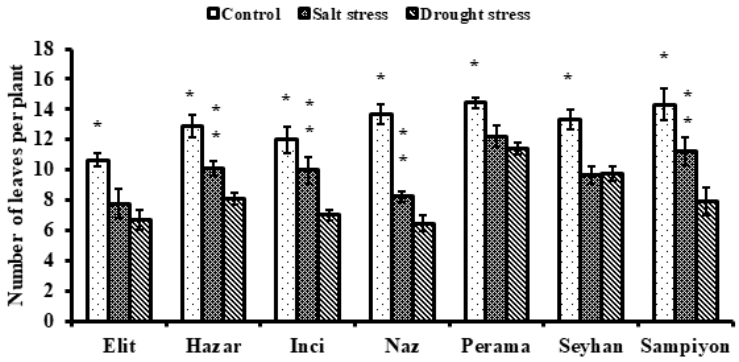


Figure 4.16. Number of leaves per plant of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.3.2 Diameter of leaves

The reduction in leaf diameter was noted in all stressed plants. In drought stress, Naz and Sampiyon showed a 40% decrease and, in salt stress, Inci and Naz showed a 30% decrease; while a higher leaf diameter was observed in Perama under both stress conditions (Figure 4.17). Both stresses resulted in a marked reduction in the number of leaves per plant and diameter of a leaf which was supported by a study in which onion seedlings exhibited reduced leaf growth when onions were deprived of water (Metwally, 2011). Similar results regarding reduction in onion leaf diameter were also reported in response to salt and drought stress in onion, moreover, it was also concluded it is a useful indicator of stress tolerance in onion (Hanci and Cebeci, 2015). The results of this study are also in accordance with the earlier study that showed that with the increase in salt stress a significant reduction in onion leaf diameter was noticed (Hanci and Cebeci, 2019).

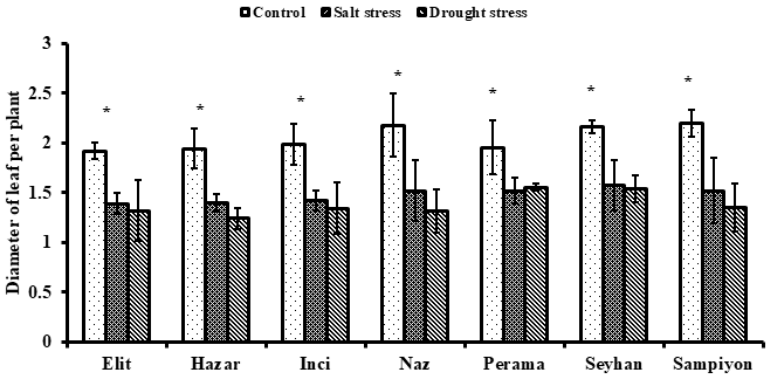


Figure 4.17. The diameter of leaves (cm) of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.3.3 Length of leaf

Hazar cultivar exhibited stunted leaf length under SS and Elit cultivar under DS with a decrease of 24% and 28%, respectively, whereas the cultivars ‘Perama’ and ‘Seyhan’ showed the least reduction in leaf length. (Figure 4.18). The differences in vegetative growth among the cultivars found in this study can be attributed to a disruption in physiological characteristics. The sensitive cultivars might have experienced a decrease in turgor pressure limiting the expansion of the leaf (Fahad et al., 2017). Metwally (2011) also demonstrated the negative effects of stress on the growth of onion. Stunted leaf length observed in this study is in agreement with the earlier report of water deficit growth conditions for onion growth (Zheng et al., 2013). It also depends upon the onion inbred ability to overcome stress environment at early growth stages during cell multiplication as validated by earlier studies on onion (Abbey and Joyce, 2004; Bekele and Tilahum, 2007).

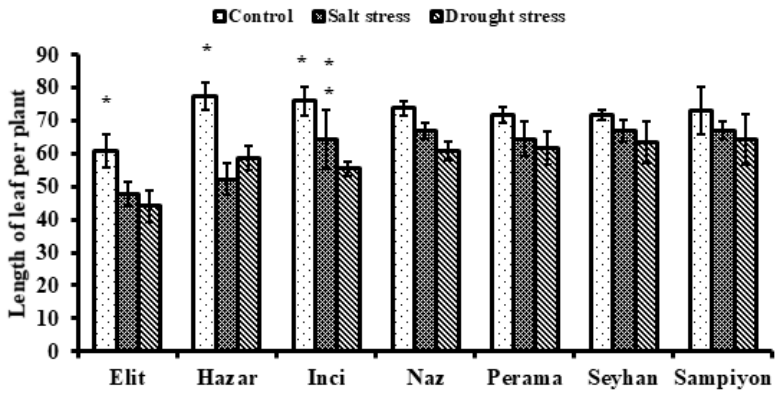


Figure 4.18. Length of leaves per plant of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.4 Yield Related Parameters

Bulb characteristics are important economic traits to evaluate bulb yield. As expected from physiological and morphological responses, onion bulb length and bulb diameter were affected remarkably with stress conditions.

4.4.1 Bulb diameter

The reduction in bulb diameter was more compared to the decrease in bulb length. The reduction trend was similar for all the cultivars under both stress conditions. The cultivar Sampiyon showed a maximum decrease in bulb diameter under SS and Elit cultivar under DS with a decrease of 23% and 33% respectively, whereas the cultivar Inci, Perama, and Seyhan exhibited the least decrease under both stress conditions (Figure 4.19). Decreased bulb diameter was reported in response to drought stress which is in accordance with the results of this study (Ghodke et al., 2018). Bulb diameter is associated with the water availability to onion especially during bulbification stage, as the plants were deprived of water for a total 20 days obvious reduction in bulb diameter was noticed which was corroborated by the study in which difference in bulb diameter was observed with the different irrigation schedulings (de Santa Olalla et al., 1994).

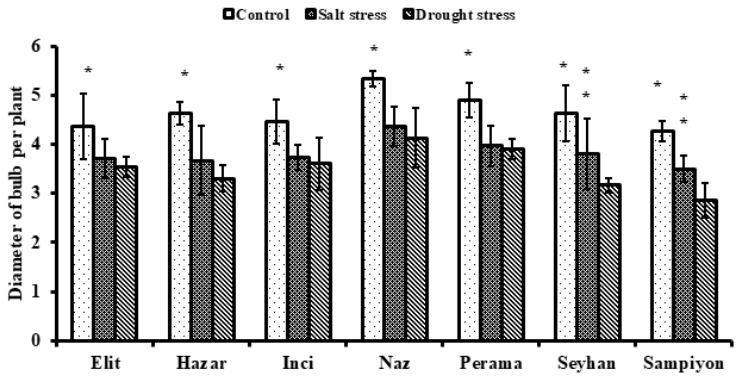


Figure 4.19. Diameter of bulb per plant of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.4.2 Bulb length

Onion bulb length decreased in all the cultivars irrespective of stress treatments. No significant difference was noticed among salt and drought groups. However, Elit, Hazar, Inci, Naz, and Sampiyon cultivars under control conditions showed a significant differences from their counterparts in stressed groups (Figure 4.20). The cultivar Hazar showed a decrease of 20% under SS and 18% under DS. The cultivar Perama and Seyhan showed the least decrease with the exposition to SS and DS conditions. Decreased bulb length was also reported in response to drought stress which is in accordance with the results of this study (Ghodke et al., 2018).

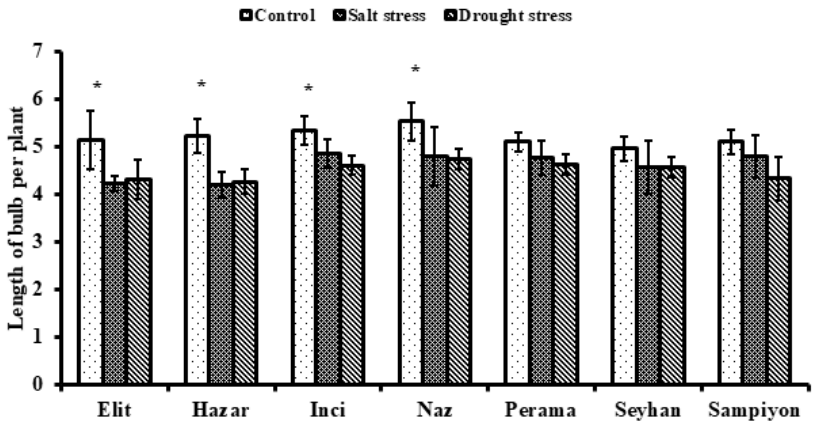


Figure 4.20. Length of bulb per plant of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.4.3 Bulb weight

The significant difference regarding total bulb weight ($P \leq 0.05$) was noticed among all cultivars as well as the plants that were under stress compared to their control. The cultivar 'Perama' showed the highest bulb weight under SS (50.6 g) and DS (44.2 g) conditions, respectively. The lowest bulb weight was observed from cultivar 'Hazar' with a reduction of 51% under SS and 53% in cultivar 'Sampiyon' under DS (Figure 4.21). Stress at the bulbification stage significantly reduced the yield traits in all cultivars tested in response to the SS and DS, as expected based on the previous reports (Metwally, 2011; Zayton, 2007; Pelter et al., 2004). The highest bulb weight of the cultivar 'Perama' is attributed to the tolerance in response to stress conditions. Bulb characteristics are known to show a reduction in response to stress among the cultivars due to the difference in soil water intake and evapotranspiration flux (Pelter et al., 2004; Lipiec et al., 2013). In the present study, cultivars 'Elit' 'Hazar' and 'Sampiyon under DS having lower photosynthetic activity, higher leaf temperature, and lower chlorophyll contents compared to other cultivars, showed the lowest bulb weight in response to SS and DS, which can be explained as the reduction in morphological and physiological characteristics of susceptible cultivars resulting into smaller cell size (Tisne et al., 2010).

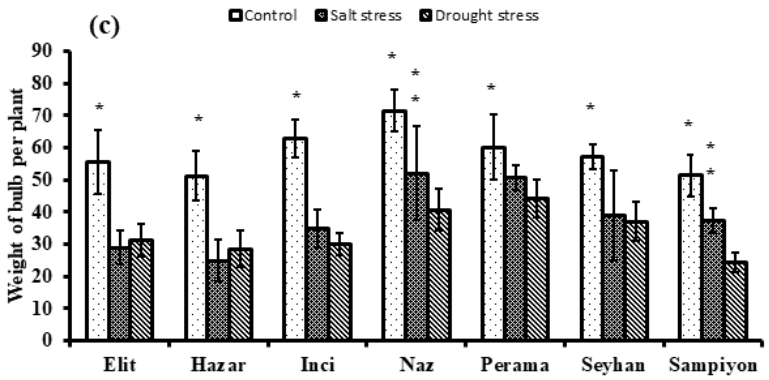


Figure 4.21. Weight of bulb per plant of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.5 Root Morphological Parameters

Roots response towards SS resulted in retarded growth as compared to the DS group, which showed an increase in root characteristics. Root system plays an indispensable role in plant growth and development. It is the site of nutrient uptake essential to support robust growth at every stage of the plant. Abiotic stress conditions alter root functioning and cause morphological changes. Environmental pressure on the root development of onion was unexplored before this study. It gave room to explore root morphological changes against stress in onion. Root developmental changes are generally an unobserved trait for abiotic stress breeding. To the best of my knowledge, this is the first study that focuses on the effects of SS and DS on the root morphology of onion. Drought is the utmost threat to intensified root development. The Stress puts pressure on plant roots to supply water to upper plant parts and support vegetative growth. So, under drought stress, the onion genotypes used in this study showed elongated root development except. However, in the case of salt stress, opposite results were obtained. Root morphological response under SS was different from water deficit conditions in which the root length, root diameter, surface, and root volume. This might be due to excess Na^+ around the vicinity of roots. Moreover, salt uptake by roots decreases the activity of root meristem cells.

4.5.1 Root length of onion cultivars

Salt stress suppressed the total root length by 14% in cultivar ‘Sampiyon’ contrarily least reduction by 4% in ‘Inci’, while under DS the cultivar ‘Sampiyon’ exhibited 17% increase in root length followed by 13% in cultivar ‘Inci’ (Figure 4.22). Root length is the critical factor in absorbing water, and in the current study, extensive root development was observed. Results regarding root length are in accordance with an earlier report on rice which is considered a monocot model plant (Kano et al., 2011). Eggplant roots also responded in the same way as observed the suppressed growth of onion roots (Akinci et al., 2004). The decreased root length in the cultivar ‘Sampiyon’ under SS is due to higher osmotic pressure in the vicinity of roots which prevented the uptake of water and resulted in shorter roots (Sadat-Noori et al., 2008). The results obtained regarding inhibited root length with exposure to SS are in accordance with Basu et al. (2017). The increase in root length of the cultivar ‘Inci’ indicated the plasticity of the root. It might be due to better cell division and expansion of root apical meristem. Thus, it suggests that salt stress altered root growth with enhanced and reduced cell division and cell expansion (West et al., 2004). The growth increment in root could be due to its ability to alleviate osmotic stress by maintaining osmotic potential. The absorbed ions by the root might be quickly separated into vacuoles without their higher accumulation, therefore increasing the turgor of the cell and stimulated cell elongation (Mukami et al., 2020). Increased root length of cultivar ‘Sampiyon’ under the DS is probably due to its sensitivity to moisture deficiency. It is known to force plant roots to extract water from deeper soil pockets (Fang et al., 2017).

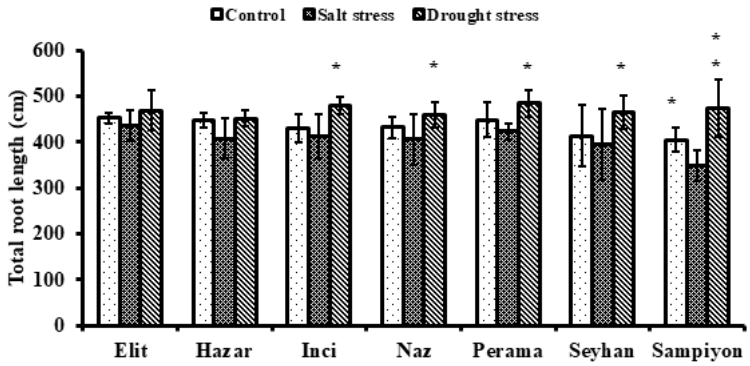


Figure 4.22. Effect on total root length of different onion cultivars subjected to salt and drought stress conditions. Asterisk (*) represents a significant difference ($p \leq 0.05$). Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.5.2 Root diameter of onion cultivars

The average root diameter showed a 15%, reduction with the imposition of SS in the cultivars ‘Elit’ and ‘Naz’. On the other side, the cultivar ‘Seyhan’ showed a 3% increase in root diameter. The cultivar ‘Perama’ showed a 16% increase in root diameter under DS (Figure 4.23). Results indicated an increase in the thickness of root as well root surface area and root volume in response to drought stress. Enhanced root surface area assists in minimizing the depletion of localized water in the vicinity of the root (Franco et al., 2006). Results were strengthened by the response of roots wheat that moisture deficiency forced wheat plant roots to extract water from deeper soil pockets (Fang et al., 2017). The decreased root diameter was observed with the application of SS in all cultivars, whereas DS resulted in increased root diameter. The reduction in root diameter of the cultivars ‘Elit’ and ‘Naz’, is due to ionic toxicity and osmotic pressure (Fricke et al., 2006).

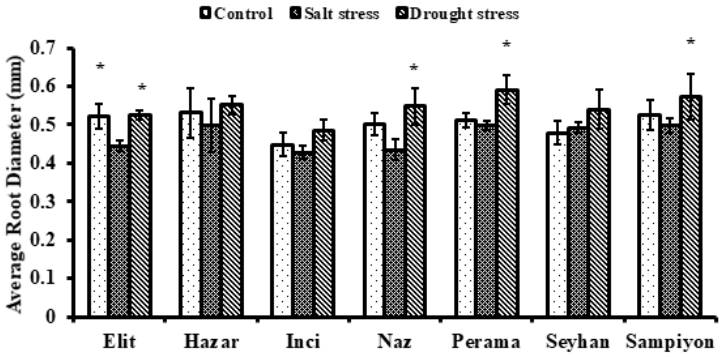


Figure 4.23. Effect on average root diameter of different onion cultivars subjected to salt and drought stress conditions. Asterisk (*) represents a significant difference ($p \leq 0.05$). Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.5.3 Root volume of onion cultivars

Root volume was decreased by 31% in cultivar 'Inci' followed by 23% in cultivars 'Elit' and 'Hazar' under SS. A reverse trend was observed with an increase in root volume by 35% in the cultivar 'Inci' under DS (Figure 4.24). Decreased root volume might be correlated with the higher accumulation of solutes in the vicinity of the plant. Numerous studies reported the same influence of SS on root architecture of rice, maize, wheat, and tomato (Ijaz et al., 2019; Karni et al., 2010; Annunziata et al., 2017).

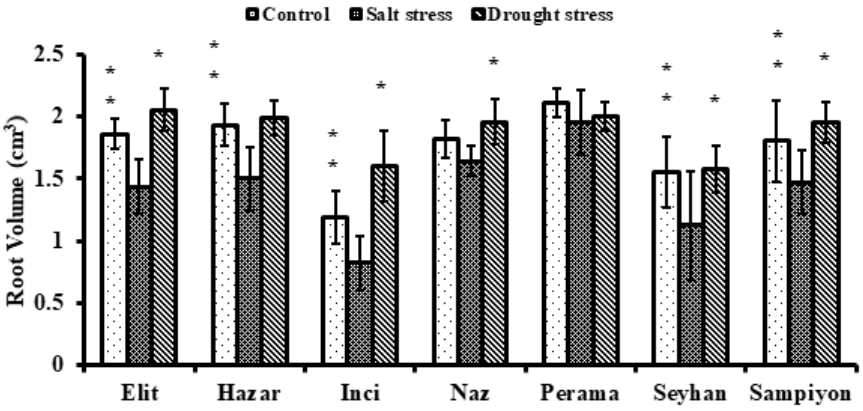


Figure 4.24. Effect on root volume of different onion cultivars subjected to salt and drought stress conditions. Asterisk (*) represents a significant difference ($p \leq 0.05$). Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.5.4 Root surface area of onion cultivars

The root surface area also decreased under SS, whereas the same variable depicted an increasing trend under DS. The cultivars 'Sampiyon' and 'Elit' showed a decline of 12% in root surface area in response to SS, while the cultivars 'Hazar' and 'Sampiyon' resulted in an increase of 16% and 13%, respectively in root surface area under DS (Figure 4.25). The root surface area decreased in response to SS while it increased under DS. The cultivars 'Elit' 'Sampiyon', and 'Hazar' showed a decline in root surface area, respectively. The reduction under SS was due to inhibited root growth due to osmotic stress and hampers root meristem size (Jiang et al., 2016). In contrast, a reverse trend was noticed in the cultivars 'Hazar', 'Sampiyon', and 'Elit' resulted in an increase in root surface area under DS. It might be due to moisture deficiency that triggers the synthesis of abscisic acid for the closure of stomata (Hussain et al., 2016). These cultivars also showed poor performance i.e., lower RWC, damage to photosynthesis, and photosynthetic pigments regarding which triggers oxidative stress. These disruptions in physiological processes exert pressure with enlarged root surface area to extract water. Findings of this study are consistent with previous studies that reported a similar influence of stress on root architecture of garlic, potato, tomato, eggplant, and pea (Akinci et al., 2004; Al-Safadi and Faoury, 2004; Karni et al., 2010; Khenifi et al., 2011; Pereira et al., 2020).

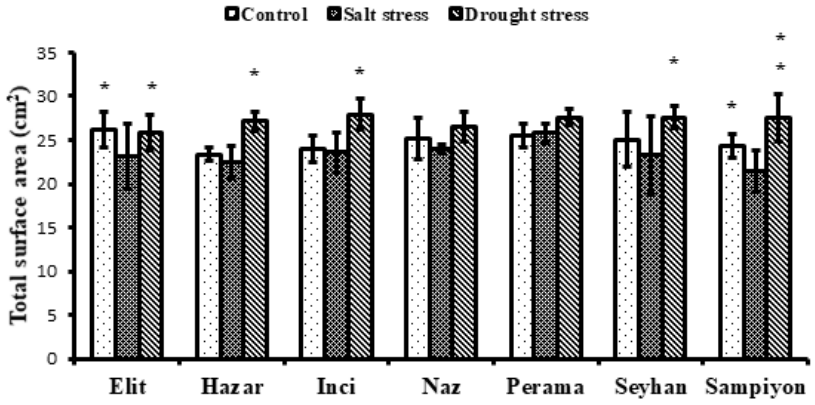


Figure 4.25. Effect on total root surface area of different onion cultivars subjected to salt and drought stress conditions. Asterisk (*) represents a significant difference ($p \leq 0.05$). Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.6 Principal Component Analysis

The interrelationship among selected onion cultivars along with the tested variables under SS and DS conditions were analyzed by biplot principal component analysis (PCA) as shown in Figure 4.24. It revealed that the first two components explained 60.78% (contributed by PC1 38.78%, and PC2 22.00%) under SS conditions. DS conditions showed a total variation of 71.36% (contributed by PC1 53.12%, and PC2 18.24%) among the onion cultivars for the measured traits. PCA biplot grouped the onion cultivars based on their response to the tested morphological and physiological variables/traits. In SS, the cultivars 'Perama', 'Seyhan', and 'Inci' depicted positive PC1 values. The cultivar 'Perama' showed the best performance for chlorophyll index, total chlorophyll contents, photosynthesis, length of leaf, and length of the bulb. The cultivar 'Seyhan', was best performing for the traits such as root surface area, average root diameter, a diameter of bulb, carotenoid content, stomatal conductance, and transpiration rate. The cultivar 'Inci', was best in total root length and RWC under SS. The cultivars 'Perama' and 'Seyhan', were referred to as tolerant based on their response to the tested variables, whereas the cultivars 'Hazar', 'Elit' and 'Sampiyon' were salt sensitive. The cultivar 'Inci' showed an average response. In DS condition, the cultivar 'Seyhan' showed maximum chlorophyll index, root diameter, and length of the bulb, while the cultivar 'Perama' indicated the maximum diameter of a leaf, number of leaves, RWC, photosynthesis, transpiration rate, and weight of bulb. The cultivars 'Elit' 'Hazar', and 'Sampiyon', were drought-sensitive according to the observed traits (Figure 4.26).

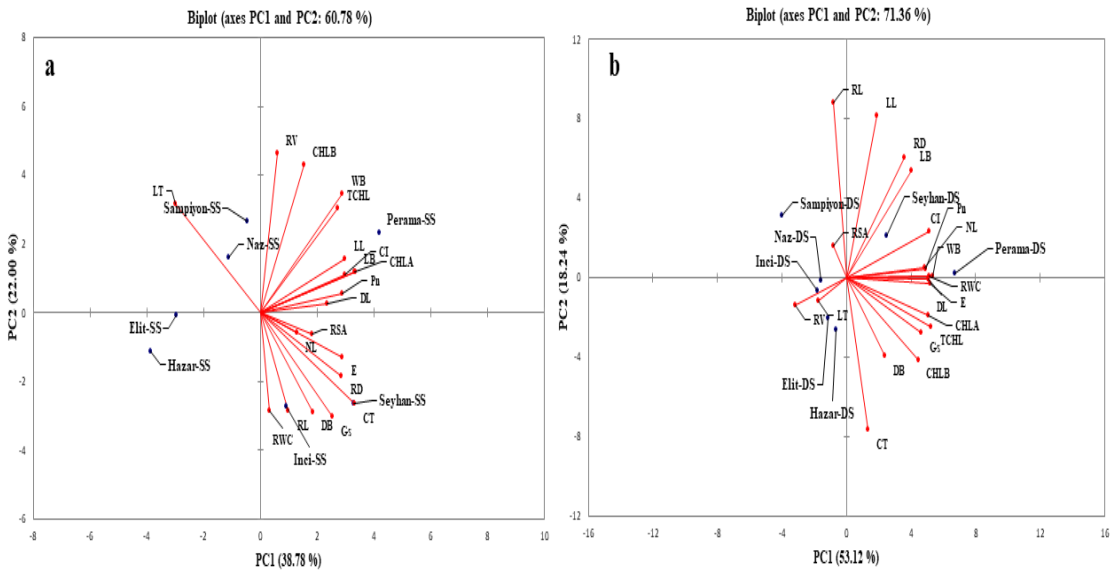


Figure 4.26. Principal component analysis biplot for morpho-physiological variables of seven onion cultivars grown under salt and drought stress conditions. PCA biplot is a combination of the score plot of onion cultivars (represented as dots) and the loading plot of variables (represented as vectors). SS: salt stress, DS: drought stress, NL: number of leaves, DL: diameter of a leaf, LL: length of the leaf, DB: diameter of the bulb, LB: length of the bulb, WB: weight of the bulb, TRL: total root length, ARD: average root diameter, RV: root volume, RSA: root surface area, RWC: relative water content, LT: leaf temperature, CI: chlorophyll index, CHLA: chlorophyll a, CHLB: chlorophyll b, TCHL: total chlorophyll, CT: carotenoid content, Pn: Photosynthesis, Gs: stomatal conductance, E: transpiration rate

4.7 Molecular Analysis of Onion Cultivars

4.7.1 RNA extraction

Plant RNA was isolated from young leaves of all the onion cultivars under investigation. After RNA extraction, RNA concentrations were checked on nanodrop (Table 4.1), and later it was checked by running on 1% gel and visualized under UV light (Figure 4.27).

Table 4.1. Concentration of RNA

Sr. No.	Name of samples	Concentration ng µl
1	Elit-C	1009.3
2	Elit-SS	544.2
3	Elit-DS	278.1
4	Hazar-C	396.7
5	Hazar-SS	822.8
6	Hazar-DS	730.6
7	Inci-C	74.0
8	Inci-SS	221.0
9	Inci-DS	173.6
10	Naz-C	412.5
11	Naz-SS	165
12	Naz-DS	191
13	Perama-C	244.6
14	Perama-SS	183.6
15	Perama-DS	298.4
16	Seyhan-C	348.8
17	Seyhan-SS	197.4
18	Seyhan-DS	152.3
19	Sampiyon-C	272.6
20	Sampiyon-SS	172.3
21	Sampiyon-DS	230.5

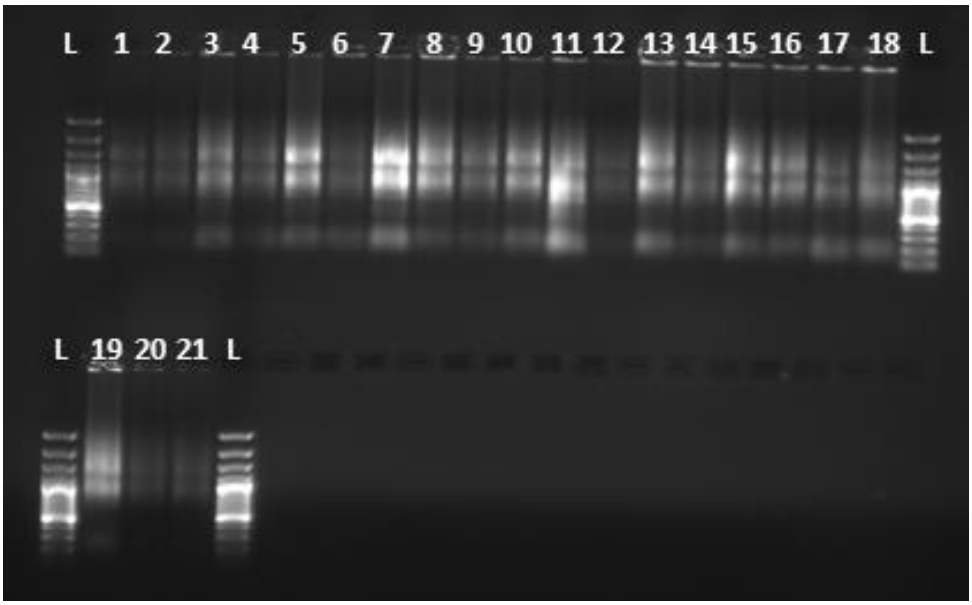


Figure 4.27. Isolated RNA from onion leaves under drought and salt stress conditions. L (100 bp ladder), 1-21 RNA of onion cultivars used in this study

4.7.2 cDNA synthesis

After measuring the quality of the RNA samples, they were used for the synthesis of cDNA for molecular analyses to observe gene expression of onion cultivars acclimatized to stress conditions.

4.7.3 Gradient degenerate PCR

Gradient degenerate PCR was conducted with two selected primers (PSII and CAT) with two control samples of randomly selected cultivars at four different temperatures. The detail of the labeled names on the gel picture is mentioned in the table at the end. The product size of Primer1 was (PSII is 527 bp), Primer 2 (CAT is 396 bp). (Figure 4.28) (Table 4.2).

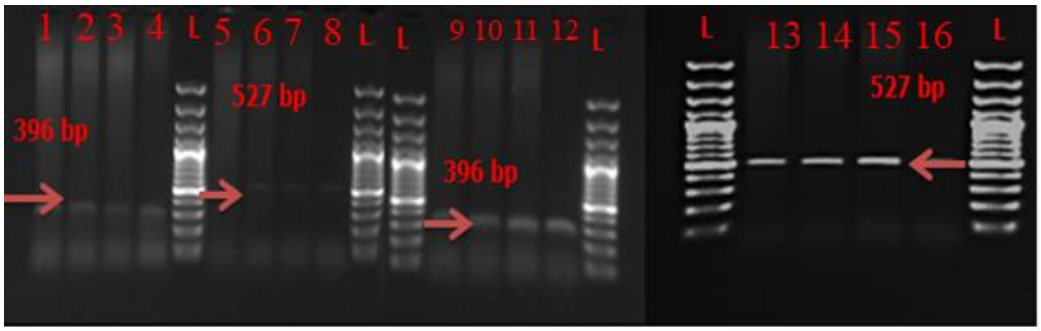


Figure 4.28. Gradient Degenerate PCR results of amplified fragments of photosystem II (527 bp) and catalase (396 bp) in onion. DNA ladder (L) 100 bp was used

Table 4.2. Acronyms detail of degenerate PCR

Samples	Acronyms	Details
1	P1C1T1	CAT primer, Cultivar 1, 44 °C
2	P1C1T2	CAT primer, Cultivar 1, 48 °C
3	P1C1T3	CAT primer, Cultivar 1, 52 °C
4	P1C1T4	CAT primer, Cultivar 1, 56 °C
5	P2C1T1	PSII primer, Cultivar 1, 44 °C
6	P2C1T2	PSII primer, Cultivar 1, 48 °C
7	P2C1T3	PSII primer, Cultivar 1, 52 °C
8	P2C1T4	PSII primer, Cultivar 1, 56 °C
9	P3C1T1	CAT primer, Cultivar 1, 44 °C
10	P3C1T2	CAT primer, Cultivar 1, 48 °C
11	P3C1T3	CAT primer, Cultivar 1, 52 °C
12	P3C1T4	CAT primer, Cultivar 1, 56 °C
13	P1C2T1	PSII primer, Cultivar 2, 44 °C
14	P1C2T2	PSII primer, Cultivar 2, 48 °C
15	P1C2T3	PSII primer, Cultivar 2, 52 °C
16	P1C2T4	PSII primer, Cultivar 2, 56 °C

4.7.4 Purification of PCR amplified fragment from agarose gel

The amplified fragment of MnSOD, CAT, and APX was purified from agarose gel with GeneJET Gel extraction in such a way that the required band of a gene was cut with the help of sterilized blade from the gel.

4.7.5 TA cloning

Gel eluted fragments were ligated by inserting them into a TA vector solution. Later it was transformed into *E. coli* strain Top 10 and incubated at 37 °C overnight.

4.7.6 Colony PCR

Positive clones were selected by picking ten random colonies to conduct colony PCR with M13 primers (Figure 4.29).

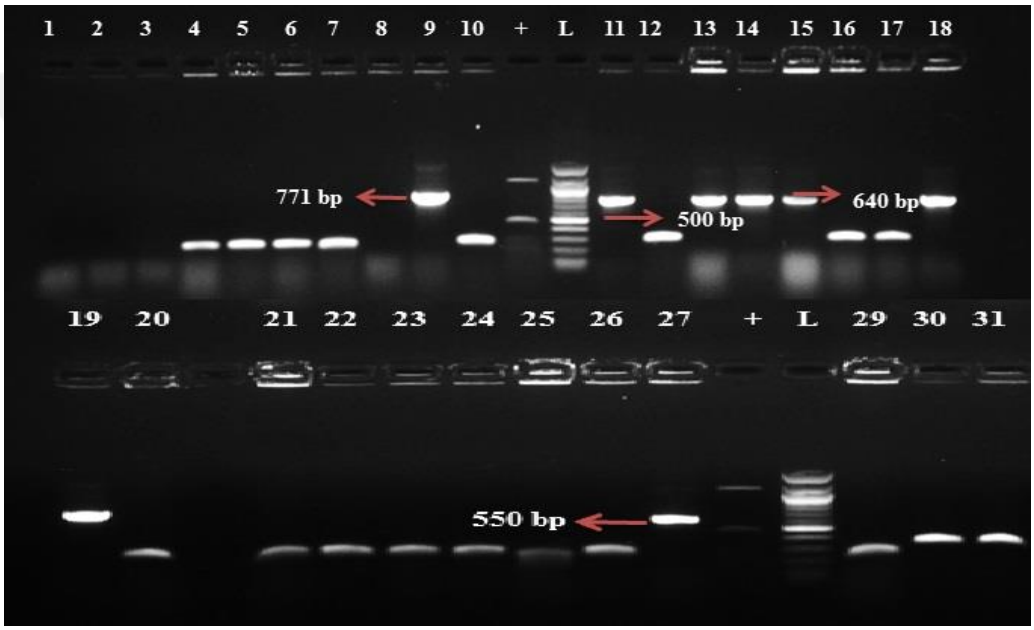


Figure 4.29. Colony PCR results. L (100 bp ladder), 1-31 clones that were selected for confirmation, + positive control

4.7.7 Plasmid DNA isolation from positive clones

The positive clones were confirmed by colony PCR after that colony culture was conducted to harvest plasmid. Plasmid isolation of the positive clones was done, and their concentration was observed by running agarose gel as shown in Figure 4.30

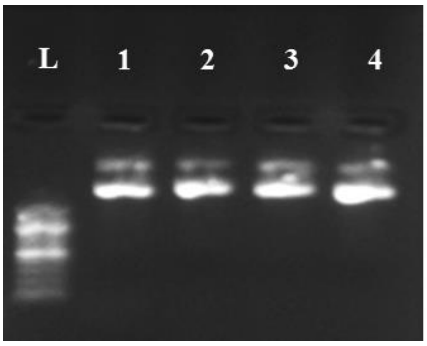


Figure 4.30. Plasmid isolation of the positive clones L (100 bp ladder), 1-4 positive clones

4.7.8 Restriction analysis of cloned DNA

Positive clones of CAT, Photosystem II plasmids were further confirmed by restriction digestion analysis with EcoRI restriction enzymes to excise fragment. Result revealed proper excision of the desired fragment (Figure 4.31).

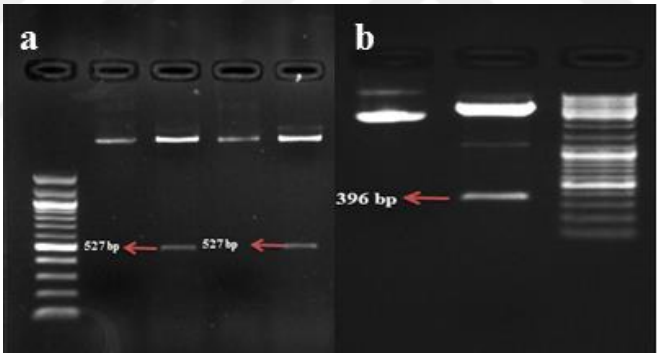


Figure 4.31. Confirmation of the cloned gene a. PSII b. CAT gene in onion by restriction digestion

4.7.9 Blast analysis of the sequencing results

After confirmation of the sequencing results by having sequenced data and blast analysis (Figure 4.32). According to the available literature and my knowledge, there is no study conducted to explore onion at a molecular level. Thereby no stress-related genes have been identified in an onion to date. To bridge this gap, isolation of antioxidant enzyme gene CAT and PSII gene from an onion using degenerate primer design approach. Later qRT-PCR primers were designed from the gene fragment obtained to quantify the

expression of the catalase gene in all the seven onion cultivars under salt and drought stress conditions.

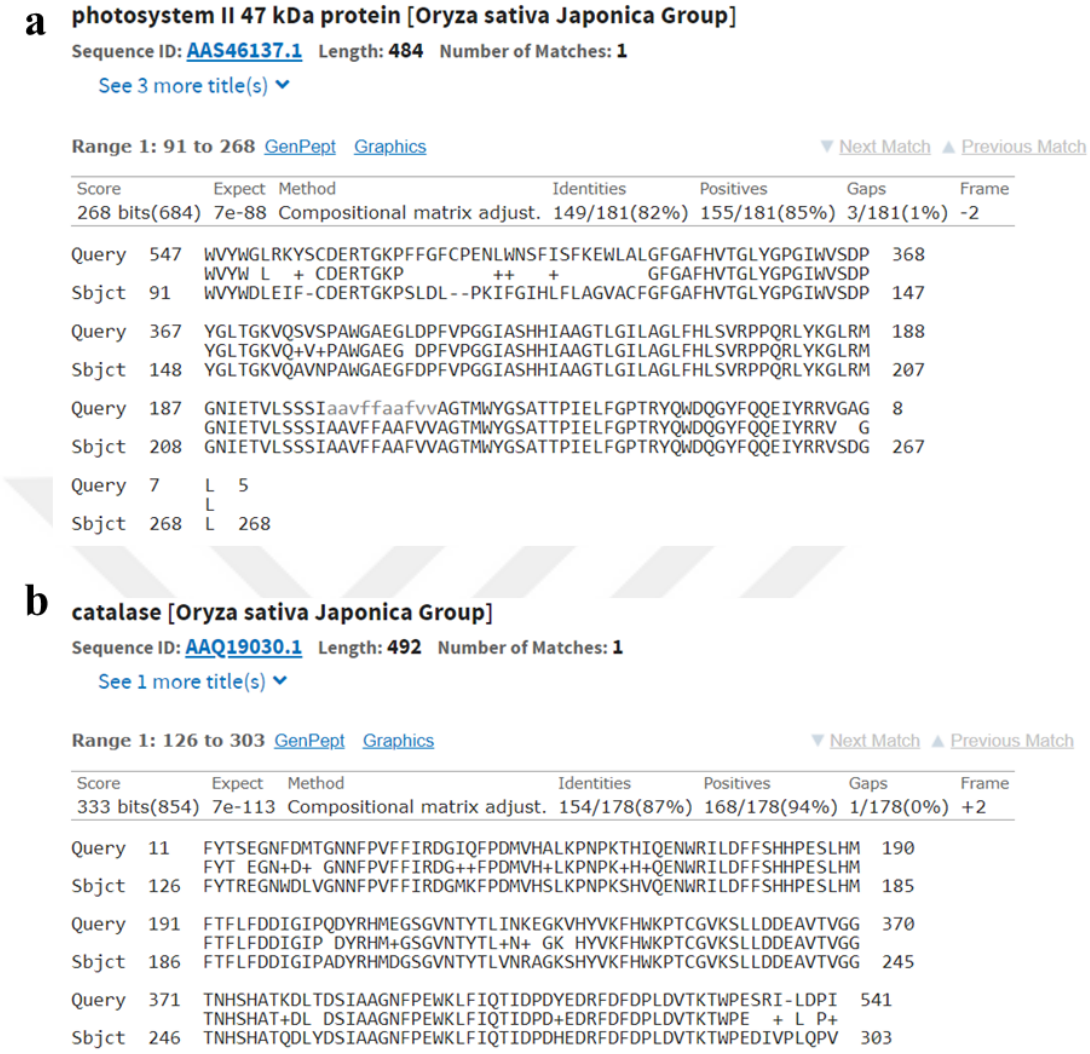


Figure 4.32. Blastx results of (a) photosystem II and (b) catalase gene fragments in onion

4.7.10 Expression of CAT gene in *A.cepa* leaves

Catalase enzyme's gene expression was observed by exploiting qRT-PCR using Cdna isolated from all the seven onion cultivars subjected to drought and salt stress conditions along with their control plants. The stress conditions triggered the upregulation of the catalase gene. All the cultivars behaved differentially to the transcript level changes of the catalase enzyme gene. It was noticed that expression level peaked in Perama (8.84) cultivar, Inci (8.04), Seyhan (6.54), and Naz (4.89) under DS condition whereas slight upregulation was noticed in Sampiyon (1.96). In case SS condition gene expression level

peaked in Seyhan (7.79), Perama (7.64), Inci (7.06), Naz (5.57), and Sampiyon (4.97) cultivars whereas minimal increase was observed in Elit (2.05) cultivar (Figure 4.33). Catalase is involved in the detoxification of ROS species H_2O_2. Catalase is one of the key enzymes involved in the alleviation of oxidative damage by converting H_2O_2 to H_2O in peroxisomes (Demiral and Türkan, 2005). Moderate concentration of H_2O_2 is essential in plants which work as a signaling molecule, but higher concentration imparts devastating effects as it is noxious for plant cells triggering oxidative damage. Catalase function is also inevitable as an enzymatic system in photorespiration for protecting photosynthesizing cells in response to oxidative stress. It was supported by the study on barley mutant lines which showed decreased catalase activity responsible for diminished growth due to photorespiratory conditions (Fath et al., 2001). Results showed that Perama, Seyhan, and Naz exhibited a higher expression level of catalase gene as compared to other cultivars under investigation (Figure 4.29). It might be because of the ability of these cultivars to regulate transcript levels in response to stress. Higher gene expression also stimulated increased synthesis of catalase enzyme in these cultivars as discussed above (Figure 4.13). Furthermore, higher expression levels suppressed the H_2O_2 concentration in onion. Current study showed similar results regarding upregulation of transcript level of catalase gene in stressed plants as it was earlier reported in rice, which is also in accordance with a study on wheat under drought stress conditions (Joo et al., 2014, Moloudi et al., 2013). Catalase gene response under salt stress was also reported for the confirmation of the function of this gene. It conferred tolerance to the sensitive rice cultivar with the transformation of CAT gene with a higher expression level in transgenic rice as compared to wild type rice. As this study strengthened the role of CAT enzyme in scavenging ROS towards tolerance of oxidative stress (Nagamiya et al., 2007).

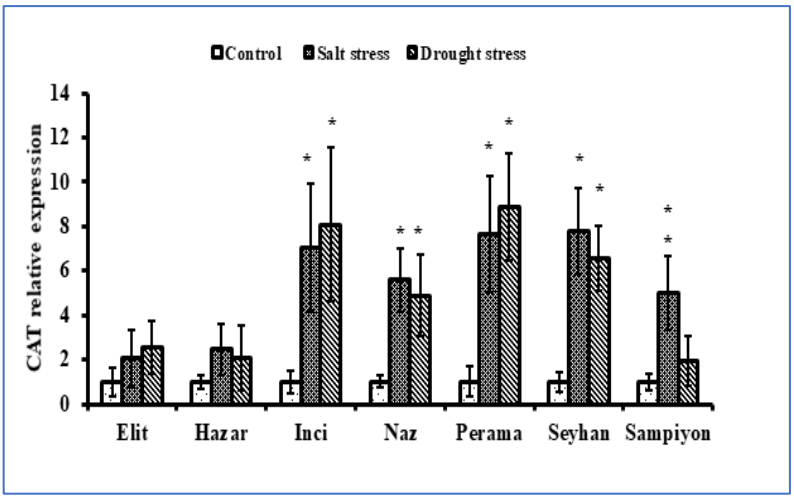


Figure 4.33. Relative expression level of CAT gene of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.7.11 Expression of photosystem II gene in onion leaves

To evaluate the disruption in the photosynthetic performance of the onion cultivars changes in transcript level of photosystem II gene were measured by qRT-PCR. Downregulation in the transcript levels of all the cultivars was observed. The decrease in transcript levels was noticed more in drought-stressed plants contrary to salt-stressed plants in all cultivars. The least downregulation in the expression was quantified in Perama cultivar (0.92) under DS and (0.85) under SS conditions followed by Seyhan cultivar (0.87) and (0.73) with no significant ($P \leq 0.05$) difference with the control. Conversely, the significant ($P \leq 0.05$) downregulation was measured in Elit and Hazar cultivars under both stress conditions, while Sampiyon (0.43) exhibited decreased gene expression level under DS (Figure 4.34). In this study characterization of PSII photochemistry response pattern to drought and salt stresses in onion was also done. PSII is the key component involved in the photosynthesis process. It consists of multiple subunit complexes embedded in the thylakoid membrane of plants (Umena et al., 2011). Results indicated that the gene expression level of PSII was pronouncedly lower in Elit and Hazar under both stress conditions, while Naz and Sampiyon showed a marked decrease under drought stress. However, no significant ($P \leq 0.05$) difference was noticed in the remaining cultivars compared with the control (Figure 4.30). Photosynthesis and photosynthetic pigments are sensitive to salt and drought stress that generally suppresses

that photosynthesis rate and even damages the photosynthetic machinery of the plant. Results regarding lower transcript abundance of PSII are in accordance with the study that demonstrated the devastating effect of drought stress on barley (Yuan et al., 2005). It was further confirmed from the data of another study on wheat reported similar results as observed in onion regarding PSII expression level in stressed plants (Wang et al., 2011). The lower PSII expression level was resulted due to damaged photosynthetic activity influencing steady-state content of protein complexes essential for primary functions of photosynthesis (Liu et al., 2006). Drought and salt stress also inhibit the swift replacement of impaired PSII with the degradation of mRNA which changes the function of the protein compounds directing the detachment and relocation of PSII proteins (Yuan et al., 2005). Thereby it is logical to observe the inhibition in the transcription levels of onion leaves in response to salt and drought stress conditions as noticed in this study.

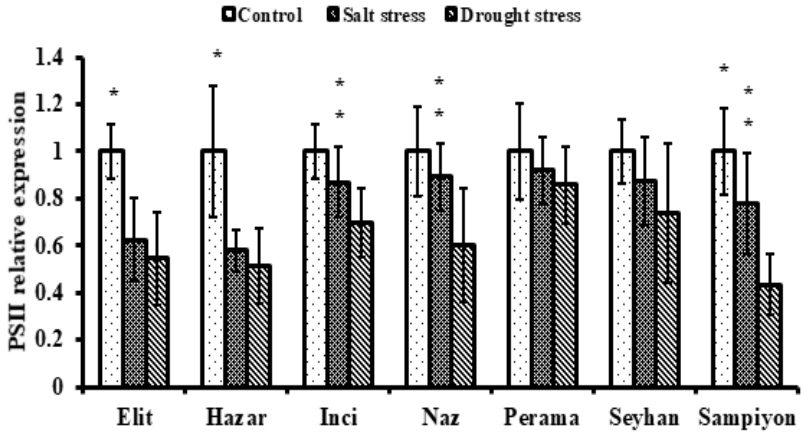


Figure 4.34. Relative expression level of PSII gene of different onion cultivars subjected to drought and salt stress conditions. Asterisk (*) represents a significant difference. Two vertical asterisks (**) shows the significant difference among stressed counterpart (salt or drought stress)

4.7.12 Correlation and principal component analysis

Principal component analysis (PCA) explains the variance in data by considering the first few components, to reduce the dimensionality of data sets (Wold et al., 1987). PCA analysis was conducted for seven onion cultivars by considering biochemical variables, i.e., MDA, PRO, SOD, CAT, and APX, in response to salt stress conditions. PCA biplot

of the first two components (PC1 and PC2) explained 95.95% variance for the biochemical variables tested among seven onion cultivars (Figure 4.35).

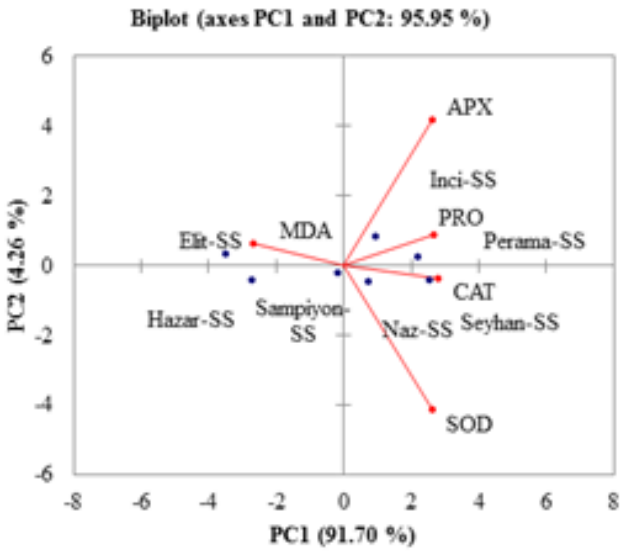


Figure 4.35. PCA biplot of the first two principal components (95.95%) for biochemical variables of seven different onion cultivars grown under salt stress conditions. PCA biplot is a combination of the score plot of onion cultivars (represented as dots) and the loading plot of biochemical variables (represented as vectors). SS, salt stress; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; PRO, proline, and MDA, malondialdehyde

The first principal component (PC1) can be identified as the variation in seven different onion cultivars under salt stress. The second principal component (PC2) was considered for the distribution of five biochemical variables to a salt stress response. PCA biplot grouped onion cultivars having the same differential response to biochemical variables. Cultivars Inci, Naz, Seyhan, and Perama appearing to have positive PC1 values have a similar response to biochemical variables (CAT, SOD, APX, and PRO). It showed that these cultivars accumulated antioxidants (CAT, SOD, APX) and proline (PRO) as a response towards salt stress. Contrarily, Elit, and Hazar showed negative PC1 values with a higher accumulation of MDA contents. Therefore, these biochemical attributes of Elit and Hazar lead to the formation of a separate sensitive group of cultivars depicting oxidative stress when exposed to salt stress. Sampiyon's response under salt stress is close to the origin and revealed moderate sensitivity to SS. The correlation study (Table 4.4) among the biochemical variables of PCA showed significant positive correlations between superoxide dismutase with catalase ($r=0.93^{**}$), ascorbate peroxidase ($r=0.79^{*}$)

and proline (r=0.90**), whereas strongly negative correlation to malondialdehyde (r=−0.87**). The correlation scatters matrix displaying the graphical illustration of the pairwise scatter plot is presented in Figure 4.36.

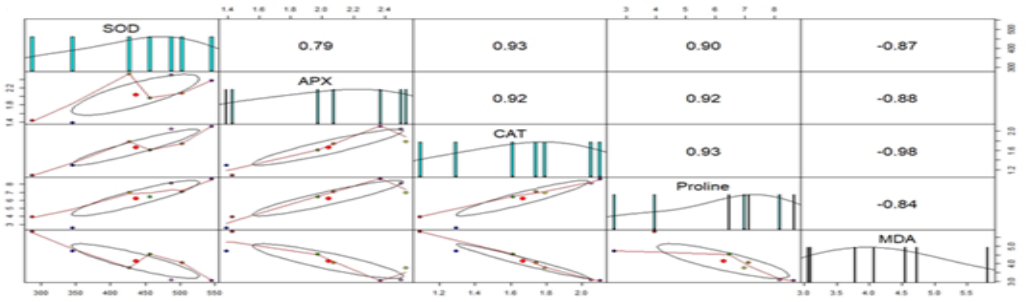


Figure 4.36. Scatter plot matrices of the biochemical response of onion cultivars under salt stress conditions. SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase, and MDA, malondialdehyde

The PCA analysis of the seven onion cultivars subjected to drought stress was also conducted. PCA biplot of the first two components (PC1 and PC2) explained 96.65% variance for the biochemical variables tested in response to drought stress (Figure 4.37).

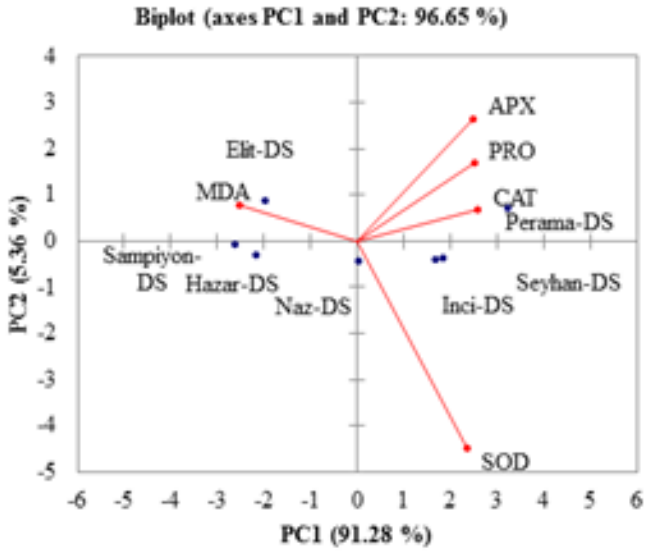


Figure 4.37. Principal component analysis biplot of the first two principal components (96.65%) for biochemical variables of seven different onion cultivars grown under drought stress conditions. PCA biplot is a combination of the score plot of onion cultivars (represented as dots) and the loading plot of biochemical variables (represented as vectors). DS, drought stress; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; PRO, proline and MDA, malondialdehyde

The first principal component (PC1) can be identified as the variation in seven different onion cultivars under drought stress. The second principal component (PC2) was considered for the distribution of five biochemical variables to drought stress. In case of drought stress, the cultivars Inci, Seyhan, and Perama appearing to have positive PC1 values exhibited a similar response to biochemical variables (CAT, SOD, APX, and PRO). Contrarily, Elit, Hazar, and Sampiyon showed negative PC1 values with a higher accumulation of MDA contents. It leads to the formation of a separate sensitive group of cultivars depicting oxidative stress when exposed to drought stress. Naz was close to origin and showed slightly inappreciable sensitivity to drought conditions. The correlation study (Table 4.4) among the biochemical variables for drought stress response of PCA showed significant positive correlations between superoxide dismutase to catalase ($r=0.87^{*}$), ascorbate peroxidase ($r=0.76^{*}$) and proline ($r=0.81^{*}$), whereas strongly negatively correlated to malondialdehyde ($r=-0.89^{**}$). The correlation scatters matrix displaying the graphical illustration of the pairwise scatter plot is presented in Figure 4.38.

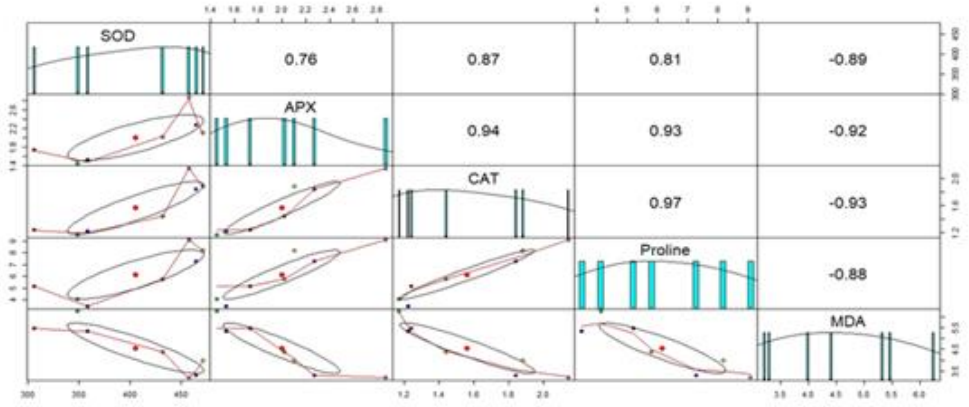


Figure 4.38. Scatter plot matrices of the biochemical response of onion cultivars under drought stress conditions. SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase, and MDA, malondialdehyde

The PCA analysis of the seven onion cultivars subjected to drought stress was also conducted. PCA biplot of the first two components (PC1 and PC2) explained 93.57% variance for the antioxidant enzymes tested in response to drought stress (Figure 4.39).

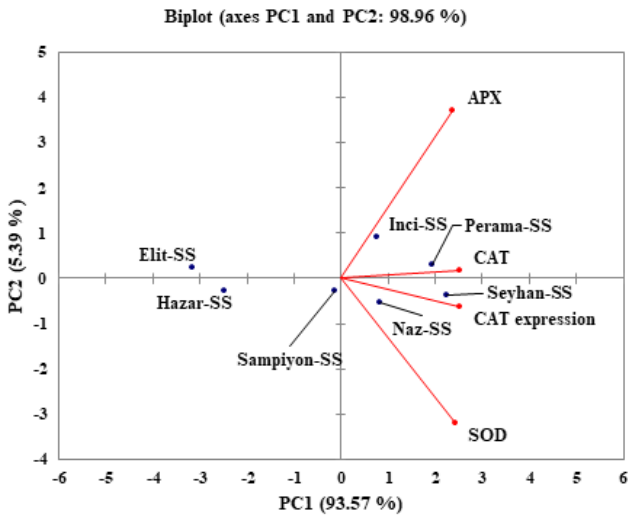


Figure 4.39. Principal component analysis biplot of the first two principal components (93.57%) for antioxidant enzymes of seven different onion cultivars grown under salt stress conditions. PCA biplot is a combination of the score plot of onion cultivars (represented as dots) and the loading plot of biochemical variables (represented as vectors). SS, salt stress; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase

The PCA analysis revealed that the cultivars Perama, Seyhan, and Naz had higher CAT activity and elevated expression of the CAT gene in case of salt stress conditions. These genotypes constituted a group of tolerant cultivars to salt stress. The cultivar Inci had accumulated higher APX in response to saline conditions whereas Sampiyon showed moderate sensitivity to salt stress. In comparison to other cultivars, Elit and Hazar showed less antioxidant accumulation and hence termed as sensitive cultivars. Catalase activity and gene expression of catalase were positively correlated (Figure 4.40).

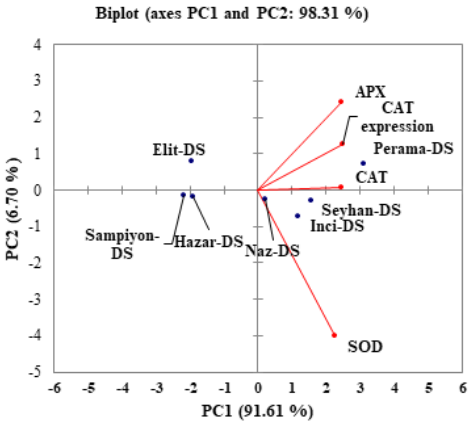


Figure 4.40. Principal component analysis biplot of first two principal components (98.31%) for antioxidant enzymes of seven different onion cultivars grown under salt stress conditions. PCA biplot is the combination of score plot of onion cultivars (represented as dots) and the loading plot of biochemical variables (represented as vectors). DS, drought stress; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase

The cultivars Inci, Perama, Seyhan had higher CAT expression which might result in higher CAT activity under drought stress conditions. Since two variables (CAT and CAT expression) were positively correlated, it might be interpreted that higher transcript abundance resulted in higher accumulation of catalase in these cultivars as expected. Naz exhibited moderate tolerance to drought stress conditions whereas Elit, Hazar, and Sampiyon showed lower transcript abundance and did not accumulate antioxidants in response to drought stress hence constitute a group of sensitive cultivars. The PCA analysis for catalase activity with respect to cultivars' response to both stress conditions was also conducted. The PCA biplot of the first two components (PC1 and PC2) explained 90.94% variance for the catalase enzyme (Figure 4.41).

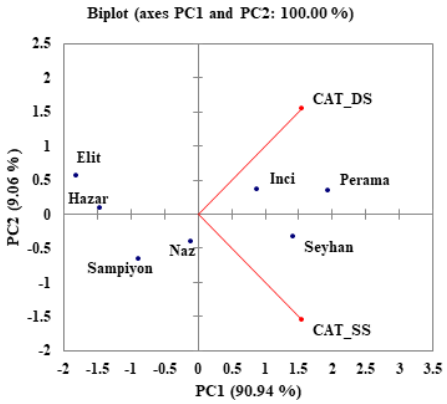


Figure 4.41. Principal component analysis biplot of first two principal components (90.94%) for catalase enzyme of seven different onion cultivars with respect to salt and drought stress conditions. PCA biplot is a combination of the score plot of onion cultivars (represented as dots) and the loading plot of biochemical variables (represented as vectors). DS, drought stress; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase

It was noticed that the cultivars Inci and Perama had higher CAT under drought stress while Seyhan had higher CAT under salt stress conditions. The other cultivars Elit, Hazar, and Sampiyon had comparatively less accumulation of CAT enzyme. The correlation study (Table 4.3) among the antioxidant enzymes for salt and drought stress response showed significant positive correlations between antioxidant enzymes whereas the correlation of CAT expression was strongly correlated to catalase enzyme under salt (r=0.98**) and drought (r=0.93**) stress conditions.

Table 4.3. Pearson's correlation coefficients between the antioxidant enzymes and CAT gene expression under salt stress (above main diagonal) and drought stress (below main diagonal) conditions

	SOD	**APX**	**CAT**	**CAT exp**
SOD	1	0.78**	0.92*	0.95**
APX	0.76*	1	0.92**	0.90**
CAT	0.86**	0.94**	1	0.98**
CAT exp	0.83**	0.98**	0.93**	1

** highly significant ($p<0.01$), *significant ($p<0.05$). SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; CAT exp, catalase gene expression.

The correlation study (Table 4.4) among the biochemical variables of all the onion cultivars revealed that superoxide dismutase, catalase, ascorbate peroxidase, and proline

are strongly correlated with each other while malondialdehyde is negatively correlated which divided the onion cultivars into salt and drought sensitive (Elit, Hazar) and tolerant (Inci, Perama, Seyhan) groups. It was confirmed by an earlier study elucidating the role of antioxidant enzymes (SOD, CAT, APX) and proline for stress tolerance in rice (Lum et al., 2014). Moreover, exogenous application of proline also elucidated higher proline accumulation concomitantly enhanced antioxidant defense mechanism in rice (Sobahan 2018), whereas decreased MDA concentration (Wu et al., 2017).

Table 4.4. Pearson's correlation coefficients between the biochemical variables under salt stress (above main diagonal) and drought stress (below main diagonal) conditions

	SOD	**APX**	**CAT**	**PRO**	**MDA**
SOD	1	0.79*	0.93**	0.90**	-0.87**
APX	0.76*	1	0.92**	0.92**	-0.88**
CAT	0.87*	0.94**	1	0.93**	-0.98**
PRO	0.81*	0.93**	0.97**	1	-0.84*
MDA	-0.89**	-0.92**	-0.93**	-0.89**	1

** highly significant ($p<0.01$), *significant ($p<0.05$). SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; PRO, proline; MDA, malondialdehyde

CHAPTER V

CONCLUSION

Drought and salt tolerance of onion depends on its performance for its growth and yield traits. Current study was conducted to evaluate the performance of onion cultivars under drought and salt stresses. It was concluded that drought and salt stresses had negatively affected all the cultivars used in the study depending on their genetic traits. The cultivars Inci, Seyhan, and Perama performed better than the other cultivars under both stress conditions. They showed the minimum decline in physiological traits as less damage to chlorophyll contents and higher RWC, while the response of Elit, Hazar, and Sampiyon under DS indicated that they were susceptible to stress conditions. Bulb weight is the most desired trait that gives a higher yield of onion. Naz and Perama showed higher total bulb weight under both stress conditions. The overall results reported deciphered the differential response of onion cultivars under two stress environmental conditions. In the current study isolation of genes from onion was done with the use of Degenerate PCR. It generated novel information for an onion that was not available before regarding CAT and PSII gene. This knowledge can be used for the screening of onion cultivars at a molecular level. Moreover, gene expression levels of these genes were calculated under salt and drought-stressed conditions. The cultivars Inci, Perama, and Seyhan accumulated higher transcript levels as compared with other cultivars under both stress regimes. The Elit and Hazar cultivars accumulated lower transcript levels under both stress conditions while Sampiyon showed higher abundance under SS than DS conditions. Abiotic stress cause the formation of ROS species and to mitigate plants activate antioxidant enzymes to alleviate oxidative stress. In this study, it was also observed that defense mechanism of Inci, Perama, and Seyhan was better to mitigate oxidative stress making them a separate group of tolerant cultivars compared with Elit and Hazar grouped into sensitive cultivars under salt and drought stress conditions. The Naz and Sampiyon cultivars depicted better response under SS conditions while Sampiyon showed sensitivity to drought stress. Naz performance was average to DS. This study can be helpful for a screening tolerant and susceptible cultivars of onion. Resilient cultivar such as Inci, Seyhan, and Perama can be used in future breeding studies against abiotic stresses.

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PUBLICATIONS PRODUCED FROM PhD RESEARCH

From this thesis 1 (one) international article was published, and this research work has been presented at 1 (one) international conference. The detail is presented below.

Chaudhry, U.K., Gökçe Z.N.O. and Gökçe, A.F., "Influence of Drought and Salt stress Regimes on Morphological, Physiological and Biochemical Parameters in Allium cepa L." as an oral presentation in ***1st International Conference on*** "***Research of Agricultural and Food Technologies***" Adana/Turkey-October 03-05, 2019.

Chaudhry, U.K., Gökçe Z.N.O. and Gökçe, A.F., "Effects of Salinity and Drought Stresses on the Physio-morphological attributes of Onion Cultivars at Bulbification Stage", ***International Journal of Agriculture and Biology***, Volume 24, 10 October 2020, Pages 1681-1691. DOI: 10.17957/IJAB/15.1611