



T.R.

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

DETERMINATION OF PHYSIOLOGICAL RESPONSES OF SHORT-DAY ONION
LINES IN GAF-MTN GENE POOL AGAINST SALT, DROUGHT, SALT AND
DROUGHT STRESSES

NAZLİCAN AYDOĞAN

AUGUST 2020

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Master Thesis

Supervisor

Assoc. Prof. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE

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Nazlıcan AYDOĞAN tarafından **Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE** danışmanlığında hazırlanan “**Determination of Physiological Responses of Short-Day Onion Lines in GAF-MTN Gene Pool Against Salt, Drought, Salt and Drought Stresses**” adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Tarımsal Genetik Mühendisliği (İngilizce) Anabilim Dalı**’nda Yüksek Lisans tezi olarak kabul edilmiştir.

Danışman : Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE, Niğde Ömer Halisdemir Üniversitesi, Ayhan Şahenk Tarım Bilimleri ve Tek. Fak., Tarımsal Genetik Müh. Bölümü (Raporlu olduğu için sınava katılmamıştır.)

Başkanı : Dr. Öğr. Üyesi Ali Fuat GÖKÇE, Niğde Ömer Halisdemir Üniversitesi, Ayhan Şahenk Tarım Bilimleri ve Tek. Fak., Tarımsal Genetik Müh. Bölümü

Üye : Dr. Öğr. Üyesi Fatih HANCI, Erciyes Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü

Yedek Üye : Prof. Dr. Sedat SERÇE, Niğde Ömer Halisdemir Üniversitesi, Ayhan Şahenk Tarım Bilimleri ve Tek. Fak., Tarımsal Genetik Müh. Bölümü

ONAY (CONFIRMATION):

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Prof. Dr. Murat BARUT

MÜDÜR

THESIS CERTIFICATION

I hereby declare that this 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.

Nazlıcan AYDOĞAN

A handwritten signature in blue ink, consisting of a stylized 'A' followed by a comma.

SUMMARY

DETERMINATION OF PHYSIOLOGICAL RESPONSES OF SHORT-DAY ONION LINES IN GAF-MTN GENE POOL AGAINST SALT, DROUGHT, SALT AND DROUGHT STRESSES

AYDOĞAN, Nazlıcan

Niğde Ömer Halisdemir Üniversitesi

Graduate School of Natural and Applied Sciences

Department of Agricultural Genetic Engineering

Supervisor : Assoc. Prof. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE

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Onion is a plant sensitive to abiotic stress factors such as salinity and drought especially during bulbing phase. Therefore, the physiological responses of the lines were investigated by applying salt, drought, salt and drought stresses to ten short-day onion lines in GAF-MTN gene pool to do the thesis experiment. As a result, it was determined that the lines were mostly affected by drought stress in terms of leaf length, diameter and temperature, and when the chlorophyll index values were analyzed, all lines were mostly affected by salt and drought combined stress. In addition, there was an increase in photosynthesis rate from the initiation to the end of the stress. However, despite this increase, the photosynthesis rate was still lower in lines treated with drought, and salt and drought stresses than the control. According to the results, the line most affected by the salt treatment was K18, while the K28 was the least affected. While K20 was maximally affected by salt and drought combined stress, K25 was only slightly affected. While commercial type K51 was mostly affected by drought in terms of green parts and leaf temperature, while K52 was affected by drought in terms of leaf elongation, expanding and temperature.

Keywords: Short-day onion, abiotic stress, salt stress, drought stress, *Allium cepa* L.

ÖZET

GAF-MTN GEN HAVUZUNDA YER ALAN KISA GÜN SOĞAN HATLARININ TUZ, KURAKLIK, TUZ VE KURAKLIK STRESLERİNE FİZYOLOJİK TEPKİLERİNİN BELİRLENMESİ

AYDOĞAN, Nazlıcan

Niğde Ömer Halisdemir Üniversitesi

Fen Bilimleri Enstitüsü

Tarımsal Genetik Mühendisliği Anabilim Dalı

Danışman : Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE

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Soğan tuzluluk ve kuraklık gibi abiyotik stres faktörlerine özellikle baş bağlama döneminde hassas bir bitkidir. Bu nedenle tez kapsamında, tuz, kurak, tuz ve kurak stresleri GAF-MTN gen havuzunda yer alan on adet kısa gün soğan hattına uygulanarak, hatların fizyolojik tepkileri incelenmiştir. Bunun sonucunda, hatların yaprak boyu, eni ve yaprak sıcaklığı bakımından en çok kuraklık stresinden etkilendiği, klorofil indeksi değerlerine bakıldığında ise ortalama olarak tüm hatların en çok tuz ve kuraklık stresinden etkilendiği belirlenmiştir. Fotosentez hızında ise tüm hatlarda stres başlangıcından sonuna kadar bir artış yaşanmıştır. Ancak buna rağmen kurak ve tuz ve kurak uygulamalarının sonunda fotosentez hızının yine de kontrolden daha az olduğu gözlemlenmiştir. Sonuçlara göre K18 tuz stresinden etkilenirken, K28 en az etkilenmiştir. K20 hattının tuz ve kuraklık stresinden maksimum etkilendiği görülürken, K25 az etkilenmiştir. K51 ticari çeşidi yeşil aksam ve yaprak sıcaklığı bakımından, K52 çeşidi ise yaprak uzaması, en artışı ve yaprak sıcaklığı açısından kuraklıktan etkilenmiştir.

Anahtar sözcükler: Kısa gün soğan, abiyotik stres, tuz stresi, kuraklık stresi, *Allium cepa* L.

PREFACE

In this master thesis study, physiological responses of ten short-day onion lines from the GAF-MTN onion gene pool were investigated under salt, drought, salt and drought combined stresses. For this purpose, leaf width, length, number and chlorophyll index, photosynthesis rate, leaf temperature were measured at determined frequencies. It was determined that the lines were mostly affected by drought stress in terms of leaf length, width and leaf temperature, and when the chlorophyll index values were examined, on average all lines were affected mostly by salt and drought stress. There was an increase in the rate of photosynthesis from the beginning to the end of the stress on all lines.

This thesis has been carried out as part of the project entitled as “Kışlık Sebze Islahında Hat ve/veya Çeşit Geliştirme” supported by TÜBİTAK-KAMAG program with a project number of 117G023.

I would like to thank my supervisor Assoc. Prof. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE for providing valuable suggestions, competent guidance and provided me the opportunity to pursue my master degree under her kind supervision. I am deeply indebted to Dr. Ali Fuat GÖKÇE for giving me an opportunity to work in his team, for all the knowledge in the area of plant breeding that I have learned from him, and for his very helpful suggestions during my research. I would like to thank to my thesis jury members Dr. Fatih HANCI and Prof. Dr. Sedat SERÇE for their positive and constructive criticism and suggestions.

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I want to dedicate this achievement to my mother Hatice AYDOĞAN, my father Halil AYDOĞAN, my brother Erdal AYDOĞAN for their encouragement, wishes, financial and moral contribution throughout my life.



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SYMBOLS AND ABBREVIATION

Symbols	Descriptions
%	Percentage
°C	Degree celsius
cm	Centimeter
CO ₂	Carbon dioxide
da	Decare
EC _e	Electrical conductivity
g	Gram
kg	Kilogram
L	Liter
mm	Millimeter
mM	Millimole
NaCl	Sodium chloride

Abbreviations	Descriptions
ABA	Abscisic Acid
BC	Before Christ
FAOSTAT	FAO Corporate Statistical Database
IRT	Infrared Thermometer
SPAD	Single-Photon Avalanche Diode
TurkStat	Turkish Statistical Institute
UPOV	International Union for the Protection of Varieties
USA	The United States of America

CHAPTER I

INTRODUCTION

Edible *Allium* species has been cultivated for thousands of years because of its therapeutic properties, religious meanings, taste and pleasant aromas. Most of the widely grown *Allium* species evolved from wild ones that grow spontaneously in Central Asia. Nowadays, *Allium* have become one of the basic vegetables that are cultivated almost all around the world and used for consumption. *Allium* vegetables are the fourth most common commercially produced vegetable group following potatoes, cassava and tomatoes. Onion, garlic, Japanese bunching onion, shallot and leek are among the most important edible *Allium* plants.

Onion is a widely grown vegetable type in the world as well as in Turkey. World vegetable production, which is 1.094.343.707 tons in total and onion has 9% share in vegetable production with 102.997.290 tons.

The genus *Allium* was included in the *Liliaceae* family in the first taxonomic classification. However, especially British and American botanists classify this genus in the *Amaryllidaceae* family due to its flower structure. Accordingly, the genus *Allium* was reclassified as follows (Linnaeus, 1753);

- **Kingdom:** Plantae
- **Clade:** Angiosperms
- **Class:** Monocots
- **Order:** Asparagales
- **Family:** Amaryllidaceae
- **Subfamily:** Allioideae
- **Genus:** *Allium*
- **Species:** *A. cepa*

Onion is the most important *Allium* vegetable plant. Dry bulb onions can be grown in many places from semi-arctic regions to the equator. However, it is best cultivated in temperate and sub-tropical regions. Onion has an important place in international trade

due to its storage and transportation characteristics and it is exchanged about 20 million tons every year (FAOSTAT, 2017). Due to the global trade in the onion market and the development of appropriate storage and transport techniques, onions are available throughout the year in most countries.

Considering the average values of the last twenty years (1996-2016), the world's leading manufacturer has been China followed by India and United States of America (Hanci, 2018). Bulb onion production increased over the years. This increase in production is mainly due to the increase in yield and cultivation areas. World average yield of dry onion bulb is roughly 16 tons per hectare. In addition, certain growers can harvest up to 100 tons from each hectare. The reasons for this high yield are good cultivation techniques, regular irrigation and agricultural practices (Table 1.1).

Table 1.1. Table 1.1. Countries in terms of onion yield, amount and production area compared to the average of the last 20 years (1996-2016) (Hanci, 2018)

Rank	Countries	Yield (kg/da)	Countries	Amounts (ton)	Countries	Production Area (da)
1	Korea Rep.	6,190	China	18,190,997	India	9,003,386
2	USA	5,163	India	11,057,657	China	8,524,308
3	Taiwan	4,956	USA	3,255,948	Nigeria	2,542,936
4	Netherlands	4,911	Turkey	1,999,209	Bangladesh	1,364,440
5	Austria	4,812	Iran	1,751,511	Pakistan	1,179,802
6	Australia	4,784	Pakistan	1,591,255	Russia	1,009,929
7	Spain	4,769	Russia	1,463,415	Indonesia	1,006,201
8	Japan	4,755	Egypt	1,450,978	Vietnam	824,717
9	Chili	4,639	Sudan	1,333,072	Turkey	781,305
10	Germany	4,377	Brazil	1,297,058	Sudan	746,760

According to 2017 data of FAO, India, Holland, China, and Egypt are the main countries in the world onion trade and 59% of onion exports were made by these four countries (FAO, 2017). Malaysia ranks first in world onion imports with 582 thousand tons (FAO, 2017). USA, Saudi Arabia, The United Arab Emirates, Russia, Japan, England, Sri Lanka, Germany and Holland follow respectively (Figure 1.1).

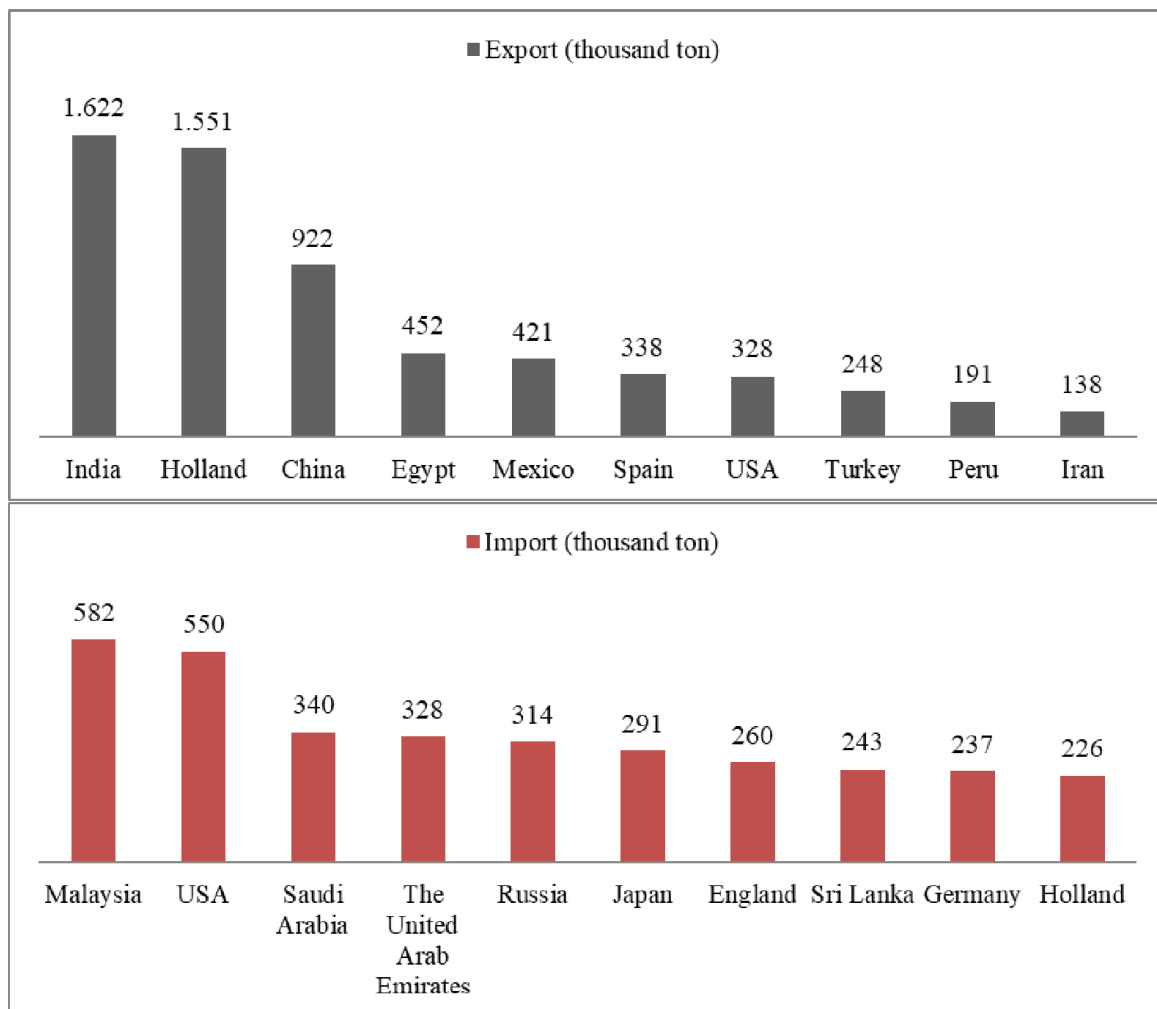


Figure 1.1. Top 10 onion importer and exporter countries

The Earth has an inclination of 23.4 degrees perpendicular to its axis. This tilt is maintained as the earth revolves around the sun for a year. As the Earth completes its rotation, the inclination of our planet towards the sun changes and, thereby, changes in day lengths occur. For half the year, the northern hemisphere is tilted to the sun, eventuating in summer. In winter, the reverse is occurred because the northern half of the globe is tilted away from the sun, and the days are shorter. The length of the day is a significant signal for the leaf formation where the vegetative growth stops in onions and initiates the bulbing phase. Short-day onions require a day length of less than 12 hours to initiate bulbing, which is why they are also called early or short-day onion. Sowing time for short-day onions is usually autumn. Therefore, this type of bulbs are cultivated mostly in the Mediterranean coastal area which is not cold in winter (Gökçe and Tekeli, 2015).

Due to change in climate conditions caused by global warming, problems have arisen in agricultural production. Plants are unable to move, so they give physiological and metabolic responses to abiotic stress conditions such as drought and salinity in such a way that their growth and development are the least impaired (Öztürk, 2015). In order to understand such mechanisms, studies have been carried out in many agriculturally important plants. Onion is one of these plants and as a result of the studies, it was found that it is prone to drought stress, especially during bulbing. The damage caused by drought is not limited to this, it also negatively affects the seed yield to be obtained in the following year. Salt is another stress factor that affects onion growth and yield considerably.

The aim of this thesis study is to investigate the physiological responses of short-day lines to salt, drought and their combination in the GAF-MTN onion gene pool, which have been maintained with breeding programs for many years, in order to determine which lines are tolerant to these stress factors, to take measures to ensure their continuity and to create a database of molecular studies that can be done in the future.

CHAPTER II

LITERATURE REVIEW

The importance of abiotic stress factors has appeared with the irregular climate observed in recent years due to global warming. Plants are the primary part of the ecosystem in terms of oxygen and nutrient production. In addition, plants cannot move away from abiotic stress conditions, so they give physiological and metabolic responses to have minimal damage possible during their growth and development. Although, these changes in abiotic stress conditions have been studied for many years, due to their complexity, they are still not fully understood. This necessitates the use of new approaches in studies aimed at understanding plant stress response and increasing stress tolerance of agriculturally important plants (Cramer et al., 2011.).

2.1 Onion (*Allium cepa* L.)

The history of onion dates back to quite old years, such that the onion figures on the pyramids in Egypt show that the onion was consumed in great quantities by the Egyptians and was a valuable food source for the Egyptian people. The mention of onion in the Bible and the Quran are the major indicators that onions have an important place in human life since the early times. Alexander the Great took the onion from Egypt to Greece and spread it to Europe thanks to the armies of conquest (Platt, 2003). The Hittites, who lived in 2000 BC, also cultivated plants such as onion and garlic, and called the onion Sum-sikil-sar. According to Gökçe and Tekeli (2015), some local geographic places, including Kayseri, Rize, and Bursa, have special names for onions. There are about 500 species of onions throughout the world and 150 of them are naturally grown in Turkey (Gökçe and Tekeli, 2015).

There are two different opinions about the origin of onion by different authors. Afghanistan, Pakistan, Tajikistan and the northern parts of Iran as the primary center (Hanelt, 1990; McCollum, 1976; Vvedensky, 1944), the mountainous parts of Turkmenistan, the north of Iran which is known as Anatolia and Near East Asia, the west of India, the east of the Caspian Sea and Azerbaijan are mentioned as secondary centers (Baytop and Mathew, 1984; Vavilov, 1951). Turkey is one of the places where

the onions produced for thousands of years and Anatolia is the geographic origin of many bulbous plants.

2.1.1 Content and human health

Onion is a pungent herbaceous vegetable whose bulbs and leaves are consumed. They are widely used in salads as fresh, in cooking foods, as dried spices and in chopped and frozen foods (Gökçe and Tekeli, 2015). The nutrients contained in the onion besides the flavoring effect on the food, vitamins and minerals are also beneficial to health (McCollum, 1966, 1968; Galmarini et al., 2001; Havey et al., 2004a, 2004b). The most important nutritional value in terms of the dry matter carbohydrates are sucrose, fructose and glucose (Havey et al., 2004a, 2004b). When the content of onion varieties was examined, it was seen that there is 89% water. In addition, it contains nearly 9% carbohydrate, 1% protein and negligible amounts of fat. Onion provide 166 kJ, in other words 40 calories of energy value, but it comprises low amount of essential nutrients. Phytochemicals, especially polyphenols, which are chemicals produced by plants against competitors and predators, are widely found in onion (Slimestad et al., 2007). Since the beginning of human civilization, herbal medicine has been the most effective way of treating diseases. Onion relieves body fatigue, supports calcium absorption and helps to remove water and urea in the body with urine (Augusti, 2000). According to several studies, tyramin protein which is found in the onion has the effect of expanding the vessels in humans (Havey et al., 2001, 2004a, 2004b, Şalk et al., 2008). It contains antiseptic effect with the presence of sulfurous substances, and provides resistance against diseases such as asthma, bronchitis and influenza (Havey et al., 2001, 2004a, 2004b, Şalk et al., 2008). It was reported that onion or its relatives can be used to prevent or reduce treatment for many health problems (Desjardins, 2008). Therefore, if the half bulb onion or three green onions are consumed as raw, it will be very useful for human metabolism.

2.1.2 Production in Turkey

The province with the largest onion cultivation area in Turkey is Ankara with 110 thousand decares (TurkStat, 2018). Ankara is followed by Amasya with 60 thousand decares, Hatay with 46 thousand decares and Çorum, Tokat and Eskişehir. In 2017, total

cultivation area was decreased by 4.49% compared to the previous year, reaching 57.7 thousand hectares in Turkey. Despite the decrease in cultivation areas, the yield increased by 7.8% (Figure 2.1).

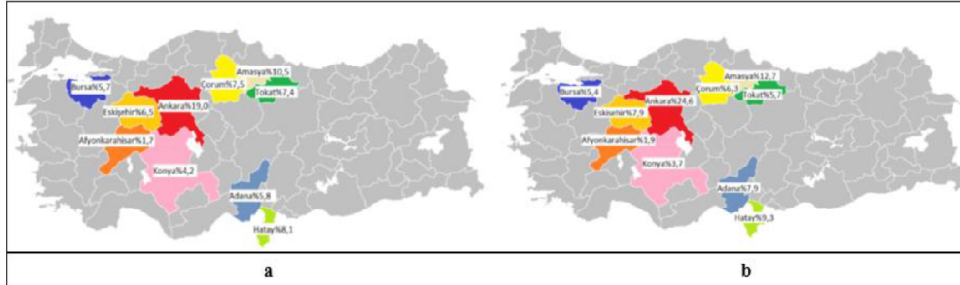


Figure 2.1. Ratio of onion cultivation areas (a) and amounts (b) in Turkey (2018)

Onion production was 2.13 million tons in Turkey accordingly 2017 FAO data. When the onion production quantities examined in the Turkey provinces, Ankara was in the first place with 523 thousand tons of onion production. Ankara was followed by Amasya with 272 thousand tons, Hatay with 198 thousand tons and pursued by Eskişehir, Adana and Çorum. Approximately, 68.6% of onion production in Turkey takes place in these provinces. Therefore, these cities have an important place in the production of onions. According to TurkStat data, onion production decreased by 7.1% and became 1.93 million tons in 2018.

According to the report published in 2019 of Agriculture and Forestry Ministry of Turkey, Iraq was a country with the highest onion export from Turkey followed by Israel, Georgia and Bulgaria (Figure 2.2 a). In addition, Syria was the leading country in imports (Figure 2.2 b).

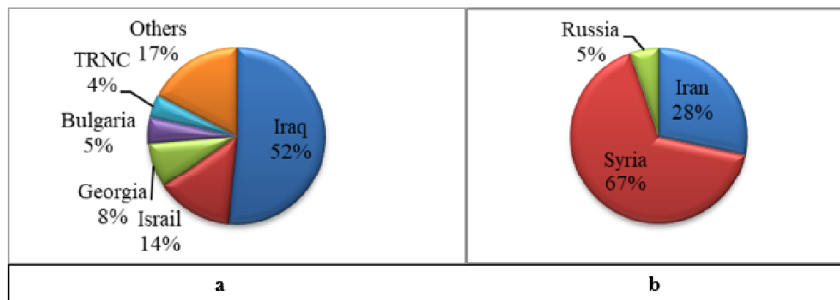


Figure 2.2. The major countries in Turkey's export (a) and import (b) of onions (2018)

According to 2016 FAO data, world onion yield was 18.81 tons / ha. Turkey onion yield was 36.9 tons / ha, which was above the world average in reference to TurkStat data in 2017. There has been an increase in the quality as well as the amount of onion production in Turkey. Although the production and export of onion has an advantageous position in Turkey, this advantage cannot be sufficiently assessed. Onion export of Turkey was 101 thousand of tons which was decreased by 58.9% compared to the previous year and onion imports was 495 tons in 2018 (TurkStat, 2018).

2.1.3 Botanical features

Onion is a species of an open pollinated plant. It is a diploid plant and has 16 chromosomes ($2n=2x=16$). Open pollination rate in the field condition is between 75% to 100% (Meer and Bennekom, 1968, 1972). Since the persistence of open pollinated populations is ensured with cross pollination by insects or bees, a high phenotypic variation occurs in onion (Picture 2.1) (Berninger and Buret, 1967).



Picture 2.1. Onion flower pollination by bees

Onions are divided into 2 groups according to their bulb characteristics. One of them is thick bulky, juicy, usually light colored and long shaped summer (short-day) bulbs. This type of onion is not storage type and has a sweet taste. The other type is winter (long-day) onions, which are firm-headed. In general, they have a lower water content and a darker color. Their shape is slightly flattened and the taste is pungent. Also, they are suitable for long-term storage.

Onion roots are self-contained in other words each root is connected to stem separately. When the roots get older, the bifurcation sometimes becomes the root of the eaves. Onion roots are weak and short-structured extending up to 30 cm and develop from the

bottom of the stem. For this reason, they are found in the first 30 cm depth and width of the soil (Brewster, 2008; Gökçe and Tekeli, 2015). As the roots enter the soil at an acute angle, it is possible to grow onions closely. Roots have generally white in color.

The stem of the onion is formed in the part where the root and leaf combine. It is impossible to see the stem from outside, but if the roots and the leaves are cut off, stem can be observed as a thick flat center. The width or length of stem may vary from 1-2 mm to 4-5 mm depending on the variety and also the growth condition (Figure 2.3).

Leaf and bulb are strongly developing organs of onion. The leaves develop in the form of sheath that enter into each other when they first came from the stem. First the outer leaves and then inner leaves develop. The leaves are fleshy, coming out from one another, tubular shape and hollow. It is usually green (Picture 2.2). Depending on the variety and environmental conditions, it can take up to 30-80 cm height. Leaf color varies from gray to dark green. The amount of wax layer on the outer surface of green leaves of onions is a source of leaf color diversity. In the absence of this layer, bright green color appears. Gökçe and Tekeli (2015) stated that twelve or thirteen leaves may form in an onion depending on the cultivation conditions and variety. The fleshy parts on the base of the leaf become swollen with storage of nutrients and form head bulbs.

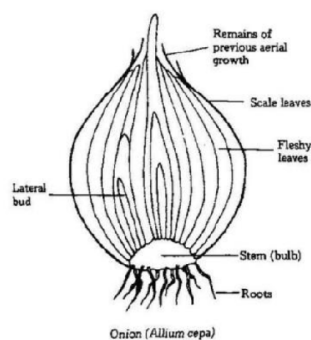


Figure 2.3. Onion (*Allium cepa*) parts



Picture 2.2. Leaf of *Allium cepa*

Onions have pear shaped, round, flattened, cylindrical, conical and spinning top forms. In addition, scale color can be brown, yellow, green, purple and red in color (Picture 2.3). The inner part of onion is white, green, yellow or red. One of the most important determinants in consumer preferences is the scale color of onion bulbs. However, it is very important to express the color in standard scales especially in products with high export value.



Picture 2.3. Onion bulbs with different scale color

Onion is a biennial plant that require two growing seasons to regenerate from seed to seed (Figure 2.4). The second year, leaf development period ends with bolting (Gökçe and Tekeli, 2015). The onion blossom formation begins with the rosette stems and it grows to form scape. At the end of the scape, there is a flower receptacle containing the collection of onion flowers. At the same time, the receptacle is surrounded by a thin membrane that surrounds the floral community, and when the flowers begin to develop, the thin membrane tears and the flowers begin to open. The onion flowers are bisexual. Depending on the strength of the onion, one to three flower scapes develop from each growth point. If there is more than one center in an onion, it can form up to three times more scapes than the number of centers. Thus, individual flowers between 100-2000

pieces can be found in bulk on a scape. In each individual flower, there is the innermost female organ, the ovary, which has 3 carpels where each carpel has 2 ovules. There are 6 male organs which lined up around the female organ and outside of the male organ, 6 petals and 6 sepals can be found. Protandry is dominant in onion blossoms and the male organs mature and shed pollen before the female organs get receptive, which encourages cross pollination (Pike, 1986; Şalk et al., 2008; Gökçe 2010, 2011). In this respect, the necessary importance should be given to the methods of protection from cross pollination in breeding studies and seed production and maintenance pure variety. In other words, the isolation distance must be arranged correctly and the choice of land should take into account the varieties of plants grown in the surrounding area. In addition, since some of the culture forms do not contain fertile pollen grains, it is necessary to include pollinator varieties when seed formation is required.

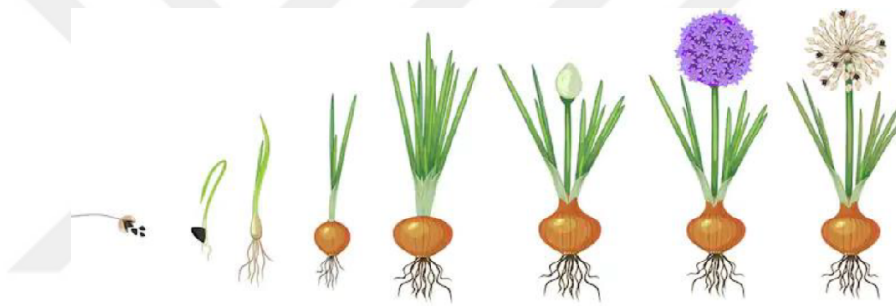


Figure 2.4. General life cycle of *Allium cepa*

Each individual female organ forms 2 to 6 seeds and the onion seeds are quite small. Seed colors are generally pale black and wrinkled. As many seeds form from 6 seed ovule, they take the shape of spheres separated by slices and when the seed loses moisture, the shell collapses inward and becomes wrinkled (Picture 2.4). There are 200-275 seeds in 1 g. The weight of a thousand grains ranges from 2.7 to 4 g. The seeds retain their germination power for 2-3 years and can remain intact for 4-6 weeks after sowing (Gökçe and Tekeli, 2015).



Picture 2.4. Seed formation of onion

2.1.4 Cultivation and climate requirements

Onion is cultivated in between all areas of the 50th parallel of the southern hemisphere and 65th parallel of the northern hemisphere. In other words, onion may adapt to climate in all areas from Argentina and New Zealand to Canada and Poland. Although onion is a cool season vegetable that is cold hardy, climatic condition is also very important. Optimum temperature for plant growth is 13-24 °C, while this rate is 20-25 °C during seedling period. Important factors in onion cultivation are day length and temperature. Onion that require a cool climate in the early development period require high temperatures during the bulbing phase (Butt, 1968). There are varieties that are well adapted to different climates in the world, and these varieties are best grown in their climates. Short-day, mid-day and long-day varieties are determined according to day length requirements. Onion adapted to different climates are called short-day onions (early) for those who need day length less than 12 hours and these onions are sown in the autumn in the Mediterranean coastal region where it is not cold in winters. Mid-day onions need day length between 12-14 hours during bulbing phase and require early spring cultivation in places where the winters are cold. Long-day (late, winter or storage) onions require the maximum day length with 14 hours and are sown in March and April in the interior and Northern regions (Pike, 1986; Gökçe, 2010, 2011). In this period, the ideal temperature should be 25-30 °C (Şalk et al., 2008).

Soil moisture should not exceed the field capacity during the germination of onion seeds and should be around 80-85%. Considering that the minimum germination temperature

of the seed is 0 °C, the optimum germination temperature is 20-25 °C and the maximum germination temperature is 35-40 °C, seed sowing should not be done until the soil temperature reaches 8-10 °C (Gunay, 1992). According to Gökçe and Tekeli (2015), onion does not like sandy, clay and hard soils as it will make the bulb formation difficult. Loose, high water retention capacity, easily workable fertile soils are preferred for well root growth. Onion is highly sensitive to high acidity, so the pH should be between 6.0 and 6.5. It is very sensitive to salt stress, so manure and over-fertilization should be avoided in particular. Fertilization should be done according to the soil analysis in the place where the cultivation is performed, since there are different nutrients in each soil. It is also very sensitive to sodium and boron toxicity and, therefore, irrigation water should not contain high amounts of boron and sodium (Brown, 2000).

Shalk et al. (2008) reported that onion needs water during the green development period and requires the abundance of water and humid weather. If there is not enough rainfall during this period, 450-500 mm water should be given to the plant. In contrast, it is more convenient to stop irrigation when the bulb onions are close to the harvest time. In order to produce seeds, onion vernalization requirements must be met. This period varies depending on the type, but can be met by keeping at 8-13 °C for one to two months (Kimani et al. 1993). During the flowering period, flower powders are sensitive to moisture and rain, and rainfall on the days following flowering adversely affects the pollination. In this period, it is desired that the air is warm and the humidity is low.

Onion can be grown in four ways from seed, seedling, sets (shallot) and peak bulbils. Seedling is used in climatic conditions where vegetation period is short. It is a method used in the production of green onion and short day onion. Onion seeds are grown in viols under low plastic tunnels or greenhouse conditions. Then, according to the climatic conditions of the region, seedling is transplanted to the land in February or March. Seeds of onion varieties that are not planted in climates suitable for the variety cannot form bulb in that year. In the first year, small bulbs with a diameter of 1-3 cm can be obtained. These are called onion sets in worldwide and especially called 'arpacık' or 'kıska' in Turkey. They are planted in the second year to obtain bulb onion. Onion seed is called 'karaca' in some regions of Turkey. Since the onion seeds are very small, the land should be prepared very well. For this, a deep ploughing should be done

in autumn. Onion seed planting is carried out between September-November in short-day cultivars and between January-March in long-day cultivars. Soil temperature is desired to be between 10-15 °C. Seeds germinate with the moisture of soil. They are thrown in depth of 2-3 cm. Under favorable weather conditions (15-20 °C) the outlet is achieved within seven to ten days by seeds (Voss et al., 1999).

2.2 Stress Factors

Plants are the most susceptible organisms to changes in environmental conditions and adverse conditions due to their inability to move. Conditions that affect or prevent growth, development and metabolism in plants are called stress (Gürel and Avcıoğlu, 2001). Stress often show its effects on plants simultaneously or in combination (Kalefetoğlu and Ekmekçi, 2005). Stress factors can be divided into two groups as abiotic and biotic according to their origin. Abiotic stress due to environmental changes such as drought, salinity, excessive rainfall, temperature or cold throughout the life cycle directly affect plant growth and development (Taiz and Zeiger, 2010). Plants can stretch their growth and development mechanisms to achieve the least damage from these changes in environmental conditions, and even adapt to the least impact from environmental factors when grown in the same climatic conditions for extended periods of time. The distribution of plants belonging to the same species in regions whose climate characteristics change in the world is the best indication that they can adapt to very different environmental conditions (Dolfeus, 2014). In this context, it is not difficult to predict that many changes can occur not only physiologically but also metabolically in a plant experiencing stress. When they encounter abnormal conditions, plants can restructure their metabolism dramatically depending on the severity and duration of stress (Öztürk, 2015).

2.2.1 Drought stress

Drought is divided into four sub-categories as meteorological, agricultural, hydrological and socio-economic. It begins as a meteorological drought, develops as agricultural and hydrological drought, then its effects as socio-economic drought become visible. Meteorological drought is defined as the decline of rainfall below the normal values for a long time and agricultural drought is defined as the lack of water in the soil to meet

the needs of the plant (Mengü et al., 2011). While hydrological drought is the decrease in the amount of water in rivers, lakes and underground water resources, socio-economic drought includes deterioration of economic balances due to other drought types (Mishra and Singh, 2010).

Water constitutes 50% of the fresh weight of the trees and 89-90% of the other plants (Anjum et al., 2011). The developmental stage of the plant is very important during the drought stress. Numerous physiological characters that determine yield in plants are also affected by drought conditions. Plants are most susceptible to drought in the generative period, so yield losses reach up to 95% with the drought experienced in seed formation period. It even leads to infertility when experienced during the flowering phase (Farooq et al., 2009). Plant growth under drought conditions is also significantly affected. This change in growth depends on the time of water shortage. In the early periods of drought, plant slows the stem elongation and triggers root growth to reach more water. However, if dry conditions last long enough to cause damage to the plant, both stem and root development cease, leaf area and number of leaves decrease, and even some leaves turn yellow. Decrease in plant growth develops due to cessation of cell division and expansion of shoot and root meristems. Stopping cell division or expansion is directly related to the decrease in photosynthesis rate due to water deficiency (Anjum et al., 2011).

Water use efficiency of onion was investigated with the work done by Kadayıfçı et al. (2005). In this study, eight different irrigation (I) levels (1, 2, 3, 4, 5, 6, 7 and 8) were tested and three replications were performed. All growth media consisted of homogeneous substances containing equal amounts of soil, sand and fertilizers. Irrigation treatments were established as I1 full irrigation and I2, I3 and I4 with no irrigation in the vegetative growth period, yield formation and ripening, respectively, and I5, I6, I7 and I8 accepted 0.0, 0.25, 0.50 and 0.75 times the soil water depletion in the treatment I1 on the same day. Irrigation was carried out two times a week. Five seedlings were transferred to per pot while irrigated at field capacity. The findings showed that the onion plant is susceptible to drought during the total life and yield period, whereas it is not sensitive during vegetative and ripening periods. In addition, it was observed that water use efficiency raise in increasing irrigation stages and vegetative stage without irrigation.

In a field study conducted by Zayton (2007), the most critical period for onion to drought stress was tried to be determined and irrigation was stopped at four significant times at initial, development, mid-season and late-season growth stages. Stress was applied in three replicates in a random full block trial pattern. In addition, some quality properties were investigated to observe the effect of drought stress. According to the results obtained, lack of water causes small bulb formation, while the lack of water in the early period causes the formation of broad and shallow bulb. As a result of drought, the amount of water soluble dry matter increased.

Pelter et al. (2004) have studied how drought affects yield and quality in four different developmental processes. Irrigation was stopped in 3, 5, 7 and 9 leaf stages and control continued to be watered normally. It is desirable on the market that the single center bulb formation should be at least 70%. In the control, single center bulb formation was obtained above the ideal and found to be 75%. While this rate decreased to 45% during the early period of water stress, that was 3-leaf period, the number of growth points was not affected by the water stress experienced during the 7-9-leaf period. On the other hand, the yield decreased to 26% in plants which exposed to drought in both 3 and 7 leaf periods compared to control.

Ghodke et al. (2018) evaluated morphological, physiological and biochemical properties of onion in order to evaluate bulb yield and quality under drought conditions. Drought was applied continuously for 40 days initiated 45 days after transplantation. It was observed in this study that the relative water content, which was calculated as approximately 80-85% in the control, decreased to 70-73% in the stressed plants. Leaf length and leaf area decreased significantly after stress application. As the cultivar used in this study was a relatively more tolerant variety, proline and dry matter accumulation under stress increased while maintaining the chlorophyll content.

Hancı and Cebeci (2014) conducted the study to determine the physiological characteristics of three onion cultivars (Kantartopu-3, Akgun-12 and Texas Early Grano) with 3 different irrigation applications (90%, 70% and 40%) under greenhouse conditions. As a result, the amount of proline in the seedlings increased, whereas chlorophyll and carotenoid amount decreased.

2.2.2 Salt stress

Salt stress is defined as the presence of different salts in soil or water in concentrations that may inhibit plant growth and create toxic effects. These salts are generally chlorides, sulphates, carbonates, bicarbonates and borates. However, the most common salt form in nature is sodium chloride (Hancı and Cebeci, 2015b).

Salinity negatively affects both cultivated soils and causes many adversities in the plants grown in soils under salinity threat. Approximately 1.5 million hectares of our country's land (of which 32.5% are irrigable areas) face the problem of salinity (Ekmekçi et al., 2005). On the earth, more than 800 million hectares of terrestrial land is affected by salinity, which is more than 6% of all terrestrial areas in the world (Sudha and Riazunnisa, 2015). It is predicted that such salt accumulation in arable areas will reach even more devastating dimensions in the global framework. This will result in major economic losses due to the decrease in product yield and quality (Mahajan and Tuteja, 2005). Excess amounts of soluble salts in soil solution reduce the water usability of plants. The reduction in water potential, which is a common response in such cases, can be offset by a reduction in osmotic potential as a result of increasing the solute content to maintain turgor potential. Increased salinity makes plants' osmotic potential more negative. The low osmotic potential of the dissolved substances in the rooting zone reduces the water potential of the soil and thus affects the general water condition of the plants. Thus, salt stress affects many biological phenomena such as growth, development, germination, cell division, and photosynthesis in plants depending on the intensity and duration, and salinity limits crop productivity and crop quality in agricultural areas (Bressan, 2008).

Salt stress affects cell division and elongation, causing a decrease in the number of cells in the root and stem, mitotic activity and cell division in plants (Bursens et al., 2000). Accordingly, a decrease in the length and weight of the plant root and stem, shrinkage and thinning of leaves, thinning of the waxy layer and cuticle layer on the leaf surface, reduction in vascular tissue differentiation and development occur. Direct growth of the primary root system from the root systems directly exposed to NaCl is inhibited as a result of cell expansion and suppression of the cell cycle (Wang et al., 2009). Root hairs lose their activity due to increasing salt concentration and disappear (Ali et al., 1999).

Although the root system is directly exposed to salinity, leaf growth is more susceptible to salt stress than root growth, and therefore, in salt stress, the root to shoot ratio of plants increases (Munns and Tester, 2008). Although salt stress affects all growth stages of the plant, the most affected stage is seed production and thus seed yield (Khatun and Flowers, 1995). In addition, salinity in the reproductive stage causes a decrease in the number of productive flowers and changes in flowering time (Munns, 2002). In addition to reducing water potential, NaCl also affects plant growth by disrupting the ion balance in the cell. High amounts of NaCl intake in the cell Na^+ and Cl^- level increases, Ca^{+2} , K^+ and Mg^{+2} concentrations cause a decrease (Parida and Das, 2005). In addition to reduce water potential, NaCl affects plant growth by disrupting the ion balance in the cell. While the Na^+ and Cl^- level is increasing, Ca^{+2} , K^+ and Mg^{+2} concentrations decrease (Parida and Das, 2005).

In terms of the salinity tolerance threshold, there are significant differences between crop plants. It is known that there are differences at different levels between varieties belonging to the same species as well as differences between family, genus and species. Vegetables include spinach and asparagus are salt tolerant (EC_e 5–10 dS m^{-1}), cabbage, potato, tomato, cauliflower, sweet potato, head salad, celery, watermelon, melon, cucumber and pepper are medium tolerant (EC_e 3-5 dS m^{-1}), and beans, radishes, carrots and onions are grouped as sensitive species (EC_e 1.5–3 dS m^{-1}) (Rampino et al., 2006). Yılmaz et al. (2011) stated the threshold value of onion is 1.2 dS m^{-1} and there is a relative yield decrease of 16% per dS m^{-1} due to salt increase.

The effects of salt stress on onion vary according to variety and salt source. Hancı and Cebeci (2011) evaluated the effects of NaCl and CaCl_2 salts on germination performance of some onion cultivars. Kantartopu-3, Akgün-12, İmrâli Kirmasi-15, Metan-88 and Beyaz Bilek were used in this study reported by Hancı and Cebeci (2011). Increased salinity in both types of salt adversely affected germination and seedling growth on all cultivars. However, the negative effect of CaCl_2 was found to be greater than NaCl.

Joshi and Sawant (2012) applied salt levels from about 43 mM (0.25%) to 171 mM (1%) in six varieties (N-2-4-1, B-780, Phule Samarth, Bhima Red, Bhima Raj, and Bhima Super). At the 1% concentration, the decrease in seedling development in B-780

was the least. In addition, the most proline accumulation was again in variety B-780, from which it was found that there may be an attribute between salt tolerance and proline deposition.

In the study conducted by Sudha and Riazunnisa (2015), four different onion cultivars were used which include Agrifound dark red, Agrifound white, LINE-28, Krishnapuram and four different amounts (50 mM, 100 mM, 150 mM and 200 mM) of salt were applied. Morphological characteristics such as germination percentage, germination rate, seed vigor, length of plumule and radicle were evaluated 10 days after salt stress application and parameters reported to be decreased with increasing amount of salt. Germination percentage, germination rate and seedling vigor were highest at 50 mM, and lowest at 200 mM. While Agrifound white was found to be the most tolerant than the others, LINE-28 was found to be sensitive to salt stress.

2.2.3 Combined stresses (Salt and drought)

Climate change caused by global warming adversely affects crop quality and yield. Drought is expressed as not having enough moisture to grow and develop in the root area of the plant. Salinity occurring mostly due to drought emergence is another source of stress that affects plant development and yield in especially arid and semi-arid climates (Lopez et al., 2011; Eisa et al., 2012). Insufficient precipitation, high evaporation, natural salt rocks, salty irrigation water and insufficient drainage lead to salinity in the soil. Many products of economic importance can be significantly affected by drought and salinity (Solmaz et al., 2011). Combined salinity and drought affect many morphological features in the plant and also affect many physiological processes which are related to plant growth and development (Siddiqui et al. 2008). Despite the changing climatic conditions, it is necessary to understand the physiological and biochemical mechanisms of plants under stress to obtain resistant genotypes in order to increase productivity in agriculture. It was stated that the reactions of the plants to the combined stresses are very different from the responses of the individual stress (Grzesiak et al. 2016).

Hancı and Cebeci (2015a) investigated the physiological and morphological characteristics of four onion cultivars (Texas Early Grano, Kantartopu-3, Besirli-77 and

Akgun-12) under drought and salinity conditions. The seeds were germinated in viols and after three weeks the seedlings were transferred to the pots. Drought and salinity stress was started 10 days after transplanting. A 90%, 70% and 40% field capacity irrigation was performed and a 70% field capacity was considered the starting point of drought in onion cultivars studied. Salt stress was started by increasing from 25 mM concentration to 50 mM and 125 mM concentrations. While all morphological features decreased with increasing salinity and drought, all other physiological properties except leaf waxiness and proline content decreased.



CHAPTER III

MATERIALS AND METHODS

3.1 Material

Ten elite short-day onion lines to be used in this study have been provided by GAF-MTN gene pool which was developed by Dr. Ali Fuat Gökçe as a result of long-term onion breeding program. They were morphologically identified before (Table 3.1).

Table 3.1. Some basic features of the lines to be used in the study

Line No	Color	Seed Increase Cage No	Cage Year	Generation
K18	Yellow	15137 (S)	2019	(Hazar1x24)S1M3
K20	Yellow	15141 (S)	2019	(Hazar3x24)S1M3
K25	Yellow	37x154	2019	(37xHazar1)M3
K28	Yellow	37x160	2019	(37xHazar4)M3
K35	Yellow	101-1 (N)	2019	S1MS4M2
K37	Yellow	101-54	2019	S1MS4M2
K39	White	101-36	2019	S1MS4M2
K41	Yellow	101-14	2019	S1MS4M2
K51	Yellow	201-1 (N)	2019	Commercial variety
K52	Yellow	201-2	2019	Commercial variety

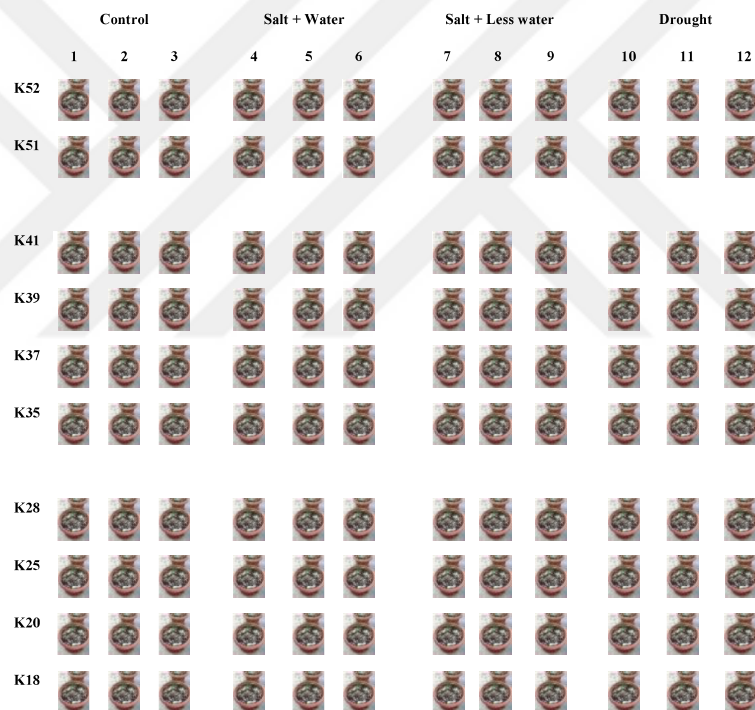
3.1.1 Plant growth

The short-day seeds were planted in 7 liter pots containing perlite in a 3:1 ratio in November the 22th (Picture 3.1). At this stage, each short-day onion line was planted in 9 pots with 3 pots for each abiotic stress application (salt, drought, salt and drought) (Picture 3.2). In addition, 3 control plants were planted in each genotype. All plants were grown in semi-controlled greenhouse conditions. The greenhouse temperature, which was controlled daily during the winter season, was kept constant in the range of 15-20°C. In April, when stress treatment started, cooling system was used to prevent excessive increase of greenhouse temperature due to air temperature and the

temperature was kept at around 25 °C during the bulbing period. Greenhouse humidity was also controlled and kept below 50%, because high moisture could cause fungal diseases.



Picture 3.1. Sowing preparations by mixing peat and perlite in 3:1 ratio



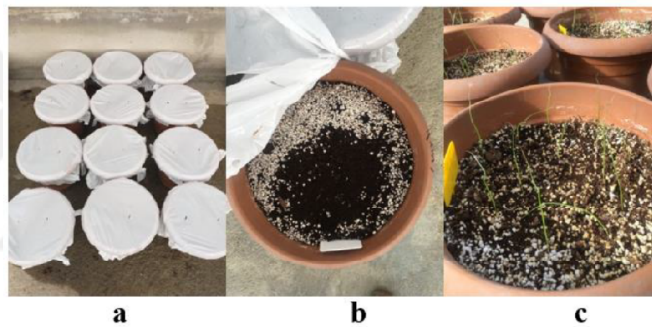
Picture 3.2. Greenhouse layout after sowing onion seeds

Twelve seeds were sown per pot and the pots were covered with stretch for 1 week in order to maintain the moisture of the pots during germination period (Picture 3.3 and Picture 3.4a). While eight of the genotypes used from the GAF-MTN gene pool were breeding lines, two were commercial varieties. Germination was observed in some genotypes 9 days after planting (Picture 3.4b and c). Although the risk of disease was low due to the cold weather, fungicide was applied during the seedling period in order

to prevent problems due to moisture (Picture 3.5a and b). While avoiding excessive watering for the first 2 months, moistening with a back pump was provided, then normal irrigation was continued until stress application (Picture 3.6).



Picture 3.3. Sowing of short-day onion seeds



Picture 3.4. Stretching of pots (a), observation of germination (b) and plant output (c)



Picture 3.5. Pesticide application of plants against mildew by manual (a) and back pump (b)



Picture 3.6. Irrigation of short-day onion lines

3.2 Method

3.2.1 Stress application

Onion is a plant very sensitive to stress factors in the bulbing phase, so stress applications were initiated in this period (Pelter et al., 2004; Krasensky et al., 2012; Rattin et al., 2011). The short-day lines sowed in November started to bulb in the sixth leaf stage. When the plant development reached this period, stress application was started in April the 11th. It continued during the bulbing phase about 20 days. After the stress application was over, the plants continued to be watered normally.

3.2.1.1 Control application

No stress was applied to the control group and grown in the same greenhouse conditions. The daily water requirement of a normal onion plant was given. Three pots from each onion genotype were grown as a control.

3.2.1.2 Salt stress application

The amount of saturation capacity of pots was determined as 1.5 L and salt stress was applied by giving salt with water in saturation capacity. NaCl was used as salt and the salt concentration was started from 75 mM and continued by increasing 25 mM in each

watering until 150 mM (Hancı and Cebeci, 2015a) (Table 3.2) (Figure 3.1). After this stage, irrigation was done twice with 150 mM salt water and the stress application was terminated. After salt treatment, plants continued to be watered normally two more months until harvest once in three days.

Table 3.2. Salt concentrations and amounts applied per pot

Stress Application	Salt Type	Salt Concentration (mM)	NaCl gr (gr/1.5 L)
1.	NaCl	75	6.57
2.	NaCl	100	8.76
3.	NaCl	125	10.95
4.	NaCl	150	13.14
5.	NaCl	150	13.14

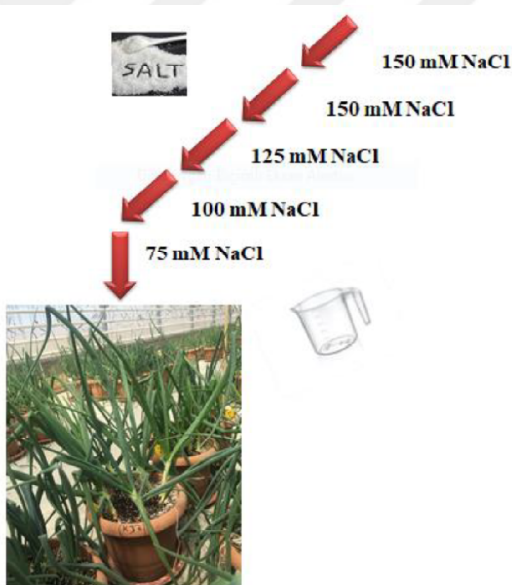


Figure 3.1. Increasing the amount of NaCl step by step during salt stress

3.2.1.3 Salt and drought stress application

Combination of salt and drought stress was performed by limited irrigation with salty water to expose the plants both to water and salt stress. For this purpose, the application of stress on short-day onion lines started with 75 mM NaCl, and 25 mM increasing once in three days until reaching the concentration 150 mM during the period of bulbing (Hancı and Cebeci, 2015a) (Table 3.3). It was carried out by irrigation with 70% of

the soil saturation capacity (Hancı and Cebeci, 2015a). After this stage, irrigation was continued with 150 mM NaCl. After salt and drought treatment, plants continued to be watered normally 2 more months until harvest once in three days.

Table 3.3. Salt concentrations and amounts applied per pot in combined stresses

Stress Application	Salt Type	Salt Concentration (mM)	NaCl gr (g/1 L)
1.	NaCl	75	4.38
2.	NaCl	100	5.84
3.	NaCl	125	7.30
4.	NaCl	150	8.76
5.	NaCl	150	8.76

3.2.1.4 Drought stress application

Drought stress was applied by stopping irrigation during the bulbing phase under the greenhouse conditions. Before starting the drought treatment, the pots were watered at saturation capacity and 20 days of drought stress was applied without any watering. After drought treatment, plants continued to be watered normally 2 more months until harvest once in three days.

3.2.2 Parameters of general view of plant

General condition of the plants in stressed (salt, drought, salt and drought) and control plants (untreated pots) were monitored every day. In order to evaluate the difference in leaf development that occurs after stress, leaf number, diameter and length measurements were made before stress started. Control and stress pots were photographed before and after stress to see the current status of genotypes.

3.2.3 Physiological measurements

Physiological measurements were performed starting from the first day of abiotic stress accepted as the zero day. In order to evaluate the physiological effects of stress conditions in varieties with different tolerance and to determine the changes, the

following physiological measurements were taken from the stress applied and control plants during stress treatment.

3.2.3.1 Measurement of SPAD value (Leaf chlorophyll index)

The chlorophyll content in the leaf is an indicator of the physiological state of a plant. Pigments are compulsory in the conversion of chlorophyll light energy to chemical energy. The amount of radiation absorbed from the sun also depends on the amount of photosynthesis in the leaf. Therefore, the content of chlorophyll is related to the photosynthetic activity and primary production (Curran et al., 1990). The SPAD values were measured by the Minolta SPAD 502 Chlorophyllmeter starting from the initiation of abiotic stress application in the third or fourth leaves of plants which have completed development at noon time on the day 0th, 5th, 10th, 14th, 17th and 20th (Picture 3.7). Measurements were taken from three different plants belonging to each application and the average value was calculated as SPAD value.



Picture 3.7. Chlorophyllmeter model used in leaf chlorophyll index measurement

3.2.3.2 Leaf temperature measurements (°C)

Plant temperature is an important indicator of plants response to many environmental factors. In addition, plant temperature is related with the occurrence of many physiological events within the plant. Many studies have reported that leaf temperature changes in response to stress factors (Downes, 1970; Liu et al., 2011; Dalil and Golezani, 2012). In plants under stress, light energy reaching the leaf is wasted in order to be protected from the negative effects of high energy coming from the sun. For this purpose, light energy usually affects the change of leaf color. It is reflected back without being absorbed, therefore, increases in leaf temperature occur.

In this thesis, leaf temperature was measured once in two days from the start of stress application with the help of Infrared Thermometer (IRT) in the middle of the day (Picture 3.8). In each measurement, five plants and two measurements from each plant were taken and an average value was obtained.



Picture 3.8. Infrared Thermometer (IRT)

3.2.3.3 Photosynthesis rate

It was made using LICOR-6400 portable photosynthesis device under $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ constant light intensity, $400 \mu\text{mol mol}^{-1} \text{CO}_2$ and $500 \mu\text{mol s}^{-1}$ air flow conditions (Picture 3.9). Three measurements were taken from each plant's 4th leaf and all measurements taken were calculated as the average photosynthesis value.



Picture 3.9. LICOR-6400 portable photosynthesis device

CHAPTER IV

RESULTS

4.1 Determination of Morphological Features

Morphological measurements and observations of ten short-day lines in GAF-MTN onion gene pool were evaluated in two groups as green parts and general plant views.

4.1.1. Evaluation of green parts

Leaf lengths were measured before the stress application on the short-day onion lines, which came to the bulbing stage approximately 4.5 months after seed sowing. For this purpose, the lengths of three of the seven plants in each pot were measured and the average of these values was calculated. Under normal conditions, plant development was expected to continue and leaf length would increase. Leaf length was re-examined after twenty days of stress application period to determine the effects of stress on leaf length (Figure 4.1).

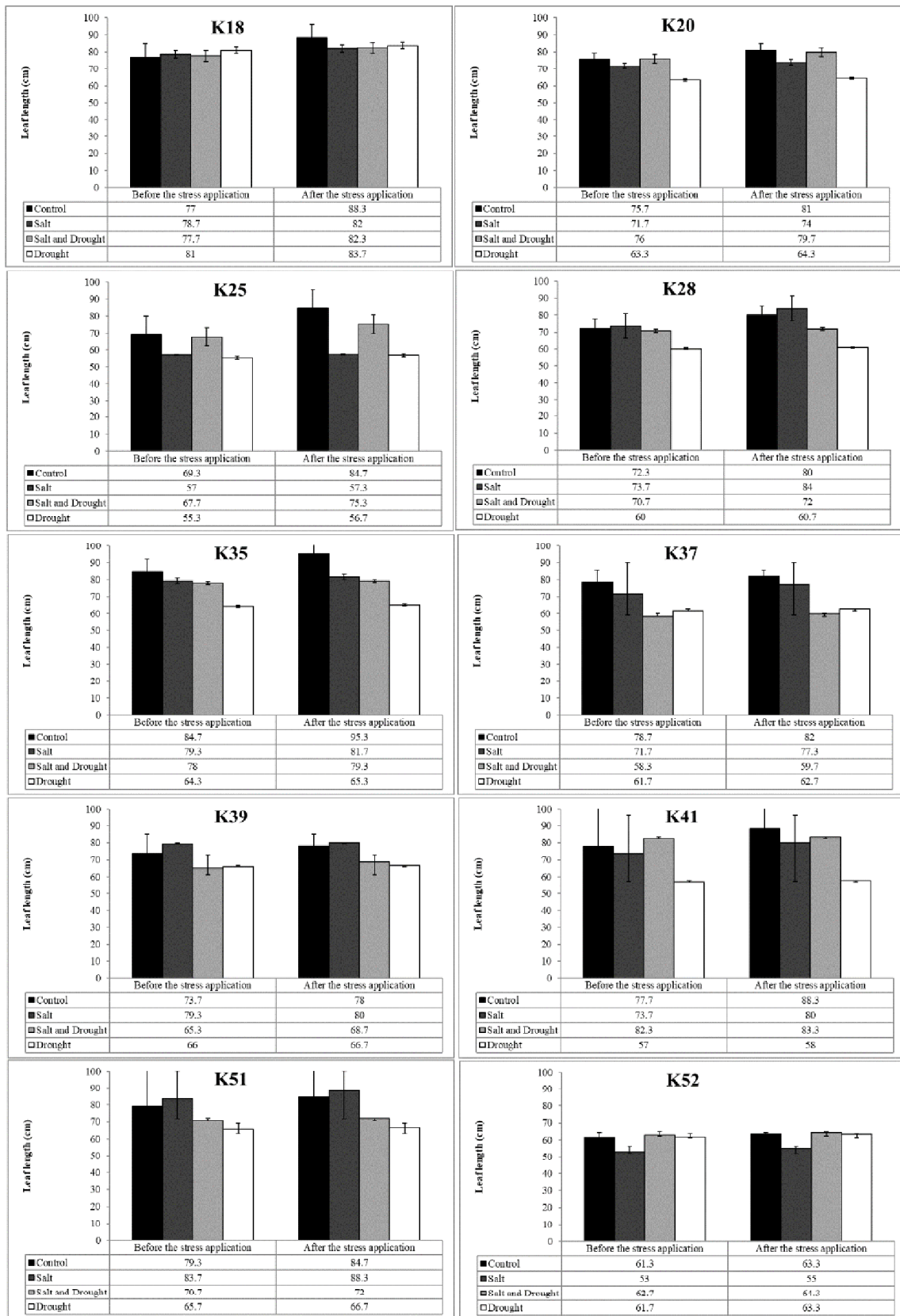


Figure 4.1. Leaf length change graphs of ten lines before and after stress treatment

During the stress of the K18 and K20 onion lines, although the leaf length increase in salt and combined stress (salt and drought) was less compared to their control, the lowest value was detected in drought stress. Although there was an increase in the control group of K25 as expected, the leaf elongation was not occurred in response to salt and drought stress, whereas in the case of salt and drought combined stress, the leaf length partially increased. In the K28 line, the length increase in salt stress application was 13.9%, while the control was observed as 10.6%. The increase of leaf length was partly less in drought and salt and drought combined stress compared to its control. There was a 12.5% elongation in the control group of K35 line as expected, while in the case of salt, drought and combined (salt and drought) stresses, increase of leaf length percentage were shown differences between 1.5-3%. In response to salt treatment in the K37 line, elongation was more than its control as an unexpected situation. There was a less elongation than the control in the case of drought and combined (salt and drought) treatment. After the stress in the K39 line, the leaf length in control increased by 5.8% and there was a 5.2% increase in the combined stress nearly as much as its control. In addition, leaf elongation was 0.8% in salt stress treatment and 1% in drought stress treatment. Even though there was an increase in the control groups of K41 and K51 lines as expected, leaf length increase was not occurred in response to drought and combined (salt and drought) treatment, whereas in the case of salt stress, elongation almost increased as much as the control. When all lines were compared, there was a minimum leaf elongation in K52 line in the control group and leaf elongation was occurred higher than its control after salt stress treatment. The leaf length elongation was equal and slightly less than control in drought and combined (salt and drought) treatment in K52 line.

In the K25 line, the minimum elongation was observed in salt stress with 0.5% elongation rate, while in the same line, the highest elongation was observed with 11.2% in combined stress (salt and drought) among all the genotypes. The percentage of elongation in drought stress varies between 1.0 and 3.3, and the line with the maximum elongation in drought stress was K18 line.

The widest part of the longest leaf was taken as the basis for the measurement of leaf diameter in onion in accordance with UPOV criteria. This measurement was performed twice, at the beginning and end of the stress period. After the measurement was made

from three plants, it was calculated as an average value and the effects of stress factors were examined on the leaf diameter (Figure 4.2).



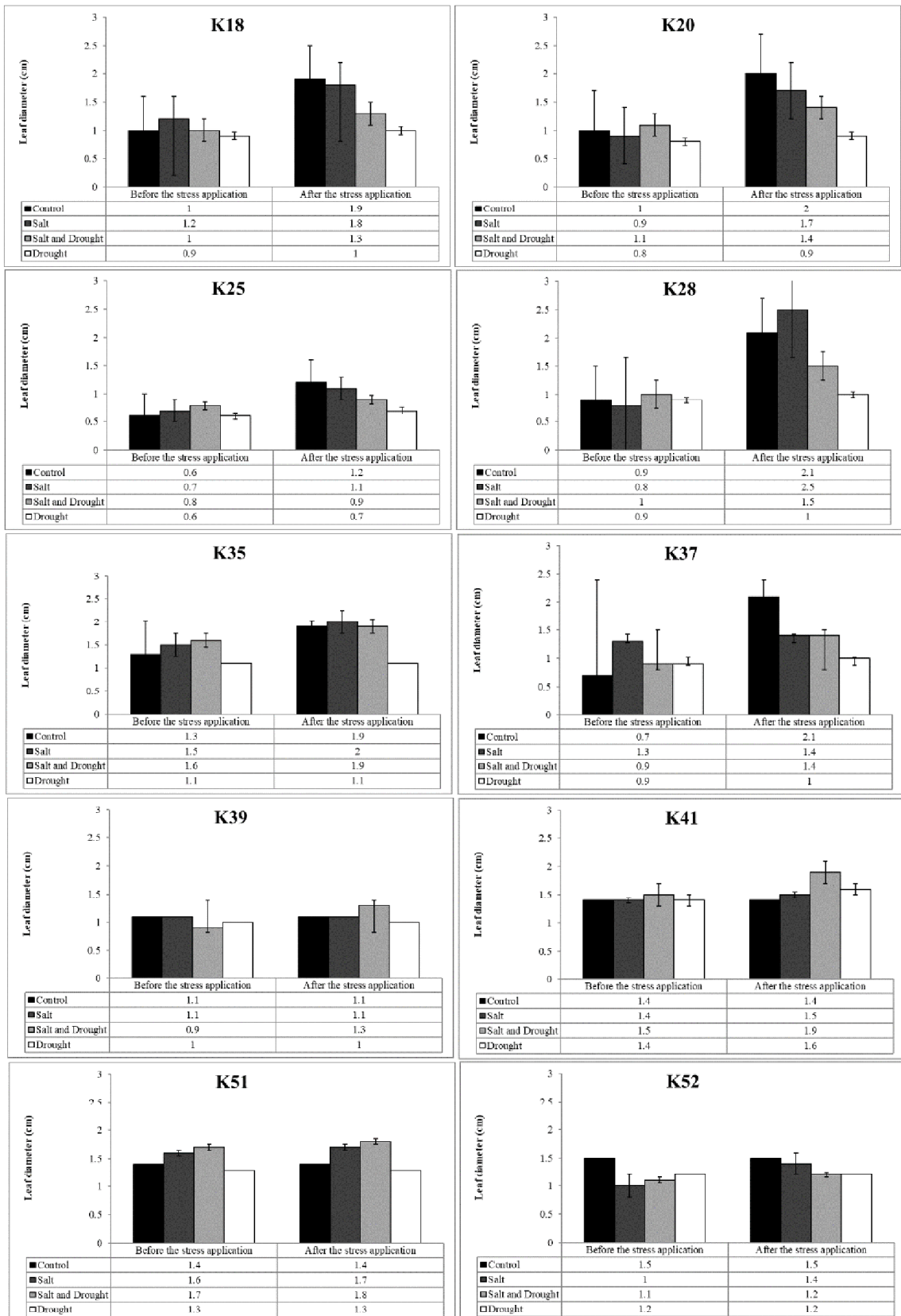


Figure 4.2. Leaf diameter change graphs of ten lines from GAF-MTN onion gene pool before and after stress treatment

Although there was an increase in the control group of K18 as expected, the leaf diameter increase was not occurred in response to drought stress, while in the case of salt and combined stress (salt and drought), the leaf diameter increased. While the increase ratio in salt treatment was close to the expected increase in the control of K20, there was less leaf diameter expansion in terms of drought and combined (salt and drought) treatment than its control group. The leaf diameter increase was not occurred in response to drought and combined (salt and drought) treatments, even though there was an increase in the control of K25 line as expected. Furthermore, leaf diameter increase was observed nearly as much as control group after salt treatment. There was a higher increase after salt treatment than control group of K28, but leaf diameter increase was not occurred in drought treatment. Moreover, leaf diameter partially increased under salt and drought combined treatment. Although, there was no increase in K35 in response to drought treatment, leaf diameter was expanded after salt and combined (salt and drought) treatments in comparison with control group. There was an increase in the salt and drought combined stress treatment in K37 as much as its control group, whereas there was less expansion in diameter of leaf under salt and drought stress compared to the control. In K39, there was no increase in the control, salt and drought treatments, leaf expansion was only occurred in response to salt and drought combined stress. There was no increase in the control group in K41, but leaf diameter expansion was partially occurred in response to salt, drought and combined (salt and drought) treatments. Although there was no increase in the control and drought group of K51, leaf diameter slightly increased in the case of salt and combined (salt and drought) treatment. In the K52 line, there was no increase of leaf diameter in the control and drought groups similar to K51, but enhancement was comparatively occurred in the salt and combined (salt and drought) treatments.

In the control groups during the 20-day period in the bulbing phase, the maximum leaf diameter increase was observed in the K37 line, while the leaf diameter did not increase in the K39, K41, K51 and K52 lines. After salt stress application, the most expansion was observed in the K28 line. The least increase of leaf diameter was in the K51 line (5.8%) and the highest was in the K37 line (55.5%) in combined (salt and drought) stress group. The increase in leaf width of the plants, where drought stress is applied without giving water for twenty days, varies between 0 and 16.6 percent.

Observations on the number of leaves were made twice, at the beginning and at the end of stress period. Considering the number of leaves in general, different levels of increases occurred in all stress groups (Figure 4.3).



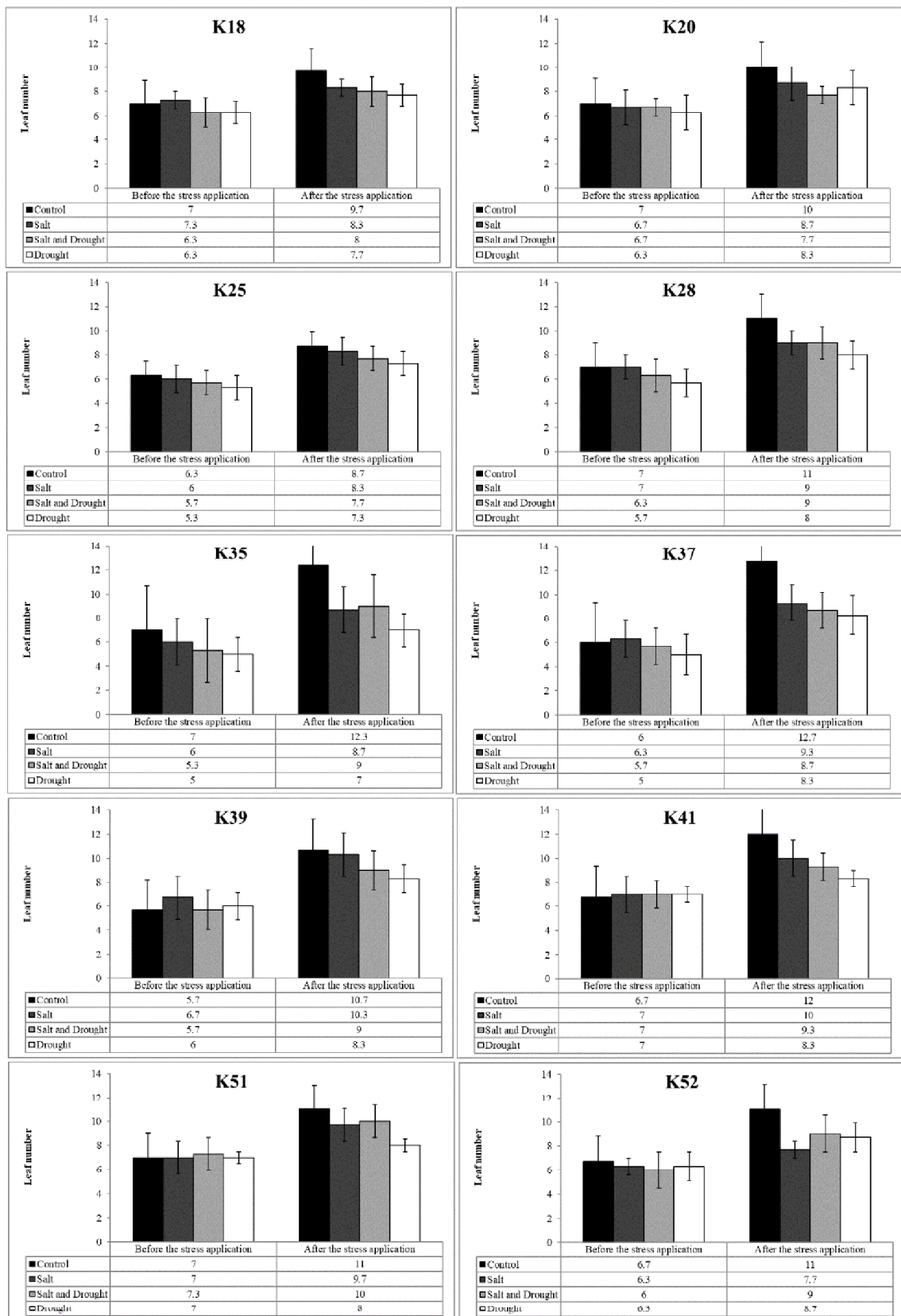


Figure 4.3. Leaf number change graphs of ten lines from GAF-MTN onion gene pool before and after stress treatment

There was a change of the number of leaves in control groups as expected in K18, while the minimum increase was observed in response to the salt treatment, the leaf number increase was occurred in drought and combined (salt and drought) stress as much as its control group. Although there was an increase of leaf number in response to salt, drought and combined (salt and drought) stress in K20, minimum increase was occurred in salt and drought combined treatment compared to its control group. Increase of leaf number was almost the same as in the control group of K25 in terms of salt, drought and combined (salt and drought) stresses. There was an increase of number of leaf in the control group of K28 as expected, minimum change was occurred in response to salt treatment, while in the case of drought and salt and drought combined stresses, the leaf number almost increased as much as its control group. Even though increase of number of leaf was occurred in the salt, drought and combined (salt and drought) group of K35, there was minimum increase in drought treatment according to its control group. There was an increase in the leaf number of drought group of K37 nearly as its control, whereas in the case of salt and combined (salt and drought) stress leaf number increased less. The increase rates of salt, drought and combined (salt and drought) treatment groups of K39 were close to each other and similar to its control group. Although there was a change in control group of K41 as expected, the increase in the leaf number was less in response to drought treatment, while in the case of salt and combined (salt and drought) stresses, the leaf number increased. Leaf number increase was not occurred in response to drought treatment, though there was an increase in the control group of K51 as expected. Leaf number partially increased in terms of salt and drought stress treatment in K51. There was an increase in salt, drought and combined (salt and drought) stress groups of K52. Minimum increase was occurred in response to salt treatment, leaf number comparatively increased in drought and combined (salt and drought) stresses as much as its control group.

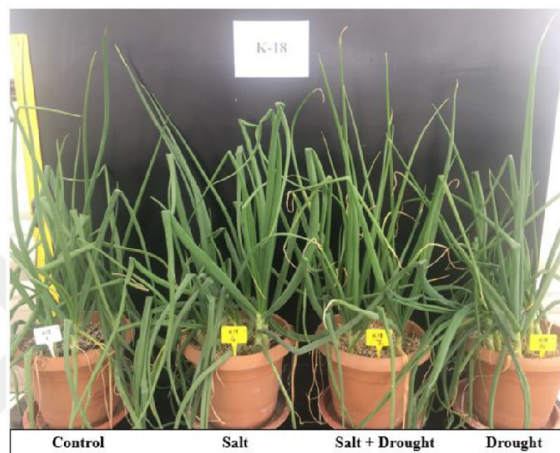
When the control groups were evaluated within themselves, the line with the highest leaf increase in the 20-day bulbing period was K37. The genotype with the highest number of leaves after the salt stress was K39 (53.7%). After the salt and drought stress application, K35 was the line with the highest leaf number increase with 69.8%. Leaf number increase rates in the drought group were between 14.2 and 66%. While the genotype with the highest increase in the number of leaves was K37, the least one was K51 (Table 4.1).

Table 4.1. Leaf length, diameter and number values and standart deviations before and after stress treatment

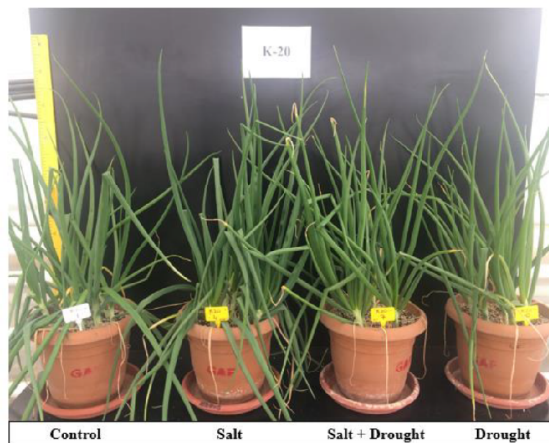
Leaf Length Values (cm) and Standart Deviations Before and After Stress Treatment								
	Control		Salt		Salt and Drought		Drought	
	Before	After	Before	After	Before	After	Before	After
K18	77.0 ± 3.6	88.3 ± 5.6	78.7 ± 7.0	82 ± 4.0	77.7 ± 0.5	82.3 ± 3.0	81 ± 1.0	83.7 ± 3.0
K20	75.7 ± 3.0	81 ± 3.6	71.7 ± 3.7	74 ± 5.2	76 ± 3.6	79.7 ± 4.9	63.3 ± 12.0	64.3 ± 13.0
K25	69.3 ± 7.7	84.7 ± 5.0	57 ± 2.6	57.3 ± 3.0	67.7 ± 3.2	75.3 ± 5.0	55.3 ± 3.7	56.7 ± 4.1
K28	72.3 ± 6.5	80 ± 2.0	73.7 ± 8.6	84 ± 6.9	70.7 ± 3.2	72 ± 3.6	60 ± 3.6	60.7 ± 3.0
K35	84.7 ± 3.7	95.3 ± 7.5	79.3 ± 5.5	81.7 ± 7.6	78 ± 4.0	79.3 ± 3.0	64.3 ± 0.5	65.3 ± 1.1
K37	78.7 ± 1.1	82 ± 2.0	71.7 ± 7.7	77.3 ± 2.3	58.3 ± 2.0	59.7 ± 2.5	61.7 ± 2.8	62.7 ± 2.5
K39	73.7 ± 3.2	78 ± 2.0	79.3 ± 2.5	80 ± 2.0	65.3 ± 1.5	68.7 ± 1.1	66 ± 5.5	66.7 ± 6.1
K41	77.7 ± 2.0	88.3 ± 17.0	73.7 ± 0.5	80 ± 4.0	82.3 ± 3.5	83.3 ± 3.0	57 ± 1.7	58 ± 2.0
K51	79.3 ± 5.7	84.7 ± 8.6	83.7 ± 5.5	88.3 ± 5.6	70.7 ± 3.2	72 ± 2.0	65.7 ± 4.0	66.7 ± 4.1
K52	61.3 ± 3.0	63.3 ± 3.0	53 ± 2.6	55 ± 2.0	62.7 ± 1.1	64.3 ± 1.5	61.7 ± 8.0	63.3 ± 7.0
Mean	74.9	82.5	72.1	75.9	70.9	73.6	63.6	64.8
Leaf Diameter Values (cm) and Standart Deviations Before and After Stress Treatment								
	Control		Salt		Salt and Drought		Drought	
	Before	After	Before	After	Before	After	Before	After
K18	1.0 ± 0.1	1.9 ± 0.3	1.2 ± 0.1	1.8 ± 0.2	1.0 ± 0.05	1.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
K20	1.0 ± 0.05	2.0 ± 0.2	0.9 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	1.4 ± 0.2	0.8 ± 0.1	0.9 ± 0.2
K25	0.6 ± 0.05	1.2 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	0.8 ± 0.2	0.9 ± 0.1	0.6 ± 0.05	0.7 ± 0.1
K28	0.9 ± 0.1	2.1 ± 0.1	0.8 ± 0.1	2.5 ± 0.05	1.0 ± 0.1	1.5 ± 0.05	0.9 ± 0.2	1.0 ± 0.2
K35	1.3 ± 0.1	1.9 ± 0.1	1.5 ± 0.1	2.0 ± 0.05	1.6 ± 0.2	1.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
K37	0.7 ± 0.05	2.1 ± 0.1	1.3 ± 0.4	1.4 ± 0.1	0.9 ± 0.05	1.4 ± 0.05	0.9 ± 0.1	1.0 ± 0.1
K39	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.05	1.3 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
K41	1.4 ± 0.05	1.4 ± 0.05	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	1.4 ± 0.1	1.6 ± 0.05
K51	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.3 ± 0.3	1.3 ± 0.3
K52	1.5 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	1.4 ± 0.05	1.1 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Mean	1.0	1.6	1.1	1.6	1.1	1.4	1.0	1.0
Leaf Number Values and Standart Deviations Before and After Stress Treatment								
	Control		Salt		Salt and Drought		Drought	
	Before	After	Before	After	Before	After	Before	After
K18	7.0 ± 0.0	9.7 ± 1.1	7.3 ± 0.5	8.3 ± 0.5	6.3 ± 0.5	8.0 ± 0.5	6.3 ± 0.5	7.7 ± 0.5
K20	7.0 ± 0.0	10.0 ± 0.0	6.7 ± 0.5	8.7 ± 1.5	6.7 ± 0.5	7.7 ± 0.5	6.3 ± 0.5	8.3 ± 0.5
K25	6.3 ± 0.5	8.7 ± 0.5	6.0 ± 0.0	8.3 ± 0.5	5.7 ± 0.5	7.7 ± 0.5	5.3 ± 0.5	7.3 ± 0.5
K28	7.0 ± 0.0	11.0 ± 1.0	7.0 ± 1.0	9.0 ± 1.0	6.3 ± 0.5	9.0 ± 1.0	5.7 ± 0.5	8.0 ± 0.0
K35	7.0 ± 0.0	12.3 ± 2.0	6.0 ± 0.0	8.7 ± 2.0	5.3 ± 0.5	9.0 ± 0.0	5.0 ± 0.0	7.0 ± 0.0
K37	6.0 ± 0.0	12.7 ± 2.0	6.3 ± 0.5	9.3 ± 0.5	5.7 ± 0.5	8.7 ± 1.5	5.0 ± 0.0	8.3 ± 0.5
K39	5.7 ± 0.5	10.7 ± 1.1	6.7 ± 0.5	10.3 ± 0.5	5.7 ± 0.5	9.0 ± 1.0	6.0 ± 0.0	8.3 ± 0.5
K41	6.7 ± 0.5	12.0 ± 1.7	7.0 ± 0.0	10.0 ± 0.0	7 ± 0.0	9.3 ± 0.5	7.0 ± 0.0	8.3 ± 0.5
K51	7.0 ± 0.0	11.0 ± 1.0	7.0 ± 0.0	9.7 ± 1.1	7.3 ± 0.5	10.0 ± 1.0	7.0 ± 0.0	8.0 ± 1.0
K52	6.7 ± 0.5	11.0 ± 1.7	6.3 ± 0.5	7.7 ± 0.5	6.0 ± 0.0	9.0 ± 1.0	6.3 ± 0.5	8.7 ± 0.5
Mean	6.6	10.9	6.6	9.0	6.2	8.7	5.9	7.9

4.1.2. Evaluation of general plant views before and after stress

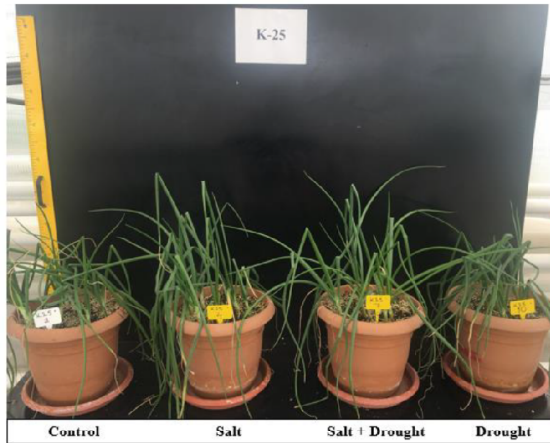
There were three repeat pots from each line, each stress application and their control group. In order to see the effect of stress treatment on the overall plant view, before the stress started; pots saved for control, salt, combined (salt and drought), drought treatments were placed side by side and photographed. Photographs of all genotypes were given in Picture 4.1 - 4.10.



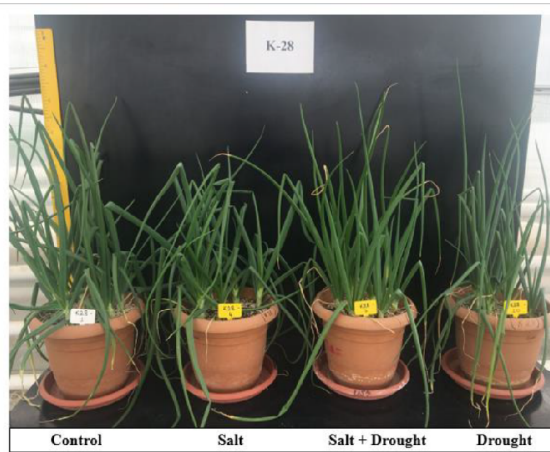
Picture 4.1. K18 short-day onion line before stress treatment



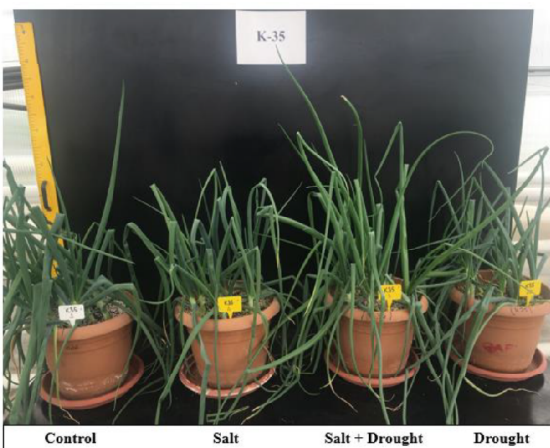
Picture 4.2. K20 short-day onion line before stress treatment



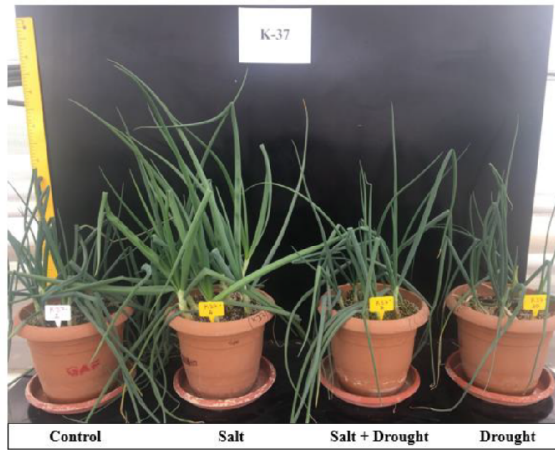
Picture 4.3. K25 short-day onion line before stress treatment



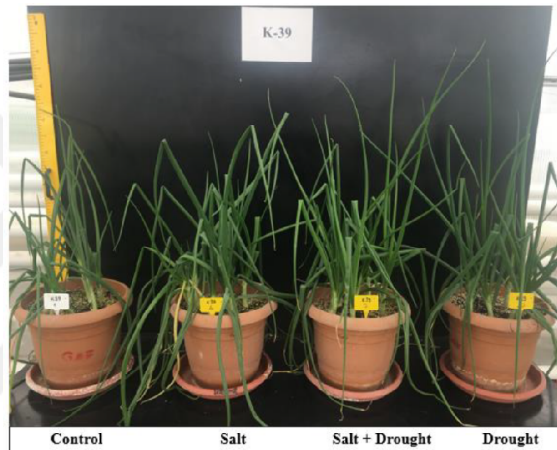
Picture 4.4. K28 short-day onion line before stress treatment



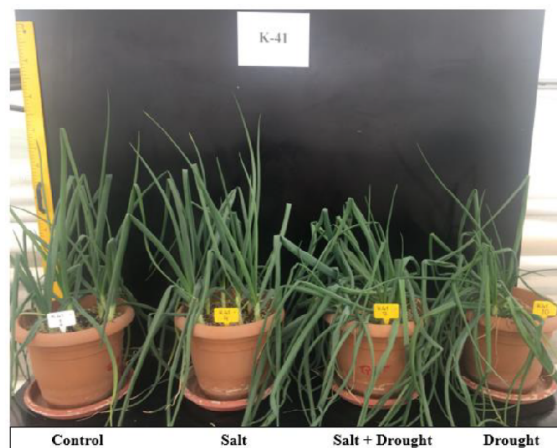
Picture 4.5. K35 short-day onion line before stress treatment



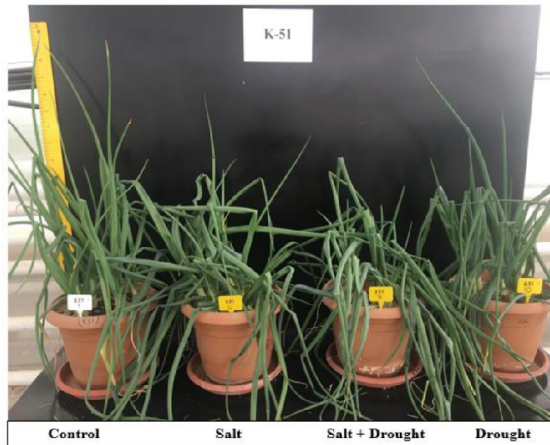
Picture 4.6. K37 short-day onion line before stress treatment



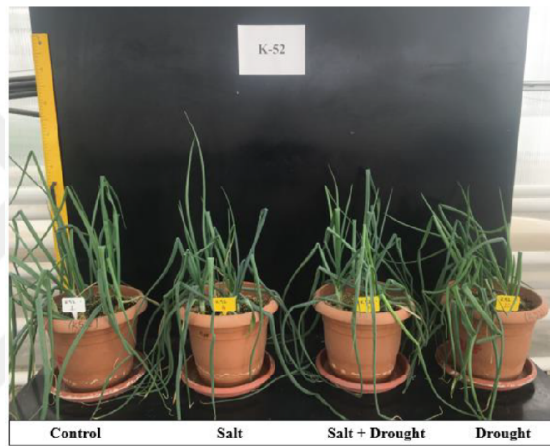
Picture 4.7. K39 short-day onion line before stress treatment



Picture 4.8. K41 short-day onion line before stress treatment



Picture 4.9. K51 short-day onion line before stress treatment

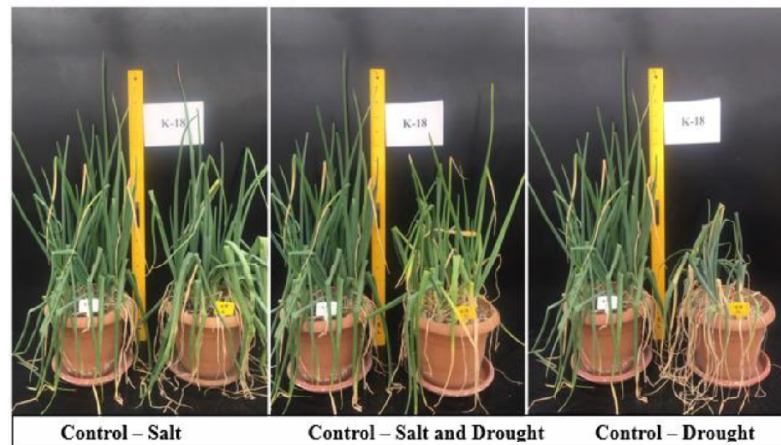


Picture 4.10. K52 short-day onion line before stress treatment

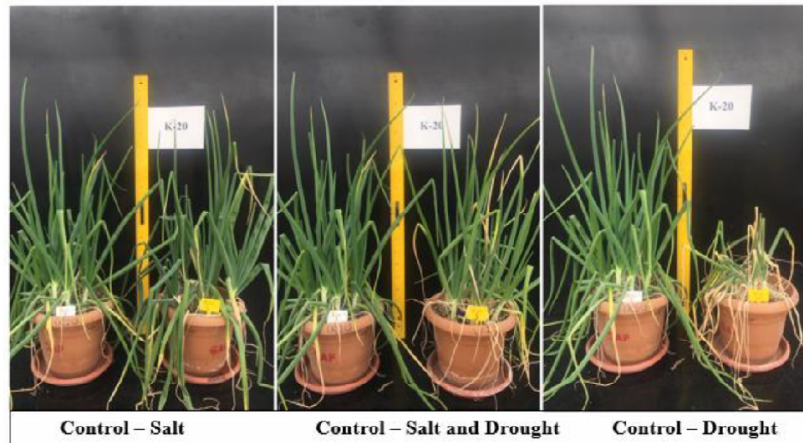
When stress was initiated, plants gave some morphological reactions. Since these reactions can be observed as paleness due to yellowing and increased water loss in plant leaves, physiological differences also occur between control and stress groups. Ten lines of short-day GAF-MTN onion gene pool were grown for approximately 4.5 months in the same greenhouse environment under equal conditions and with the same amount of irrigation. After the plants reached the bulbing stage, the stress initiated and continued for a 20-day period. Yellowing and wilting occurred in some plant leaves after stress application. This has been more apparent in drought stress in the later stages of stress period. After 20 days, stress applications were terminated and control and each stress treatment were compared with photography in Pictures 4.11-4.20.

K18 was not affected by salt stress of 20 days, but it was significantly affected with drought and combined (drought and salt) stresses as observed as morphological

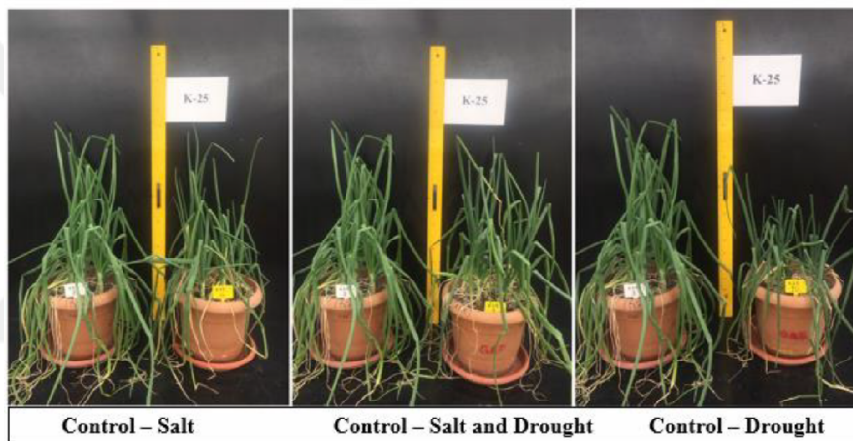
damages compared to its control. Although there were some damage by drought and combined (salt and drought) stresses in K20, injuries were less in response to salt treatment as compared with its control. Leaf damage was not occurred in response to salt and combined (salt and drought) stress , even though there was lots of damage in the drought group of K25. There was some yellowing of leaves by salt and drought treatments and wilting by drought treatment of K-28, it was not affected by salt stress. K35 was not affected by salt stress, but it was considerably affected with drought and combined (salt and drought) stresses as observed by morphological damages compared with its control. While the K37 line was not affected by salt stress, it was affected morphologically by drought and combined (salt and drought) treatment after 20 days compared to its control. K39 was not affected by salt treatment, however it was significantly affected by drought and combined (drought and salt) stresses as compared to its control. Although there was no damage by salt treatment of K41, leaf damage was occurred and growing was considerably limited by drought and combined (salt and drought) treatments. K51 was significantly affected with salt, drought and combined (salt and drought) stresses. Even though there was less damage in the salt, drought and combined (salt and drought) stress of K52, in the case of drought stress, wilting significantly occurred.



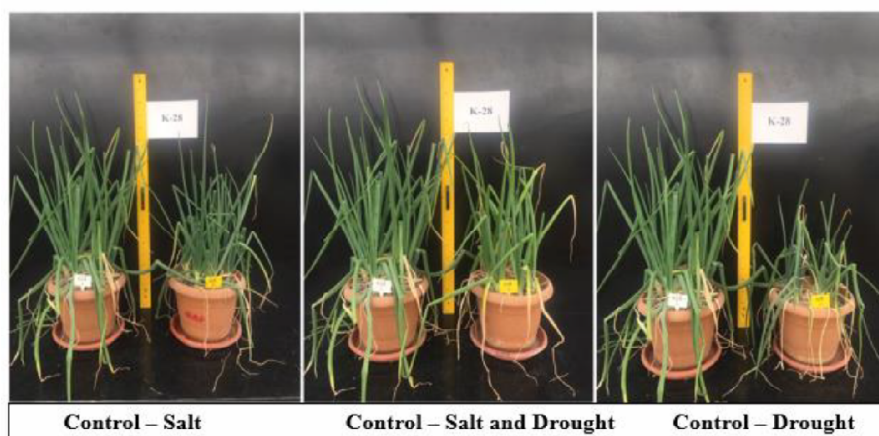
Picture 4.11. Morphological view of the K18 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



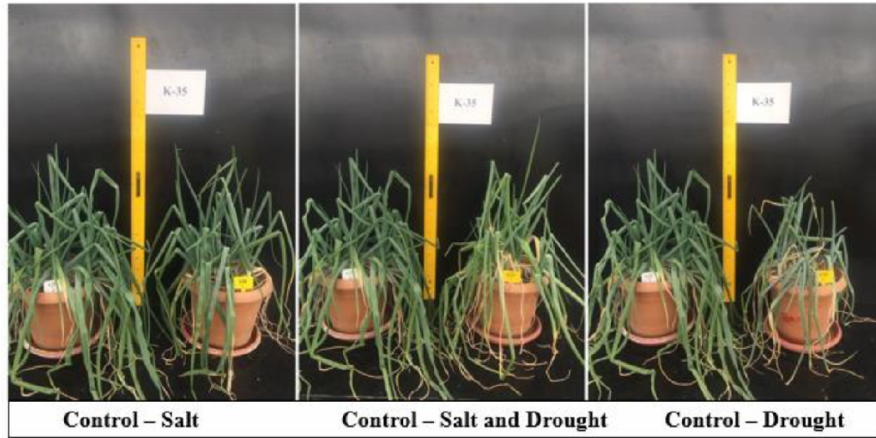
Picture 4.12. Morphological view of the K20 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



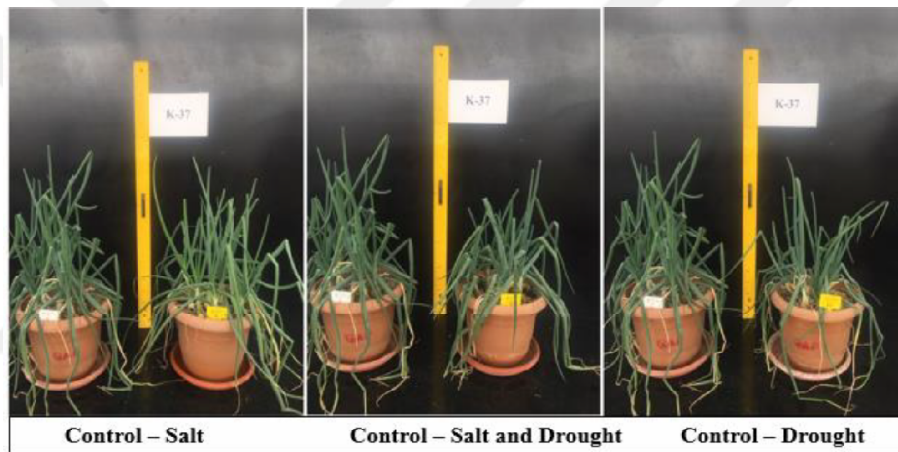
Picture 4.13. Morphological view of the K25 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



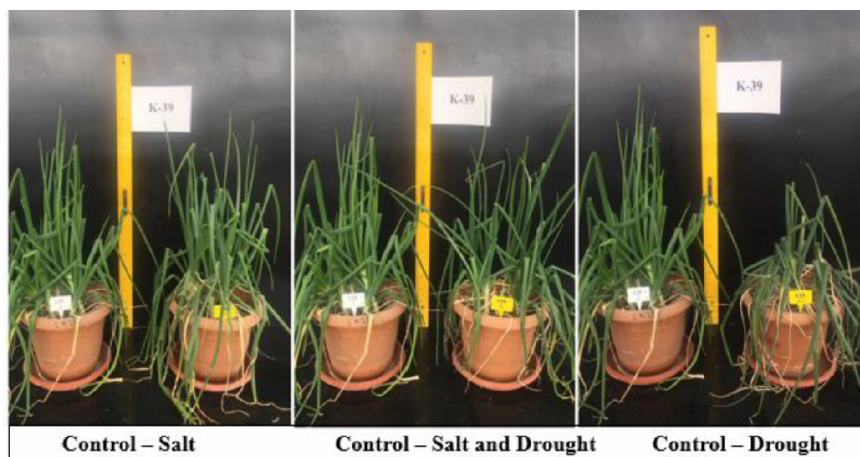
Picture 4.14. Morphological view of the K28 in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



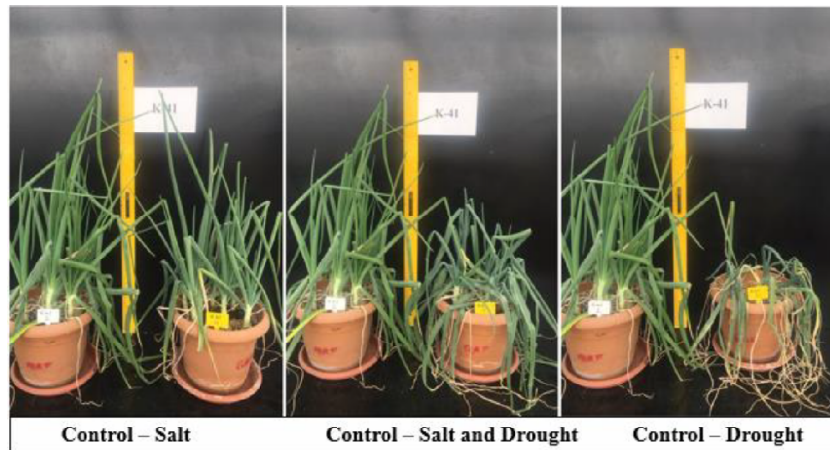
Picture 4.15. Morphological view of the K35 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



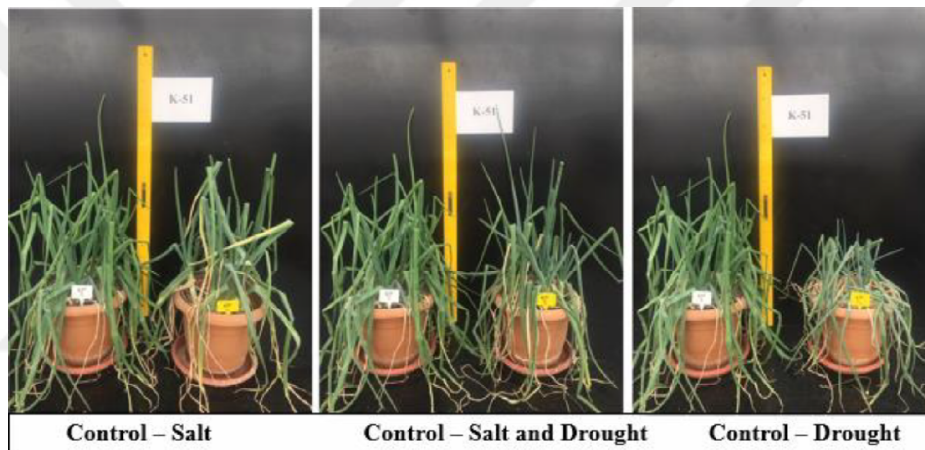
Picture 4.16. Morphological view of the K37 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



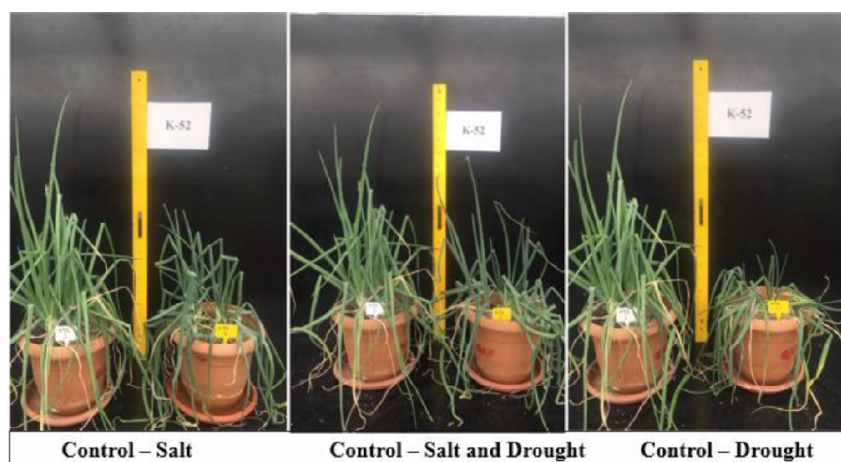
Picture 4.17. Morphological view of the K39 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



Picture 4.18. Morphological view of the K41 lines in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



Picture 4.19. Morphological view of the K51 in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control



Picture 4.20. Morphological view of the K52 in the GAF-MTN onion gene pool at the end of 20 days of stress treatment compared to its control

4.2 Determination of Physiological Features

4.2.1. SPAD value (Leaf chlorophyll index)

SPAD measurement was taken to show the amount of chlorophyll in the leaf. Chlorophyll index measurements were taken totally 6 times on day 0, day 5, day 10, day 14, day 17, and day 20 for all stress treatments and their control groups (Figure 4.4).



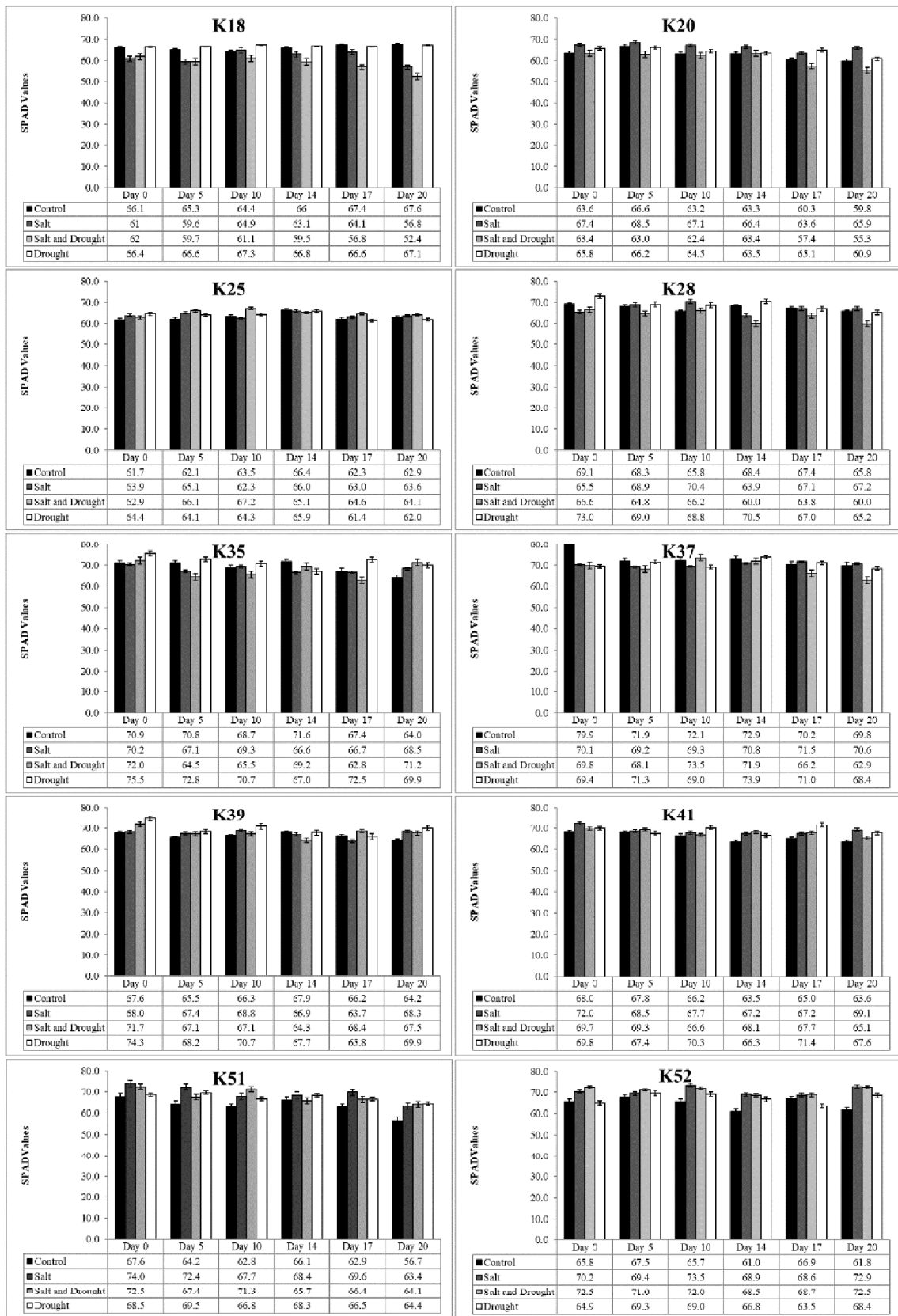


Figure 4.4. The graphs of change of chlorophyll index during stress factors applied at the bulbing stage of ten lines from GAF-MTN onion gene pool

SPAD values were found high in the drought group of K18 on day 0, day 5, day 10 and day 14, while control group values were higher than drought group on day 17 and day 20. Although the values of the salt and drought groups of K20 were close to one another, the values of the salt treatment were higher on all days except the 17th day than its control group. It was affected mostly by salt and drought groups of K25 on the day 14, but higher SPAD value was occurred than its control group on the 10th day of treatment. There was an increase by salt, drought and combined (salt and drought) stress treatments in K28 on the 10th day compared to its control, but it was especially affected by salt stress.

The highest SPAD value of K35 was on the 14th day in the control group, while the highest value was observed in the salt and drought combined treatment on the day 20. Although in response to drought chlorophyll index decreased from the start of stress until the 14th day, there was an increase on the 17th day and a decrease again on the 20th day. While there were highest SPAD values in K37 on the 0th and 5th day in its control group, a higher value was observed in response to salt and drought combined treatments on day 10, by drought treatment on day 14, by salt treatment on day 17 and 20 compared to its control group. Chlorophyll index was the highest by drought group of K39 on 0th, 5th, 10th and 20th days compared to its control group. There was a decrease between the day 0 and 20 in all stress groups, except in response to salt group. Although control group of K41 decreased until the 14th day, there was an increase on the 17th day, and the last measurement decreased again. In all stress and control groups, there was a decrease in day 20 compared to the stress initiation day.

SPAD values of salt, drought and combined (salt and drought) treatments were higher than their control in the all the measurements. Maximum values were occurred in salt group of K51 line on the 0th, 5th and 17th days. While the highest chlorophyll index was on the 0th day in the control group, it was decreased on the 20th day. In all stress groups, the values decreased from the stress initiation day to the last measurement as expected. Although there was maximum decrease in the control group, minimum decrease was occurred by drought treatment between day 0 and 20. The higher chlorophyll index of K52 line was occurred by salt and combined stress (salt and drought) on all days than its control group. SPAD value was less than control on 17th day by drought treatment.

4.2.2. Leaf Temperature (°C)

In drought stress, plants close their stomata to reduce water loss, so transpiration in the leaves is reduced which causes the leaf temperature to increase (Dolferus, 2014; Farooq et al., 2009). Leaf temperature measurements were taken to prove the temperature increase in the leaves of plants under stress conditions. Leaf temperature were measured 7 times, from the time of stress initiation, on day 0, day 3, day 6, day 10, day 14, day 17 and day 20 (Figure 4.5).



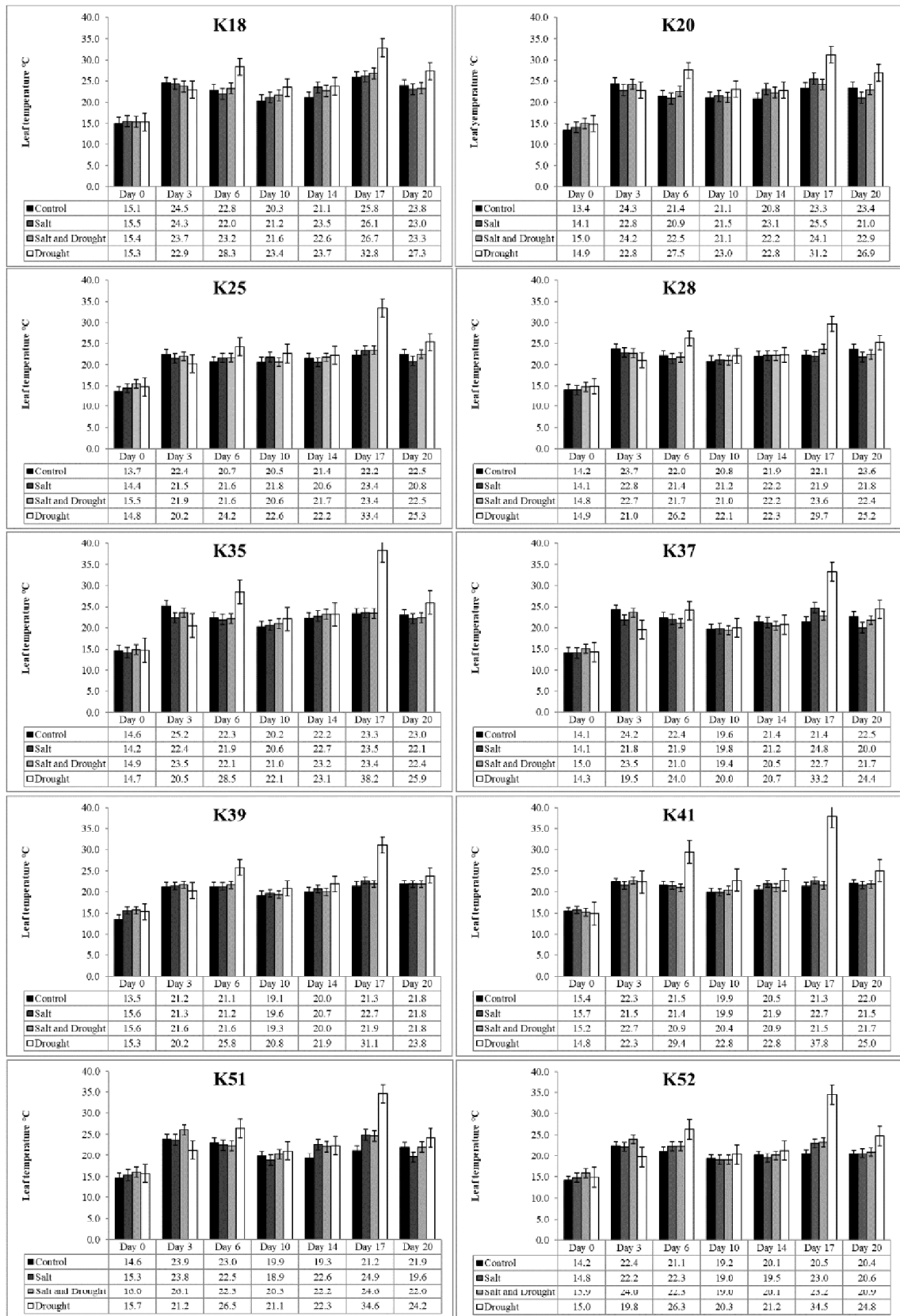


Figure 4.5. Leaf temperature (°C) measurement graphs of ten lines from GAF-MTN onion gene pool during stress treatments

The temperature was higher by the drought treatment of K18 on the 6th, 10th, 14th, 17th, 20th days compared to other stress groups. Although values were very close in all stress treatments, drought group was slightly higher than the control group except day 3. Temperature values were close to control group of K20 by salt and combined (salt and drought) treatments. Temperature increase was considerably higher than the control by drought treatment, especially on the 6th, 17th and 20th days. After all stress treatments, temperature increased from day 0 to day 20 as expected.

Temperature values of salt and combined (salt and drought) stress group of K25 were found close to their control group. In contrast, after the 6th day, all of the temperature values in the drought group were higher than the control group. Especially on the 17th day, there was excessive increase of leaf temperature. Temperature values were close to control group of K-28 by salt treatment. Higher values were occurred by salt and drought combined group with very little difference on days 0, 10, 14 and 17 than its control, but the values were very close to each other in general.

The temperature values in drought treatment were higher than the control in all measurements except the 3rd day, especially in the 17th day, the temperature difference reached the maximum between the control and drought. According to leaf temperatures of K35 line, all values were very close to each other on day 0, while all stress groups increased from day 0 to day 20. On all other days except for the 3rd day, the temperature values were higher than the control in the drought group, but on the 17th day, the temperature increased from 23.3 °C to 38.2 °C in the drought group and the maximum increase was experienced in the drought group of K35 line. All leaf temperature values were close to each other and its control group by salt and combined (salt and drought) stresses. There was an increase of leaf temperature in all stress groups from the first measurement to the last measurement of K37.

Temperature values were close in salt, drought and combined (salt and drought) groups to its control until 17th day. On the 17th day, a serious temperature increase was occurred by drought treatment compared to other groups. While leaf temperature values of K39 were quite close in salt and combined (salt and drought) treatments, the highest values were occurred in all measurements by drought treatment except day 0 and 3. Moreover, the day when the temperature difference was the highest between the control and the

drought group was day 17. Leaf temperature showed close values in the control, salt and combined (salt and drought) groups of K41 and K51, while temperature difference was maximum on the 17th day in both lines by drought treatment compared to its control. The temperature values of K52 were more than its control in response to salt, drought and combined (salt and drought) treatments on day 0th, 6th, 17th and 20th. While leaf temperature was lowest in control group on day 17th, it increased to 34.5 °C and it was 40.5% higher than control.

4.2.3. Photosynthesis measurement

The increase in the amount of salt causes the amount of chlorophyll in the plant leaves and the photosynthetic activity to decrease. In drought stress, due to stomata closure, the entrance of CO₂ to the leaves decreases and photosynthesis reduces. Because of all this, photosynthesis rate decreases in salt and drought treatments. Therefore, since the rate of photosynthesis was expected to decrease in all stress groups, for this purpose, photosynthesis measurement was taken on days 0, 5, 10, 14, 17, 20 (Figure 4.6.).

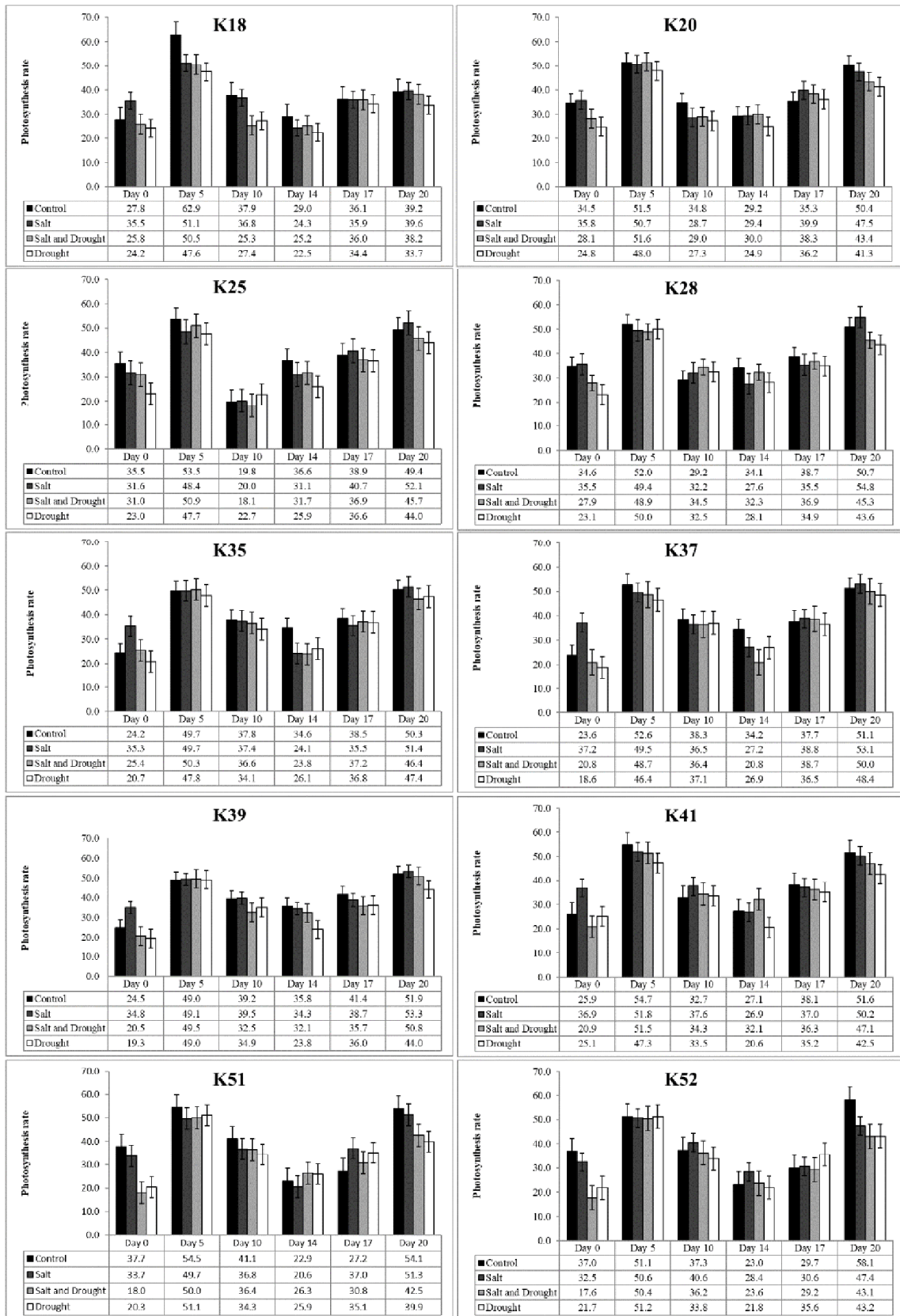


Figure 4.6. Photosynthesis rate ($\mu\text{molm}^{-2}\text{s}^{-1}$) measurement graphs of of ten lines from GAF-MTN onion gene pool during stress treatments

When the rate of photosynthesis of the K18 line was examined, the lowest value in the control was occurred on day 0, while the lowest value was obtained on the 14th day in salt, drought and combined (salt and drought) groups. While there was increase in all stress groups in the measurements taken at the beginning and end of the stress, the minimum increase was by salt treatment. While the lowest values were on the 0th day by drought and combined (salt and drought) stress of K20, it was mostly affected by salt treatment on the 10th day and there was a 19.8% decrease compared to the measurement on the first day.

In the drought group, the photosynthesis rate was lower than the control in all days except the 17th day. The lowest photosynthesis rate of K25 was occurred on 10th day by salt and drought treatment compared to control, but it was mostly affected by drought treatment on 14th day. The K-28 line was mostly affected by salt treatment on the 14th day compared to control. On the same day, less photosynthesis rate was occurred of the drought group than the control plants.

There was an increase in the photosynthesis rate by salt, drought and combined (salt and drought) stress groups which were 54.3%, 88.7% and 62.3%, respectively from day 0 to day 20. Minimum increase was occurred by salt treatment. The photosynthesis rate was mostly affected by salt and combined (salt and drought) stress of K35 on the 14th day, while salt group was 30% less and the (salt and drought) group was 31.2% less than its control. Moreover, photosynthesis rate difference was maximum on day 14 by drought treatment compared to its control. K37 line was mostly affected by salt and drought stress on 14th day, and the lowest value was occurred compared to its control.

The photosynthesis rate increased in all stress groups and control groups from day 0 to day 20. The least increase was by salt treatment with a rate of 42.7%, while there was a 116.5% increase in control, maximum increase (160.2%) was observed by drought treatment. K39 line was mostly affected by drought stress on the 14th day. On other days, values in all stress groups were close to each other and to its control. Although close values were occurred by salt and combined (salt and drought) groups of K41 compared to its control group, minimum photosynthesis rate was occurred by drought treatment on day 14. While photosynthesis rate was close by salt treatment compared to its control of K51 on the 0th day when stress treatment was started, the values were quite

lower by the drought and combined (salt and drought) treatment groups compared to its control, but all stress groups considerably decreased on day 14. There was an increase in all stress groups and its control group, while the least increase was occurred by salt treatment from day 0 to 20. The photosynthesis rate was especially affected by salt, drought and combined (salt and drought) treatments of K52 on day 20, the salt stress treated group was 18.4% less, while drought and combined (salt and drought) group was about 25% less than its control (Table 4.2.).



Table 4.2. Chlorophyll index, leaf temperature and photosynthesis rate values and standart deviations of day 0 and 20

Chlorophyll Index Values and Standart Deviation of Day 0 and Day 20								
	Control		Salt		Salt and Drought		Drought	
	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20
K18	66.1 ± 2.6	67.6 ± 4.4	61.0 ± 3.0	56.8 ± 2.8	62.0 ± 3.9	52.4 ± 3.6	66.4 ± 2.1	67.1 ± 3.7
K20	63.6 ± 1.8	59.8 ± 3.4	67.4 ± 3.8	65.9 ± 3.2	63.4 ± 3.6	55.3 ± 2.2	65.8 ± 1.4	60.9 ± 5.6
K25	61.7 ± 4.6	62.9 ± 3.7	63.9 ± 1.9	63.6 ± 2.3	62.9 ± 2.0	64.1 ± 2.8	64.4 ± 2.6	62.0 ± 6.3
K28	69.1 ± 2.9	65.8 ± 0.7	65.5 ± 1.0	67.2 ± 5.1	66.6 ± 2.3	60.0 ± 6.4	73.0 ± 4.6	65.2 ± 7.5
K35	70.9 ± 6.0	64.0 ± 4.9	70.2 ± 3.3	68.5 ± 2.7	72.0 ± 6.4	71.2 ± 5.8	75.5 ± 0.8	69.9 ± 2.3
K37	79.9 ± 2.1	69.8 ± 7.4	70.1 ± 2.3	70.6 ± 2.6	69.8 ± 1.9	62.9 ± 5.0	69.4 ± 2.7	68.4 ± 2.4
K39	67.6 ± 2.2	64.2 ± 1.9	68.0 ± 2.1	68.3 ± 1.4	71.7 ± 6.3	67.5 ± 4.4	74.3 ± 6.2	69.9 ± 3.1
K41	68.0 ± 4.7	63.6 ± 4.2	72.0 ± 2.2	69.1 ± 6.1	69.7 ± 3.6	65.1 ± 2.0	69.8 ± 2.8	67.6 ± 6.5
K51	67.6 ± 7.1	56.7 ± 4.3	74.0 ± 1.6	63.4 ± 1.2	72.5 ± 3.1	64.1 ± 1.8	68.5 ± 3.4	64.4 ± 3.9
K52	65.8 ± 4.5	61.8 ± 1.0	70.2 ± 1.7	72.9 ± 4.4	72.5 ± 6.2	72.5 ± 1.7	64.9 ± 0.9	68.4 ± 3.2
Mean	68.0	63.6	68.2	66.6	68.3	63.5	69.2	66.3
Leaf Temperature Values and Standart Deviation of Day 0 and Day 20								
	Control		Salt		Salt and Drought		Drought	
	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20
K18	15.1 ± 0.2	23.8 ± 1.3	15.5 ± 0.2	23.0 ± 1.1	15.4 ± 0.6	23.3 ± 1.9	15.3 ± 0.3	27.3 ± 1.0
K20	13.4 ± 0.1	23.4 ± 1.4	20.9 ± 0.1	21.0 ± 0.5	15.0 ± 0.3	22.9 ± 0.1	14.9 ± 0.1	26.9 ± 1.1
K25	13.7 ± 0.6	22.5 ± 0.3	14.4 ± 0.6	20.8 ± 0.2	15.5 ± 0.8	22.5 ± 0.5	14.8 ± 0.1	25.3 ± 0.8
K28	14.2 ± 0.5	23.6 ± 1.0	14.1 ± 0.4	21.8 ± 0.9	14.8 ± 0.6	22.4 ± 0.8	14.9 ± 0.2	25.2 ± 0.5
K35	14.6 ± 0.7	23.0 ± 0.5	14.2 ± 0.2	22.1 ± 0.6	14.9 ± 0.7	22.4 ± 0.05	14.7 ± 0.7	25.9 ± 0.8
K37	14.1 ± 0.4	22.5 ± 1.1	14.1 ± 0.4	20.0 ± 1.0	15.0 ± 0.6	21.7 ± 0.5	14.3 ± 0.4	24.4 ± 0.7
K39	13.5 ± 0.4	21.8 ± 0.2	15.6 ± 0.3	21.8 ± 0.1	15.6 ± 0.2	21.8 ± 0.5	15.3 ± 0.3	23.8 ± 0.1
K41	15.4 ± 0.5	22.0 ± 1.1	15.7 ± 0.6	21.5 ± 0.9	15.2 ± 0.2	21.7 ± 0.3	14.8 ± 0.1	25 ± 1.3
K51	14.6 ± 0.1	21.9 ± 0.8	15.3 ± 0.6	19.6 ± 0.7	16.0 ± 0.1	22.0 ± 0.2	15.7 ± 0.6	24.2 ± 0.8
K52	14.2 ± 0.1	20.4 ± 1.4	14.8 ± 0.1	20.6 ± 0.5	15.9 ± 0.1	20.9 ± 0.7	15.0 ± 0.2	24.8 ± 0.4
Mean	14.2	22.4	15.4	21.2	15.3	22.1	14.9	25.2
Photosynthesis Rate Values and Standart Deviations of Day 0 and Day 20								
	Control		Salt		Salt and Drought		Drought	
	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20
K18	27.8 ± 8.7	39.2 ± 1.1	35.5 ± 5.4	39.6 ± 1.0	25.8 ± 1.3	38.2 ± 1.7	24.2 ± 1.3	33.7 ± 0.3
K20	34.5 ± 6.9	50.4 ± 1	35.8 ± 1.7	47.5 ± 3.3	28.1 ± 0.06	43.4 ± 2.5	24.8 ± 2.9	41.3 ± 1
K25	35.5 ± 4.7	49.4 ± 1.5	31.6 ± 1.1	52.1 ± 3.4	31 ± 2.6	45.7 ± 0.5	23 ± 4.1	44 ± 1.4
K28	34.6 ± 6.8	50.7 ± 1	35.5 ± 3.6	54.8 ± 2.2	27.9 ± 4.0	45.3 ± 1.5	23.1 ± 1.4	43.6 ± 2.4
K35	24.2 ± 2.8	50.3 ± 1.1	35.3 ± 1.3	51.4 ± 1.1	25.4 ± 3.2	64.4 ± 3.4	20.7 ± 2.4	47.4 ± 3.1
K37	23.6 ± 1.4	51.1 ± 1.9	37.2 ± 1.5	53.1 ± 0.9	20.8 ± 4.0	50.0 ± 1.3	18.6 ± 1.6	48.4 ± 1.1
K39	24.5 ± 2	51.9 ± 1.7	34.8 ± 0.8	53.3 ± 2.4	20.5 ± 2.4	50.8 ± 1.3	19.3 ± 2.6	44.0 ± 3.0
K41	25.9 ± 2.5	51.6 ± 2.3	36.9 ± 3.1	50.2 ± 2.0	20.9 ± 1.5	47.1 ± 1.5	25.1 ± 5.0	42.5 ± 1.3
K51	37.7 ± 1.4	54.1 ± 1.5	33.7 ± 1.0	51.3 ± 4.8	18 ± 2.1	42.5 ± 1.5	20.3 ± 1.9	39.9 ± 2.9
K52	37.0 ± 5.2	58.1 ± 1	32.5 ± 1.2	47.4 ± 2.3	17.6 ± 0.2	43.1 ± 0.9	21.7 ± 1.5	43.2 ± 1.1
Mean	30.5	50.6	34.8	50.0	23.6	47.	22.0	42.8

CHAPTER V

DISCUSSION

Drought stress occurs when the water lost by transpiration within a certain period of time is more than the amount of water taken from the environment. For this reason, water balance is disturbed in plant tissues. Stress can be daily or prolonged. In the event of stress, the cells remain small, as cell growth is negatively affected by loss of turgor. While chlorophyll is negatively affected, photosynthesis and respiration slow down or stop. The decline in the cell growth causes the leaves to shrink and the photosynthesis rate to decrease further. The stoma of the plant is closed at the time of drought to prevent water loss by transpiration (Ghodke et al., 2018).

Salt stress occurs in different countries of the world, especially in crops grown in arid and semi-arid regions. In rainy areas, salts are washed and mixed with groundwater and then with rivers is moved to the sea. For this reason, salification does not generally occur in rainy regions. In plants which are not resistant to salty environments, when the salt concentration threshold is exceeded, growth pause, paleness in the leaf and a decrease in plant dry weight occur. Onion is one of the plants that are highly sensitive to salinity. Salt stress usually occurs in plants for two reasons. When there is an excess of dissolved salts in the root zone due to the increase in concentration, a plant has difficulty in taking water and some ions with toxic effects due to the increase in the amount. Excessive salt stress causes stunting in plants and regression in root growth. Aboveground development is negatively affected and the leaves remain small. Cells die causing yellow spots on the roots, buds, leaf edges and growth tips (necrosis). In plants, chloroplasts and other cellular structures are damaged by the decrease of water and increase of salinity ions (Flowers et al., 1989).

This thesis was aimed to compare the physiological responses of ten short-day onion lines in GAF-MTN gene pool against salt, drought, salt and drought stresses. Groups and ranges of leaf length, leaf diameter and leaf number according to UPOV criteria were given in the Table 5.1. It was expected that plant development will slow down or even stop after stress treatments, and therefore there would be no increase in leaf length and diameter and new leaf formation would be delayed compared to control groups.

Table 5.1. Number of groups in all lines according to UPOV criteria after stress treatment (UPOV, 2014)

Character	UPOV groups	Group ranges	Control	Salt	Salt+Drought	Drought
Leaf length (cm)	Very short	17,25-25,41	0	0	0	0
	Short	25,42-33,58	0	0	0	0
	Middle	33,59-41,75	0	0	0	0
	Long	41,76-49,92	0	0	0	0
	Very long	49,93-58,05	10	10	10	10
Leaf diameter (cm)	Narrow	2,70-5,42	10	10	10	10
	Middle	5,43-8,14	0	0	0	0
	Large	8,15-10,85	0	0	0	0
Number of leaves (number)	Less	2,55-5,17	0	0	0	0
	Medium	5,18-7,80	0	1	2	3
	More	7,80-10,40	10	9	8	7

There was increase in all stress groups in terms of leaf length, leaf diameter and leaf number, but this increase was less occurred in drought condition with respect to leaf length and diameter (Table 5.2). Although leaf number was increased by salt, drought and combined (salt and drought) treatments, leaf number was mostly affected by salt and drought stress (Table 5.3). The lines that was mostly affected by drought stress in terms of leaf length and diameter was K39, while K18 and K20 was less affected (Table 5.4).

After 20 days of stress treatment, all of the control, salt, drought and combined (salt and drought) treatments have very long leaf length and narrow leaf diameter. Since the entire control group has more than 7.8 leaves, the number of leaves falls into the more group. K18, K25 and K35 lines had medium leaf count according to UPOV criteria (UPOV, 2014).

Table 5.2. Leaf length increase values before and after stress treatments

Lines	Salt (%)	Salt + Drought (%)	Drought (%)
K18	4.1↑	5.9↑	3.3↑
K20	3.2↑	4.8↑	1.5↑
K25	0.5↑	11.2↑	2.5↑
K28	13.9↑	1.8↑	1.1↑
K35	3↑	1.6↑	1.5↑
K37	7.8↑	2.4↑	1.6↑
K39	0.8↑	5.2↑	1↑
K41	8.5↑	1.2↑	1.7↑
K51	5.4↑	1.8↑	1.5↑
K52	3.7↑	2.5↑	2.5↑

Table 5.3. Leaf diameter increase values before and after stress treatments

Lines	Salt (%)	Salt + Drought (%)	Drought (%)
K18	50↑	30↑	11.1↑
K20	88.8↑	27.2↑	12.5↑
K25	57.1↑	12.5↑	16.6↑
K28	212.5↑	50↑	11.1
K35	33.3↑	18.7↑	0
K37	7.6↑	55.5↑	11.1↑
K39	0	44.4↑	0
K41	7.1↑	26.6↑	14.2↑
K51	6.2↑	5.8↑	0
K52	40↑	9.1↑	0

Table 5.4. Leaf number increase values before and after stress treatments

Lines	Salt (%)	Salt + Drought (%)	Drought (%)
K18	13.6↑	26.9↑	22.2↑
K20	29.8↑	14.9↑	31.7↑
K25	38.3↑	35.1↑	37.7↑
K28	28.5↑	42.8↑	40.3↑
K35	45↑	69.8↑	40↑
K37	47.6↑	52.6↑	66↑
K39	53.7↑	57.8↑	55↑
K41	42.8↑	32.8↑	18.5↑
K51	38.5↑	36.9↑	14.2↑
K52	22.2↑	50↑	38.1↑

According to the Hanci and Cebeci (2015a), a significant decrease was morphologically expected in onion plants under stress. Morphological changes started to be observed 2 weeks after the initiation of stress in this thesis. Although all stress conditions, namely salt, drought, salt and drought combined treatments affected the morphological appearance of the plants, the rates of the lines affected by the stress factors were not the same. When the images taken after the stress application of all lines were examined, they were mostly affected by drought stress in terms of morphology. Although yellowing of leaf tips was more prominent by salt and drought combined treatments as expected, whereas in case of salt treatment, there was less impact on leaves than drought and combined (salt and drought) groups.

Ziska et al. (1990) demonstrated that chlorophyll content decreased due to stress. Ashraf and Arfan (2005) observed an increase in the amount of chlorophyll with stress with the study conducted in okra cultivars. Onion lines grown in salt, drought, salt and drought combined conditions have increased and decreased changes in their chlorophyll index. In this thesis study, while maximum chlorophyll index decrease was occurred in K18 in response to salt and drought, K51 was mostly affected by salt treatment. Chlorophyll index values of K28 was affected by drought treatment. A decrease in chlorophyll index was observed in all stress groups as an average. However, this decrease was more in salt and drought combined stresses. In this case, it can be deduced that most of the lines

were affected by salt and drought combined stress in terms of chlorophyll index. While the average SPAD value decrease was 2.41% in onion lines under salt stress, it was 7.08% under salt and drought stress and 3.91% under drought stress (Table 5.5.).

Table 5.5. Chlorophyll index change values between day 0 and day 20

Lines	Salt (%)	Salt + Drought (%)	Drought (%)
K18	6.8↓	15.4↓	1↑
K20	2.2↓	12.7↓	7.4↓
K25	0.4↓	1.9↑	3.7↓
K28	2.5↑	9.9↓	10.6↓
K35	2.4↓	1.1↓	7.4↓
K37	0.7↓	9.8↓	1.4↓
K39	0.4↑	5.8↓	5.9↓
K41	4↓	6.5↓	3.1↓
K51	14.3↓	11.5↓	5.9↓
K52	3.8↑	0	5.3↑

When the plants are exposed to salt stress, they have difficulty in getting water from the soil with the increase of salt concentration around the root. At the same time, excessive salt stress negatively affects its development in the root zone and root development slows down. At the same time, when plants undergo drought stress, they close their stomata, thereby reducing transpiration. As a result, leaf temperature is expected to increase (Dolferus, 2014; Farooq et al., 2009). Similar to the situation in the literature, in all genotypes, leaf temperature increased from the start of stress to the last day of stress.

As mentioned before, an increase in leaf temperature was observed in all stress groups. However, this increase was higher in drought condition. Accordingly, it can be said that most of the lines were mostly affected by drought stress in terms of leaf temperature. While the average temperature increase was 43.7% in onion lines under salt stress, it was 44.6% under salt and drought stress and 68.9% under drought stress. The maximum temperature increase was observed in K20 line by drought treatment, however K51 was

less affected by salt and drought stress treatments in terms of leaf temperature (Table 5.6.).

Table 5.6. Leaf temperature increase values between day 0 and day 20

Lines	Salt (%)	Salt + Drought (%)	Drought (%)
K18	48.3↑	51.2↑	78.4↑
K20	48.9↑	52.6↑	80.5↑
K25	44.4↑	45.1↑	70.9↑
K28	54.6↑	51.3↑	69.1↑
K35	55.6↑	50.3↑	76.1↑
K37	41.8↑	44.6↑	70.6↑
K39	39.7↑	39.7↑	55.5↑
K41	36.9↑	42.7↑	68.9↑
K51	28.1↑	37.3↑	54.1↑
K52	39.1↑	31.4↑	65.3↑

In plants that perceive drought stress, the first reaction is the narrowing or complete closure of stomata to prevent the loss of water (Osakabe et al, 2014). Plant photosynthesis rate is directly related to the amount of CO₂ taken from stomata. For this reason, in order to minimize the amount of water lost by transpiration in drought conditions, plant closes or restricts its stomata, thereby reducing CO₂ intake and the rate of photosynthesis. Since salt density also increases and water intake is negatively affected by the roots, the decrease in water potential is also experienced in salinity. As the ABA hormone secreted by the inability of the roots to absorb water, the potassium flow changes and stomata are closed and in this case, the photosynthesis is expected to decrease (Anjum et al., 2011).

Contrary to the literature, the rate of photosynthesis increased from 0 to 20 days in all stress groups. After salt, drought, salt and drought treatments, the average of increase rates were 43.8%, 98.9% and 96.7% depending on the stress treatment, however as an average the least increase was occurred by salt treatment. Despite this increase, on the 20th day, the values were mostly lower than the control especially by drought and combined (salt and drought) stress treatment. The lowest values were occurred by the

drought group compared to the control in all of the lines on the 20th day. In these lines, photosynthesis values after drought stress were between 5.2% and 26.2% less than their respective control. The line with the least photosynthesis increase with a rate of 11.5% was K18 under salt stress conditions. K51 was the line with a photosynthesis rate of 26.2% less than the control after drought application on the 20th day (Table 5.7).

Table 5.7. Photosynthesis rate increase values between day 0 and day 20

Lines	Salt (%)	Salt + Drought (%)	Drought (%)
K18	11.5↑	48.1↑	39.2↑
K20	32,6↑	54.4↑	66.5↑
K25	64.8↑	47.4↑	91.3↑
K28	54.3↑	62.3↑	88.7↑
K35	45.6↑	82.6↑	128.9↑
K37	42.7↑	140.3↑	160.2↑
K39	53.1↑	147.8↑	127.9↑
K41	36↑	125.3↑	69.3↑
K51	52.2↑	136.1↑	96.5↑
K52	45.8↑	144.8↑	99↑

CHAPTER VI

CONCLUSION

Onion is known to be very sensitive to salt and drought stress. Drought and salt stress cause yellowing and wilting morphologically on the onion plant, photosynthesis rate and chlorophyll index value are also expected to decrease. In contrast, leaf temperature is expected to increase under stress conditions.

As a result of observations and measurements taken during this thesis study, it was shown that the onion lines undergo morphological changes under stress conditions. Especially all lines were affected by drought and combined (salt and drought) stress, but they were less affected by salt treatment. Leaf temperature also increased as expected. Chlorophyll index value was expected to decrease in all stress treatments, a similar situation was occurred and decreased on average by salt, drought and combined (salt and drought) stresses. Some lines have increased SPAD values.

While the rate of photosynthesis was expected to decrease, it was completely increased from day 0 to day 20 by salt, drought and combined (salt and drought) stress treatments. Drought and combined (salt and drought) stress treatments in all of the lines was less than their respective control according to values of the day 20, but it was higher than control in some lines by salt treatment. It was clear that salt treatment did not affect plant leaf development and photosynthesis rate. While leaf length and diameter mostly affected by drought treatment, leaf number mainly affected by salt treatment. Temperature values were increased as expected, but the maximum effect was occurred by drought treatment. While SPAD value was expected to decrease, decline was mostly observed by salt and drought combined stress in terms of chlorophyll index. Although there was an increase of photosynthesis rate of all lines, the minimum increase was occurred by salt stress an average, whereas in response to drought and combined (salt and drought) stress, photosynthesis rate was lower than control on 20th day.

According to the results, the line most affected by the salt treatment was K18, while the K-28 was the least affected. While K20 was maximally affected by salt and drought combined stress, K25 was only slightly affected. The drought treatment affected all

lines negatively as morphological and physiological, but K51 was the most affected, while K37 was the least affected by drought treatment.

When the eight lines and two commercial varieties which used in the thesis study were compared, in the control groups of commercial varieties, there was less increase in leaf length and width compared to other lines, however, there was a serious increase in the number of leaves. While K51 was most affected by drought in terms of leaf elongation, K52 was most affected by drought and combined stress. While there was no increase in leaf width in the control and drought groups in both commercial varieties, there was an average increase in salt stress in K52. Considering the increase in the number of leaves, K51 was the most affected variety from drought treatment. On the other hand, K52 was not affected by drought in terms of increase in the number of leaves, but in this sense it was mostly affected by salt stress.

While the maximum decrease in K51 chlorophyll index was experienced in the control group, the least decrease was in drought. Compared to all lines and commercial varieties, chlorophyll index decreased in all lines except K18 and K52 under drought stress. When these increases were analyzed, the maximum increase in SPAD value in drought stress was also in K52, which means that it was the commercial type of K52 least affected by drought in terms of chlorophyll index. In terms of leaf temperature, K51 and K52 commercial varieties were mostly affected by drought, similar to other lines. When analyzed on photosynthesis rate, both commercial varieties were most affected by salt stress and there was a minimum increase. However, in photosynthesis rate, K51 and K52 have increased more than most of the other lines in combined stress application.

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CURRICULUM VITAE

Nazlıcan Aydođan was born on January 27, 1995 in Niđe, Turkey. She completed her higher secondary education in Niđe Anatolian High School in 2013. Afterwards she joined Department of Agricultural Engineering, Niđe Ömer Halisdemir University, Niđe Turkey for her undergraduate studies. She went to Latvia University of Agriculture for one summer semester with Erasmus program in 2016. She completed her B.Sc. in Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies in 2018. She registered in Graduate School of Natural and Applied Sciences, Department of Agricultural Genetic Engineering at Niđe Ömer Halisdemir University, Niđe, Turkey to pursue her master education under the guidance of Assoc. Prof. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE.

