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

EFFECT OF ABIOTIC STRESS APPLICATION ON BULB YIELD IN GAF-MTN LONG DAY ONION BREEDING LINES

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Supervisor

Assist. Prof. Dr. Ali Fuat GÖKÇE

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The study titled **“Effect of Abiotic Stress Application on Bulb Yield in GAF-MTN Long Day Onion Breeding Lines”** and presented by **Francis TAINGZUNAALOUNG** under the supervision of **Assist. Prof. Dr. Ali Fuat GÖKÇE**, has been accepted as Master of Science thesis by the jury at the **Department of Agricultural Genetic Engineering** of Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

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CONFIRMATION:

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....../..... /20…

Prof. Dr. Murat GÖKÇEK
DIRECTOR

THESIS CERTIFICATION

I declare that this thesis was written by myself 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.

Francis TAINGZUNAALOUNG

SUMMARY

EFFECT OF ABIOTIC STRESS APPLICATION ON BULB YIELD IN GAF-MTN LONG DAY ONION BREEDING LINES

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In this thesis, twenty long day onion breeding lines were studied to observe the effect of abiotic stress on their yield. Salt, drought and combined salt and drought stress were induced at the bulb initiation stage and continued for 20 days with a control that was irrigated normally. Thereafter, all plants were irrigated normally. Harvested bulbs were measured for bulb weight, height, and diameter. The number of split bulbs and rotten bulbs were recorded for each treatment of each genotype. Analysis of the bulb size parameters indicated that ten of the breeding lines had no significant difference after a comparison with the control and stressed groups of each genotype. U2, U9, U23 and U29 were found to be tolerant to all the stress conditions for all bulb size parameters, while U11, U17, U44 and U57 were the most sensitive genotypes.

Keywords: Allium cepa L., long day onion, abiotic stress, split, tolerant, sensitive genotypes.

ÖZET

GAF-MTN UZUN GÜN SOĞAN ISLAH HATLARINDA ABIYOTIK STRES UYGULAMASININ BAŞ VERIMINE ETKISI

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Danışman : Dr. Öğr. Üyesi Ali Fuat GÖKÇE

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Bu tezde, abiyotik stresin verim üzerine etkisini belirlemek için yirmi uzun gün soğan ıslah hattı kullanılmıştır. Tuz, kuraklık ve birleşik tuz ve kuraklık stresi soğan bitkilerinin baş bağlamaya başladığı aşamasında uygulanmış ve 20 gün boyunca devam etmiştir, kontrol grubu ise normal olarak sulanmıştır. Daha sonra tüm bitkiler normal şekilde sulanmıştır. Hasat edilen soğanların baş soğan ağırlığı, yüksekliği ve çapı ölçülmüştür. Her bir genotipin her muamelesi için çatal ve çürük baş soğan sayısı kaydedilmiştir. Baş soğan boyutu parametrelerinin analizinde, on ıslah hattı için her bir genotipin kontrol ve stresli grupları arasında yapılan karşılaştırmalarında önemli bir fark olmadığı görülmüştür. Islah hatlarından U2, U9, U23 ve U29, tüm baş soğan boyutu parametreleri için tüm stres koşullarına toleranslı bulunurken, U11, U17, U44 ve U57 en hassas genotipler olmuştur.

Anahtar Kelimeler: *Allium cepa* L., uzun gün soğanı, abiyotik stres, toleranslı, duyarlı genotipler.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYMBOLS AND ABBREVIATIONS

Symbols	**Description**
%	Percentage
°C	Degree celsius
g	Gram
mm	Millimeter
$dS.m^{-1}$	Deci siemens per meter
mM	Millimolar
$\leq$	Less than or equal to

Abbreviations	**Description**
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
CO_2	Carbon Dioxide
CT	Control Treatment
DT	Drought Treatment
df1	Degree of Freedom 1
df2	Degree of Freedom 2
F	F Value/F Statistic
FAO	Food and Agriculture Organization
GA_3	Gibberellic Acid
GAF-MTN	Gökçe Ali Fuat- MTN Seed Company Limited
HSD	Honestly Significant Difference

MD	Mean Difference
NaCl	Sodium Chloride
PC1	First Principal Component
PC2	Second Principal Component
PEG	Polyethylene Glycol
p-value	Probability Value
SD	Combined Salt and Drought Stress
ST	Salt Stress
UV	Ultraviolet

CHAPTER I

INTRODUCTION

The Allium genus comprises of over 700 wild and cultivated species (Fritsch, 2001) with the common onion (*Allium cepa L.*) and garlic (*A. sativum L.*) being the most commonly cultivated and consumed species around the world. The exact origin of onion is unknown, although Central Asia (Brewster, 2008) and the Middle East some 5500 years ago are two prevailing theories that stand out, and have been cultivated for food, flavor, health, and religious purposes since then (Hanelt, 1990; Mehta, 2017). Currently, onions are cultivated widely in the northern hemisphere, usually in warm climates although some are adapted to the arctic regions.

Onions are cool season herbaceous plants with optimum temperature range of 13°C to 24°C and reduced growth and yield at temperatures above 30°C, dry or freezing conditions. Onions thrive in varied soil types from light sands to loamy clay and need about 400-800 mm of water per crop to achieve optimum yields (Aragona, 2003; Rubatzky and Yamaguchi 1997). They are characterized by underground storage structures in the form of bulbs (Hanley and Fenwick, 1985) which may range in shape from flat to oblong shaped (Grant and Carter, 1991). Onion bulbs are made-up of scales arranged chronologically by age, with the younger ones inside and the older ones outside (Galsurker et al., 2018). At maturity, bulbs are characterized by a couple of dry scaly scales that enclose the inner fleshy scales (Brewster, 2008; Galsurker et al., 2018). The bulbs also vary in color from white, yellow, brown, red to purple (Brewster, 2008; Pareek, 2017) and usually possess a unique flavor that may vary from very pungent to mild and sweet due to the presence of beneficial organosulfur bio compounds (Dhumal et al., 2007; Keusgen, 2002; Mota et al., 2010). These compounds have been shown to possess therapeutic capabilities (Bisen and Emerald, 2016) and are only released when onion tissues are damaged, causing enzymatic action of alliinase to produce ammonia, pyruvate, and volatile sulfur compounds (Lancaster and Boland, 1990).

Onions are biennial vegetables that require two growing seasons to form seeds. The bulb is produced in the first growing season and in the second growing season, the onion re-starts, forming the peduncle and then the receptacle. Depending on the climate of the cultivation, onion sets may be used instead of seed which makes three grooving seasons, seed to set, set to bulb, bulb to seed (Gökçe, 2015). In breeding lines, production is preferred with seedlings because the seeds are valuable. (Gökçe, 2010; Gökçe, 2011).

Photoperiodism is the determinant factor for which onion variety is adapted to a certain area. Based on day length adaptation, onions are categorized into short day onions for those that need day length of less than 12 hours, intermediate day onions for those that need day length between 12-14 hours and long day onions for those that need day length of more than 14 hours. Growing a long day onion variety in short- or intermediate-day conditions will not generate bulb while growing a short-day variety in intermediate or long day conditions will result in poor vegetative growth and small bulbs (Aragona, 2003). Short day onion seeds are planted in the autumn on the Mediterranean coastline, which is not cold in winter, while long day onion seeds are planted interior and northern regions of Türkiye in March and April. For germination to occur, onion seeds need sufficient moisture and a temperature range of 10-15°C. During development and growth, temperature ranges of 10-25°C is optimum, while temperatures within 25-30°C, rather than high temperature is thought to be ideal for maturation of bulbs and drying leaves. At high temperatures, the possibility of sunburn increases (Gökçe, 2015; Pike, 1986; Şalk et al., 2008).

Edible onion can be placed into two broad categories as dry onions and green onions. Dry onions are usually allowed to grow to maturity and are characterized by larger round bulbs with dry leaves while green onions, also known as scallions, may be obtained from varieties that do not bulb such as Chinese and Welsh onions, or harvested as young regular bulb forming onions that are yet to develop bulbs.

Onions are the second, after tomatoes, the most important horticultural crops of economic value, evidenced by its global production and importance in the global food supply chain. Data from the United Nations indicates that, averaging a period of five years from 2016-2020, China has been the biggest producer of dry onions globally, followed by India, U.S.A., Egypt, and Türkiye, with the global average at approximately 98.5 million tons of dry onions. Over this period, the biggest year on year increase in global production was achieved in 2020, where an increase of about 5 million tons was recorded relative to 2019. Most of this increase can be attributed to production increase in India of about 3.9 million tons relative to 2019, a situation that caused India to be the biggest dry onion producing country in 2019 instead of China for the whole period under review. Over the same period, global green onion production averaged 4.5 million tons, led by China, Mali, Japan, Korea, and Tunisia as the five biggest producers, with Türkiye coming in at tenth with a 137,000 tons production volume. For both green and dry onions, the top five producing countries were responsible for more than half of the total global production for the stated period under (FAO, 2022a).

The combined effects of global warming, improper irrigation, and abiotic stress factors, such as drought and salinity, have become a major cause for concern in terms of agricultural productivity due to their role in causing major yield losses globally. Despite these losses, global demand for agricultural products is bound to increase, although farmland for agriculture is being lost to urbanization because of the increasing global population, which is estimated by the United Nations to get to approximately 9.7 billion people by 2050. Specific abiotic stress conditions elicit specific plant responses, which negatively affect plant growth and may even cause plant death (Zhang et al., 2020). Many plants succumb to the effects of abiotic stresses, and onion, a vegetable crop with immense health benefits, cultivated worldwide for use primarily in seasoning of dishes (Demisie and Tolessa, 2018), is not an exception. Onion has been found to be sensitive to drought (Sta-Baba et al., 2010), especially in the bulbing stage, resulting in reduced bulb yields (Pelter et al., 2004; Rattin et al., 2011) and even affecting seed yield in the second year (El Balla et al., 2013). Onion is not spared under salt stress either, as it also causes yield losses and poses major challenges to the plant during development. To meet the increasing global demand for onions, amidst reducing farmland and unfavourable environmental conditions, cultivars that can tolerate such conditions with better yields need to be developed. Therefore, the aim of this study is to determine the yield potential and bulb quality of 20 long day onion genotypes exposed to drought, salt, and combined salt and drought stresses to identify tolerant genotypes with good yield that can be developed into new cultivars.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The global population has more than quadrupled over the last century from about 1.8 billion 1915 to about 8 billion in 2022 and expected to reach about 9.7 billion in 2050 according to UN estimates. Global food production is also expected to increase between 59% to 98% to be able to meet the food demand of the growing population despite the numerous challenges facing agricultural production worldwide such as climate change driven drought, increasing temperatures and land salinity, and population increase driven urbanization (Elferink and Schierhorn, 2016). These environmental factors are the determinants in plant growth, development, and yield (Duque et al. 2013), and are referred to as stress.

2.2 Plant Stress

Plants in their natural environments are exposed to a myriad of unfavourable conditions that are either within or even exceeding the extremities of their tolerance range. These conditions are known as stress conditions and they result in plant growth deficiencies, decreased crop yields, permanent damage, and even has the potential of threatening the survival of the plant (Duque et al., 2013). Stress conditions are categorised as biotic if the factors causing them are biological such as insects or microorganisms, and abiotic if the causative factors are environmental including but not limited to excess or insufficient water, salinity or salt stress, temperature extremes, heavy metals, and ultraviolet (UV) radiation (He et al., 2018; Kranner et al., 2010). Stress may be classified as short term if plants are able to tolerate and adapt to it and long term if it causes major and irreparable damages in plants (Kranner et al. 2010; Lichtenthaler 1996). Since plants are sessile organisms, they must tolerate these stress conditions through adaptations which may be physical, physiological, molecular, or biochemical (Basu et al., 2016; Doughari, 2015; He et al., 2018) Stress conditions affect crop production from germination, vegetative growth, seed, and fruit formation, yield and storage (Duque et al., 2013). Stress response in plants is categorised into the response phase characterised by a decline in plant vitality, the restitution phase characterised by plant repair and adaptation, the end phase which is characterized by prolonged disease or may result in death, and in situations where the stress condition was not severe and there is termination of stress, a final phase known as the regeneration phase (Lichtenthaler 1996).

2.3 Biotic Stress

Biotic stress is caused by the interrelationship between living organisms and plants resulting in either survivable repairable damage or death causing irreparable damage to plants (Mosa et al., 2017). Pathogenic bacteria, fungi, or viruses as well as nematodes, insects, animals, and other plants can all induce biotic stress in plants (Kranner et al. 2010) and are responsible for major losses in agricultural yield globally every year (Agrios 2005; Baker et al., 1997; Sazzad 2007). Microorganisms usually cause biotic stress through diseases by their parasitic feeding habits. Fungal parasites cause stress by their necrotic or biotrophic activities that may induce vascular wilts and leaf spots (Doughari, 2015; Laluk and Mengiste, 2010; Sobiczewski et al., 2017). The feeding habits of nematodes cause soil-borne diseases which usually lead to nutrient deficiency, stunted growth, and wilting (Bernard et al., 2017; Lambert and Bekal, 2002; Osman et al., 2020). The relationship between pathogens and plant hosts may have detrimental biochemical and molecular effects on the plant that lead to metabolic and physiological changes resulting in reduced production efficiency, growth, and shape disorders, or even plant death (Baker et al. 1997; Sazzad 2007).

Animals including insects may cause physical damage through their feeding while plants cause biotic stress through competition and phyto-parasitism (Mosa et al., 2017; Schumann and D’Arcy, 2006). Competition stress can occur within the same species, other cultivated species, or wild plants. Within species, competition for moisture, space, light, and soil nutrients can be caused by high seeding and population density of the same and can result in poor crop yields and quality (Peterson and Higley 2000). When plants share the same growing area as weeds, it is known as weed competition, and it has been estimated to cause about 10-15% reduction crop yields worldwide (Froud-Williams, 2003).

Biotic stress affects all organizational levels of plants from molecules and cells to whole plant populations, with variations on the impact at each level (Mosa et al., 2017). Biotic stresses may affect photosynthesis through reduced leaf area caused by insects and herbivorous animals or reduce photosynthetic rate by viral infections causing chlorosis (Gull et al., 2019; Pallas and García, 2011). To survive biotic stress, plants have evolved an intricate system that involve physical, biochemical, and molecular defences against these stresses. (Saijo and Loo, 2020; Taiz and Zeiger, 2006). Physical barriers such as bark, spines, cuticles, and wax serve as first point of defence against insects and pathogens, while secondary metabolites terpenoids, alkaloids, anthocyanins, phenols, and quinones may also be released for defence (Hanley et al., 2007; War et al., 2012). For pathogens, plants may identify molecular patterns associated with pathogens using pathogen recognition receptors or the use of plant resistance proteins to identify specific pathogen receptors (Abdul Malik et al., 2020; Monaghan and Zipfel, 2012), after which effector-triggered immunity elicit apoptosis in infected and surrounding cells (Mur et al., 2008).

2.4 Abiotic Stress

Boyer (1982) stated that environmental conditions have the potential to decrease crop production by as much as 70%. It is estimated that about 97% of the global land area is affected by some sort of environmental constraint (Cramer et al., 2011). Drought, salinity, temperature, light, and suchare all abiotic stress factors with drought and salinity being the most common forms, serving as important limiting factors in plant growth, resulting in major losses in global agricultural productivity, a situation that is worsened by the effects of global warming and improper irrigation strategies. (Andre et al., 2009; Deikman et al., 2012; Tuberosa, 2012). Such stresses may result in several morphological and physiological deficiencies such as stunted growth, which can significantly reduce productivity or yield and has the potential to cause plant death (Zhang et al., 2020). Abiotic stress response is a very complex mechanism because it affects many cellular metabolic activities at both physiological and molecular levels (Krasensky and Jonak, 2012). Plants actively reduce growth to deploy adaptive measures depending on the type and duration of the stress to ensure survival of the plant by demonstrating specific responses to specific stress conditions such as growth inhibition by drought stress due to reduced cell rigidity (Zhang et al., 2020).

2.4.1 Drought Stress/Water Deficit Stress

Water, as a critical necessity for life is gradually becoming a scarce resource for crop production and as the situation deteriorates, is going to have a significant negative impact on global agricultural output (Camilia et al., 2013; Howell, 2001). Plant efficiency in terms of water relations is dependent on its ability to take up water at a rate faster than the rate at which it is being lost (Vahdati and Lotfi 2013). Water uptake efficiency by plants is determined on the lateral and depth of reach of the root system (Shinozaki and Yamaguchi-Shinozaki, 2000).

Drought, also known as water deficit, is a major abiotic stressor characterized scarce soil water within the reach of plant roots and continuous water loss by transpiration or evaporation (Atta et al., 2022), and it affects all major aspects of plant growth and development by causing dehydration which triggers metabolic and osmotic imbalance causing cell rigidity loss and stomatal closure, and may lead to poor yields or even plant death if not remedied fast enough (Sazzad, 2007;Zhu, 2001). Different crop cultivars vary in their water use, mostly when there is a water deficit. Some reduce the rate of transpiration by closing the stomata, which then limits the amount of CO_2 entering the stomata thereby effectively limiting photosynthetic activity and, ultimately, yield (Al-Jamal et al., 2000; Vahdati and Lotfi 2013).

Generally, seed germination rate is affected by drought stress although the gravity of the effect is species dependent (Li et al., 2013). Drought stress at the seedling establishment phase, thus shortly after germination negatively affects plant growth due to undeveloped root system and stomatal control (Duque et al., 2013).

Okçu et al. (2005) performed a study on three cultivars of pea to determine the effect of drought stress on germination and seedling growth using Polyethylene Glycol (PEG) to induce drought stress at four different osmotic potential levels. The study showed a significant decline in germination percentage with one cultivar having as much as a 93.5% germination reduction. All ungerminated seeds under the PEG-induced drought conditions germinated regardless of the water potential when transferred into distilled water. Seedling growth was also adversely affected under the drought stress conditions although the level of the effect was different for each of the cultivars. A similar pattern was observed in sugar beet by Sadeghian and Yavari (2004), and maize by Liu et al. (2015) who observed that drought stress severely impacted seedling emergence, growth, and establishment. Ten plant species usually used in landscaping have even been shown to be sensitive to water stress during germination (Yigit et al., 2016).

Conversely, in canola, drought stress conditions at the seedling stage caused a significant elongation of the roots (Rezayian et al., 2018), a trait that serves as an indication for drought tolerance and used as a marker for selection of drought-tolerant cultivars (Leishman and Westoby, 1994). Numerous studies as mentioned above conducted on the effects of drought stress on seed germination indicate a sensitivity of many plant species, with varying effects depending on the specific varieties and cultivars. Notwithstanding, there are some plants that have shown tolerance to drought stress even at the germination phase such as canola.

Drought stress at the early growth stage may have detrimental effects on plant growth due to underdeveloped root system and stomatal control, yet it has largely been avoided due to accurate choice of seedling dates by farmers. Young plants will continue to grow until all water within the reach of the roots is depleted, after which root hairs die and the plants begin to wilt, ultimately leading to stunted growth and possibly death in cases of extended water deficit conditions. For normal growth to resume in adequate water conditions, plants must regrow root hairs for water and nutrient uptake (Duque et al., 2013).

Plants appear to be less sensitive to drought stress during the vegetative growth period but to ensure high yield of good quality, frequent and controlled irrigation is needed throughout the growing season. Owing to extensive studies, mostly on comparing the vegetative and reproductive growth stages, it has been established that drought stress at the latter stage usually have more devastating effects on plants than the former stage although the severity of the effect may depend on the specific phase of the reproductive stage (Duque et al., 2013). In *Phaseolus vulgaris*, it has been observed that flowering was the most sensitive reproductive stage to drought stress (Mouhouche et al., 1998). In maize, drought stress at the flowering stage caused a decrease in leaf physiology and yield (Casanovas et al., 2003) while drought at the vegetative stage and panicle development stage resulted in a minimal and severe effect on yield respectively (Boonjung and Fukai, 1996).

2.4.2 Salt Stress/Salinity Stress

Minerals and nutrients present in soil are required for plant growth and metabolism, however, the presence of soluble salts such as sodium sulphate, sodium nitrate, sodium chloride, sodium carbonates, potassium sulphate, calcium sulfate, magnesium sulphate and magnesium chloride in high concentrations can cause salinity which may result in severe osmotic and ionic stress in plants (Flowers et al., 1977; Sazzad 2007). Optimal sodium chloride concentration promotes plant growth. However, in excessive amounts of 200 mM have harmful inhibitory effects on germination, seedlings establishment, vegetative growth, and flower fertility (Guo et al., 2018) as a result of ionic toxicity, oxidative damage, and nutritional shortage (Feng et al., 2014; Zhao et al., 2010; Zhao et al., 2016), and its usual occurrence together with drought stress (Ashraf and Foolad, 2007; Shabala et al. 2015; Slama et al., 2015; Tester and Davenport 2003). At this level, most plants ultimately do not survive (Flowers and Colmer, 2008; Zhao et al., 2016). Salinity is a major environmental factor responsible for poor plant growth and productivity in arid and semiarid areas. Globally, salinity is estimated to affect upwards of 3% and 6% of topsoils and subsoils respectively, with over 60% of the affected soils found in arid and semi-arid regions (FAO, 2022b) and is responsible for a yearly decommissioning of about 1.5 million hectares of farmland from production (FAO, 2022c). In irrigation dependent areas, the lack of sufficient rainfall to wash salts from the root zone is responsible for an ever-increasing salinization of global arable land (Munns and Tester, 2008).

Depending on their salt tolerance levels, plants are classified as halophytes if they can grow under high salinity levels comparable to seawater or above and glycophytes if they are sensitive to high salt concentrations cases (Sazzad 2007; Zhu 2001). Halophytes tolerate high salinity levels by limiting salt uptake and reducing the cytoplasmic and cell wall salt concentration (Munns et al. 2006). Salinity usually creates extreme osmotic and ionic stress around roots thereby reducing the water absorption ability of the plant and reduces growth and metabolism rates (Flowers et al., 1977; Sazzad 2007). Extended salt stress exposure affects photosynthetic rates by causing stomatal closure thereby effectively reducing carbon dioxide uptake capacity, which leads to senescence and death (Flowers et al., 1977; Sazzad, 2007; Zhu, 2001).

Salinity affects plants by reducing the bioavailability and absorption of nutrients such as nitrogen, potassium, and phosphorus (Camilia et al., 2013; Bartels and Sunkar, 2005) and causing an osmotic imbalance or stress which can significantly reduce yields even before visible expression of toxicity symptoms (Camilia et al., 2013).

When plant growth is affected by salinity around the roots, usually at a level of 40 mM for many plants or less for sensitive plants like rice, an osmotic phase is initiated that may results in reduction of leaf area development relative to root growth with the aim of reducing water use by the plant. On the other hand, if salinity causes the accumulation of salts to toxic levels within the plant, usually within old leaves, it causes their death at a rate higher than the production of new leaves, ultimately reducing the photosynthetic capacity of the plant and growth rate (Munns and Tester 2008).

2.4.3 Temperature Stress

Temperature is a major determinant in global species distribution since plants have different temperature requirements and tolerance levels (Sazzad 2007). Temperature stress occurs due to extreme environmental temperature fluctuations and affects plants at every growth stage leading to permanent damage or death (Kranner et al. 2010). At extremely low temperatures beyond the tolerance limit of a plant usually causes serious physical and mechanical damages. At freezing temperatures, the intercellular spaces may begin to freeze resulting in severe cell disruption from pressurized cell walls and membranes (Olien and Smith 1977; Sazzad 2007) or desiccation of cells may occur as a result of transport of fluid from the cell into the intercellular spaces causing freezing injuries. Low temperatures may also result in molecular precipitation, cell lysis or production of reactive oxygen species (Pearce, 1999; Sazzad, 2007; Thomashow, 1999).

At high temperatures stress conditions, nutrient absorption and plant growth are reduced, while stomatal evaporation increases causing wilting, permanent damage and death if extended. Extreme conditions can result in heat shock and impact negatively on the vitality of different organs, with the potential to cause reproductive challenges by reducing production and viability of pollen (Mosa et al., 2017; Peterson and Higley, 2000).

2.4.4 Light Stress

Photosynthesis is essential for plant growth and reproduction since it is the source of energy and metabolite production. Plants require light to undergo this essential activity and has thus adapted to fluctuations in light exposure by maximizing light usage under low irradiance while preventing the damaging effects of prolonged light exposure (Adamiec et al., 2008). During light stress conditions, limited light availability causes a reduction in plant photosynthetic rate and by extension, energy, and metabolite production, thereby slowing growth rates and causing reduced crop yields (Mosa et al., 2017). Extended light exposure in plants may result in photosynthetic damage and increased reactive oxygen species production (Adamiec et al., 2008; Barta et al., 2004).

2.5 Interaction Between Different Stresses

In their natural environment, plants are simultaneously exposed to several stress conditions such as heat, drought, and high light stress in summer, and this possess a difficulty in identifying and treating each stress condition effectively (Peterson and Higley, 2000). Over the years, much effort has gone into multi stress studies and the interrelationship between stresses, and how they affect plant response been documented (Higley et al., 1993). Multi stress interaction have devastating effects on plants and the effect of one stress may be dependent on another (Atkinson and Urwin 2012; Lichtenthaler 1998). The effect of one stress may have synergetic or antagonistic effects on the tolerance of plants to another stressor (Rizhsky et al., 2004). Multi stress interaction may cause the incidence of a second stress condition to change the plant response mechanism to the first stress condition, or the incidence of one stress may change the occurrence of another such as bacterial infection caused by insect wounds and can be classified into 3 main types as independence, interaction/dependence when stress response is influenced by the occurrence of other stress conditions and false relationships (Higley et al., 1993).

2.5.1 Independence

Plant responses to each stressor is not affected or influenced by the occurrence of the other stressors under a multi stress condition (Higley et al., 1993).

2.5.2 Interaction or Dependence

In this type of response to multi stress conditions, response to one stressor is dependent on the occurrence of another and this may further be classified as (a) Stress Response Interactions if the response is not equivalent to the sum of individual stress response, an indication that physiological and molecular processes affected by the stresses are interrelated with the damage. (b) Stress Incidence Interactions, which is common in biotic stresses caused by microorganisms, and it, occurs if a first stress alters the response pattern of a second stress (Higley et al., 1993).

2.5.3 False Relationships

This occurs when there is an inability to pinpoint the interaction of stresses in a multiple stress condition. This may be in the form of false independence when independent multiples stresses are identified as dependent or false interaction when multiple dependent stressors and identified as independent (Higley et al., 1993).

2.6 Plant Stress Response Phases

During stress conditions, plants undergo mainly three phases of response; the alarm, resistance and exhaustion phases, and depending on the duration and intensity of the stress, a fourth phase, the regeneration phase, if the stress condition is removed before irreparable damage is caused to the plant (Lichtenthaler, 1998; Kranner et al., 2010).

2.6.1 The Alarm/ Response Phase

Special mechanisms, depending on species, stress type or organ affected, are deployed by plants to identify changes in plant conditions during stress in a phenomenon known as stress sensing (Kranner et al., 2010). Response to stress conditions may be physiological, such as root signal recognition or photosynthetic decline, biochemical such as accumulation of stress metabolites or increase in antioxidative enzymes or molecular such as protein specific synthesis or expression of genes related to stress response (Kranner et al., 2010; Lichtenthaler, 1998; Reddy et al., 2004; Shao et al., 2009).

Thus, different stresses such as light and drought elicit different sensing mechanisms because they affect different parts of the plant, with light stress and drought stress affecting above ground and underground plant parts, respectively. Stress sensing serves as the initiation for plant stress response after exposure to stress conditions and triggers (Lichtenthaler, 1998). Mostly, multiple stresses act together, causing simultaneous triggering of sensing mechanisms.

The most common for external stimuli sensing is chemical ligand binding to specific receptor, known as the ligand-receptor model used for only chemical stresses such as heavy metal and nutrient depletion but not physical stress such as drought and salinity. For radiation stress, the analogous photon-receptor-models is used (Verslues and Zhu, 2005). In rooting systems, sensing mechanism for water concentration is unclear (Dat et al., 2004), although experimental evidence from the isolation of the transmembrane hybrid-type histidine kinase from *Arabidopsis thaliana* is an indication of sensing of cell water homeostasis in higher plants (Urao et al., 1999). Sugars produced during photosynthesis and carbon metabolism in tissues are vital in sensing, signaling, modulating growth, development, and stress responses (Rolland et al., 2006).

2.6.2 The Resistance/Restitution Phase

At this phase, plants deploy survival mechanisms through adaptation, repair and hardening processes through activation of signaling and signaling transduction cascades (Lichtenthaler, 1998; Duque et al., 2013). This phase in stress response is usually characterized by declining vitality and a higher rate of catabolism, and coping mechanisms are initiated to prevent acute plant damage or death (Lichtenthaler, 1998). These survival mechanisms, together with coping mechanisms deployed during the alarm phase results in the creation of a new normal tuned for stress conditions (Duque et al., 2103).

2.6.3 The Exhaustion/End Phase

If the duration and intensity of the stresses exceed the tolerance range of plants, there is a continuous decline in vitality and may eventually lead to severe damage and possible death. The rate of damage is species, organ, duration, and intensity dependent (Lichtenthaler, 1998).

2.6.4 The Regeneration Phase

This phase is initiated if stress conditions are removed before severe damage or death occurs, causing plants to undergo regeneration and restoration to physiological standards (Lichtenthaler, 1998; Kranner et al., 2010). This enhances plants survivability by creating a new tolerance range based on the duration and stage of exhaustion although permanent damage caused by prolonged stress is usually irreversible (Lichtenthaler, 1998).

2.7 Effects of Abiotic Stress on Onions

Onion plants have a shallow root system with about 0.76-meter maximum penetration depth, while most of the roots are found within the top 0.18 meters with a few roots beyond 0.31 meters (Camilia et al., 2013; Drinkwater and Janes, 1955). This makes them sensitive to drought stress conditions (Kutty et al., 2014) and thus, must be frequently irrigated especially during the bulb formation phase to achieve marketable yields (Kadayifci et al., 2005; Schwartz and Bartolo, 1995).

Studies evaluating the response of onion to drought conditions clearly show that onion is a plant susceptible to drought stress, especially during the bulbing and development phase (Pelter et al., 2004; Rattin et al., 2011). In terms of salt stress, onions have been documented to be sensitive, although the level of sensitivity is dependent on factors such as cultivar, duration, and growth stage of stress application (Beinşan et al., 2015; Hanci et al., 2016).

2.7.1 Germination

In onions, it was reported by Heikal et al. (1982) that germination of onion seeds under PEG-induced drought stress significantly reduced the rate of germination. It was also observed that water absorption by germinating seeds was inversely proportional to the level of stress applied. Additionally, application of gibberellic acid (GA_3) to stressed seeds did not have any beneficial effect on the final germination percentage of onion seeds.

To determine the effect of salinity on germination, Sta-Baba et al. (2010) conducted a study on Merveille de Pompeï, a white onion variety. During the treatment with five salt levels with electrical conductivities of 1.21, 1.45, 3.70, 6.21 and 9.51 dS.m^{-1} for eight days, it was observed that the rate of germination was not significantly affected although there was a reduction. The reduction in germination rate was attributed to metabolic disorders due to the reduction in water absorption into seeds.

Mangal et al., (1989) observed that sprouting decreased gradually with salinity increase but was only significant when salinity levels exceeded 4.0 dS m^{-1} and causing a 96.5% reduction at the highest concentration of 10 dS m^{-1}. Seed yield per plant decreased significantly at 4.0 dS m^{-1} and declined by over 50% at 10 dS m^{-1}.

Studies on salt stress conducted in vitro on six different onion cultivars by Joshi and Sawant (2012) indicated that salt stress has different effects on the regeneration rate of different onion cultivars.

2.7.2 Vegetative and Reproductive Growth

Singh and Alderfer (1966) observed that soil water stress at any growth stage leads to reduction in marketable yield at varying levels. They further observed that yield reduction under soil water stress was more pronounce at the bulb formation and enlargement stage than during the vegetative stage.

A study conducted was by Pelter et al. (2004) on the effect of drought stress on long day onions by inducing drought stress at the 3-, 5-, 7- and 9-leaf growth stages and compared with a control that under optimal conditions over the course of three years. The study revealed a reduction in yield for all the stress induced growth stages in comparison to the unstressed control. Stress at both 3- and 7-leaf stages showed a 26% reduction in yield, representing the most drought affected growth stages. This study thus indicates that drought stress has more significant effects on bulb yield and at the vegetative (3- to 7-leaf) growth stages in comparison to the bulb initiation (9-leaf) stage.

Both Singh and Alderfer (1966) and Pelter et al. (2004) observed a general negative effect of drought stress on bulb yield although specific growth stages produced contrasting effects on bulb yield. This could be attributed to the difference in cultivars, the intensity and duration of drought stress applied during these studies.

Chaudhry et al. (2020) also observed a reduction in the photosynthetic rate, lower leaf number, leaf length and bulb yield of onions as a result of salt and drought stress, with drought stress causing thicker and elongated root which can be attributed to drought stress tolerance.

These studies along with numerous other studies such as Casey and Garrison, (2003), Kadayıfçı et al. (2005), Shock et al. (2005), Van Eeden and Myburgh (1971) and Zayton (2007) indicated that onions exposed to drought stress suffer significant yield loss, but the extent of the yield loss is dependent on several factors such as cultivar, growth stage, duration, and intensity of drought stress.

Onions have been found to be sensitive at the flowering stage to drought stress. A study by El Balla et al. (2013) showed that the drought stress applied during the bulbing stage affected not only the bulb yield of the plant but also the seed yield in the second year.

Sta-Baba et al. (2010) tested how salt affects vegetative growth and bulb yield in both field and greenhouse conditions. It was observed that onion was sensitive to low levels of salt, affecting vegetative growth in terms of total leaf number, height, fresh and dry weight of leaves and roots. It was also observed that salinity in field conditions resulted in severe dying of transplanted seedlings in the 60 days. In the salinized plots, bulbing was initiated after 45 days of transplanting while the control plots were still undergoing vigorous vegetative growth, a prerequisite for high yields. This resulted in a significant reduction in the bulb yield of salinized plots to the control plots. The authors concluded that the significant differences in foliage and bulb yield were resultant effect of the interaction between treatments and plant age.

Similarly, Sudha and Riazunnisa (2015), under five levels of salinity of 50 mM, 100 mM, 150 mM, and 200 mM with a control for four cultivars (Agrifound dark red, Agrifound white, LINE-28 and Krishnapuram), found that all parameters measured were decreased with increasing salt levels, with a variation in cultivar behavior at different salinity levels. Rate of germination was significantly reduced in all the cultivars with the most effect observed at 150 mM and 200 mM concentration.

Hanci et al. (2016) reported that all morphological parameters including neck diameter, leaf diameter, plant length, number of leaves and leaf water potential except for leaf waxiness were reduced under drought and salt stress at the early growth phase of four onion cultivars. A similar reduction in all physiological parameters (total chlorophyll, chlorophyll-a, chlorophyll-b, carotenoids, and chlorophyll-a/b) except for amount of proline, whose accumulation is usually used as an indicator of stress in plants (Adams and Frank, 1980). Similarly, salinity caused a decrease in chlorophyll and carotenoid content and an increased proline content with increasing salinity (Hanci et al., 2016).

Interestingly, Semida (2016) observed that exogenous application of low concentrations of hydrogen peroxide enhanced tolerance to salinity in onion plants by improving the photosynthetic efficiency and plant water status, an observation which can be useful in cultivation on salinized land.

2.7.3 Bulb Quality

Single centeredness is an important bulb quality characteristic that is particularly useful in restaurants and the manufacture of onion rings and onions may be classified as bullets if they have only one growing point or small double if the bulb has a small multiple centers with diameter less than 38 mm. These are known as functionally single centers with an overall diameter from 76 mm to 114 mm and the target by producers is 75% of all bulbs although incentives are given for delivering bulbs with more than 75% single centeredness (Shock et al., 2005).

Single centeredness is an inherited trait, enabling cultivars to produce higher frequencies of single center bulbs, although it can be affected by environmental factors such as drought stress (Cramer, 2006; Pelter et al., 2004; Shock et al., 2005). Brewster, (2008) explained multiple centers as the emergence of an axillary branch adjacent to an existing one to create multiple growing points.

Pelter et al. (2004) in addition to observing reduced bulb yield in drought stressed bulbs found that, the rate of single centeredness was reduced in drought stressed bulbs. It was determined that single centeredness was most affected when drought stress was imposed in the early growth stages, with biggest effect seen in the 3-leaf stage which recorded 40% more split/multiple centers. It was observed that drought stress at the 7-leaf growth stage and above had the potential to still produce the acceptable level of single centered bulbs of 70% and above.

In consonance, Shock et al. (2005) found water stress at the 4- to 6-leaf to increase the incidence of multiple centres in onions while having no effect on the incidence of translucent scales, which is the translucent appearance of one or more internal bulb scales. Under high salinity conditions, it was observed that the pungency of onion bulbs increased (Chang et al., 2004).

2.7.4 Bulb Storage

Since onion is a seasonal plant, bulb storage is a critical to ensuring its availability all year round. It has been established that prolonged wet conditions before harvest promote scale splitting and rots during storage by some fungi (Brewster, 2008; Shock et al., 1998), as such, withholding irrigation near the end of the bulbing cycle has been the norm to improve the storage ability of the bulb (Kumar et al., 2007).

Researchers observed reduced sprouting in onion bulbs by inducing drought stress prior to harvest (Grevsen and Sorensen, 2004; Sorensen and Grevsen, 2001). Contrastingly, losses during storage were higher in drought induced plots than in irrigated plots (Assuero et al., 2007; Drinkwater and Janes 1955; Kumar et al. (2007).

Rattin et al. (2011) to settle the contrasting findings by earlier studies conducted a study by storing drought induced bulbs for six months in a 15°C ventilated chamber with a bi-weekly weight measurement. It was found that drought stress caused a significant reduction of bulb size of the cultivars used. Sprouting of bulbs was also observed to be significantly increased during storage. It was then concluded that prolonged drought stress may have significant negative impact on bulb quality during storage with a resultant effect of reduced marketable bulbs. Drought stress applied after bulb formation has also been noted to reduce marketable grade and increase storage issues by causing regrowth problems such as thick necks and scallions (Casey and Garrison, 2003).

The existing literature gives a clear indication that onions are sensitive to drought and salt stress at every stage of their growth cycle from plant development, bulb, and seed yield, and to some extent storage capacity. As the global population soars, it is imperative that onion cultivars that are tolerant to these stress conditions be developed to shore up the global onion production to meets its increasing demand. This study was therefore conducted to provide definite information on the effects of salt and/or drought stress on the bulb yield of 20 long day onion genotypes.

CHAPTER III

MATERIAL AND METHOD

3.1 Plant Materials

Twenty elite long day onion lines, thirteen of which were obtained from GAF-MTN gene pool developed through a long-term onion breeding cooperation between Dr Ali Fuat Gökçe and MTN Seed Company Limited, three lines obtained MTN Seed Company Limited, and four commercial varieties comprised of two local and two foreign varieties each.

Table 3.1. Source material used for the study

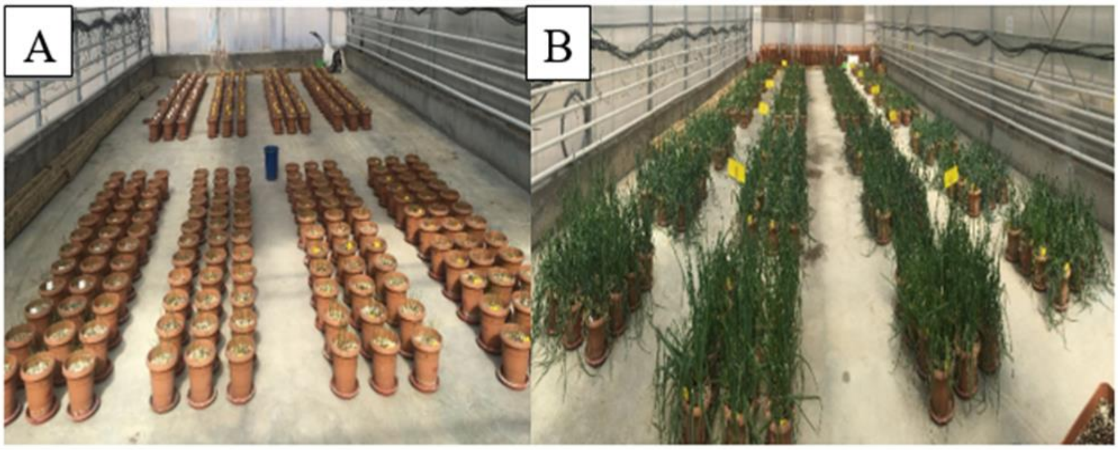
Line No.	Source	Color
U2	GAF-MTN	Yellow
U6	GAF-MTN	Red
U9	GAF-MTN	Yellow
U10	GAF-MTN	Yellow
U11	GAF-MTN	Red
U12	GAF-MTN	Yellow
U16	GAF-MTN	Yellow
U17	GAF-MTN	Yellow
U23	GAF-MTN	Yellow
U24	GAF-MTN	Yellow
U29	GAF-MTN	Red
U31	GAF-MTN	Yellow
U33	GAF-MTN	Yellow
U44	MTN	Yellow
U47	MTN	Yellow
U49	MTN	Yellow
U55	Local variety	Yellow
U57	Local variety	Yellow
U63	Foreign variety	Yellow
U65	Foreign variety	Yellow

3.2 Location

The research was conducted at the greenhouse facility of the Department of Agricultural Genetic Engineering of Niğde Ömer Halisdemir University in Niğde, Türkiye.

3.3 Seed Germination and Plant Growth

Seeds of the 20 long day lines were planted in 10-liter plastic pots containing peat and perlite (2:1) at the beginning of November and germinated by regular irrigation. After germination, plants were thinned to 10 seedlings per pot, 12 pot per line. For the 12 pots for every line, 3 pots each were designated for the control, salt stress, drought stress, and combined salt and drought stress. The plants were grown under greenhouse conditions and irrigated to the soil saturation capacity once every three days until the initiation of the bulbing stage.



a b

Figure 3.1. Pot arrangement; seedling stage(a) and vegetative growth stage(b)

3.4 Abiotic Stress Treatment

Abiotic stress treatment commenced during the bulbing phase which is the most sensitive stage to stress as indicated in the literature (Krasensky et al., 2012; Pelter et al., 2004; Rattin et al., 2011) while the control setups were continually irrigated once every three days to soil saturation capacity throughout the period of the stress treatment.

3.4.1 Salt Stress Treatment

The salt stress on long day lines started with 75 mM NaCl solution and increased by 25 mM every three days until a final concentration of 150 mM NaCl was reached, and maintained, during the period of bulbing (Hanci et al., 2016).

3.4.2 Drought Stress Treatment

For drought stress, irrigation was halted throughout the during bulbing stage.

3.4.3 Combined Salt and Drought Stress Treatment

Combination of salt and drought stress was performed by limited irrigation to expose the plants to drought stress and the application of salt stress with 75 mM NaCl and increased by 25 mM every three days until a final concentration of 150 mM NaCl is reached, which was maintained, during the period of bulbing (Hanci et al., 2016).

3.5 Bulb Harvest

When more than half of the tops in a pot were bent over and browning, the onions were harvested, left at the pots at least 10 days to cure bulbs after harvest, and thereafter put in corresponding labelled bags for measurement of bulb parameters.

Figure 3.2. Harvesting of onion bulbs

3.5.1 Number of Bulbs

For each pot, the number of bulbs was counted and recorded, taking note of rotten and split bulbs.

3.5.2 Bulb Weight

For each pot, the harvested bulbs were weighed together using an electronic balance and the average weight was taken to represent the weight of a single bulb.

Figure 3.3. Bulb weight measurement

3.5.3 Bulb Size

For each pot, the width and height of the best five bulbs was used to obtain the average width and height of the bulbs from that pot.

3.5.3.1 Diameter/Width Measurement

To measure the width, the five selected bulbs were arranged side by side with the root system facing downwards along a positioned meter rule and the corresponding value recorded and the mean calculated to represent the width for a single bulb.

3.5.3.2 Height Measurement

To measure the height, using the same five selected bulbs, they were arranged along the positioned meter rule in such a way that the root system of the next bulb was touching the neck of the previous bulb and the corresponding value on the rule was recorded and the mean calculated to represent the height of single bulb.

Figure 3.4. Diameter and height measurement of bulbs

3.5.4 Bulb Shape Determination

Bulb shapes for all the stresses were determined using the shape factor following Zayton (2007)'s formula.

Shape factor = equatorial diameter (width) / polar diameter (height)
Where spherically shaped= 1, Oblate shaped > 1, and Prolate shaped < 1

3.5.5 Statistical Analysis

Using Jamovi 2.2.5, data from the study was tested for variance among the stresses for each genotype using one-way ANOVA and followed by a multiple comparison test using Tukey HSD to determine the direction and level of the difference between control and stressed groups. A biplot analysis was made using GenStat 22.1 to determine the stability of the bulb yield parameters under the various stresses.

CHAPTER IV

RESULTS

4.1 Rotten and Split Bulbs

Rotten and split bulbs were found to be generally low, mostly falling below an acceptable 25% of the yield. The highest percentage of rotten and split bulbs were observed in the control of U63 and U12 at 56.3% and 35.3%, respectively, and the drought stress of U65 at 26.1% for the most part, it was observed that the control treatments produced the most split and rotten bulbs in comparison to the stressed plants. Even when the stressed plants produced more rotten and split bulbs, it was observed that the rate was within an acceptable limit of less than 25% with the only exception being the drought stress of U65 which recorded a 26.1% of split and rotten bulbs as compared to the 13.6% of the control. U6 and U9 were found to be the most tolerant against rot and split centers as both lines recorded no rots or split centers under any of the stresses.

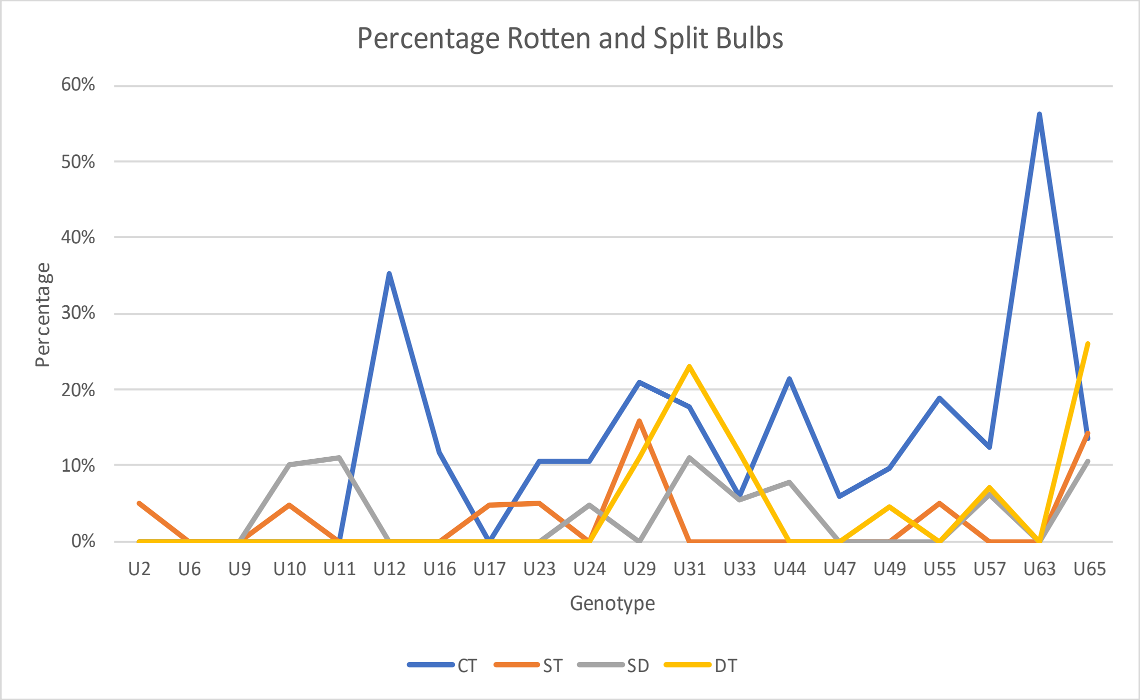


Figure 4.1. Graphical representation of rotten and split bulbs
Note: CT= control treatment, ST= salt stress, SD=combined salt and drought stress, DT=drought stress

4.2 Equatorial diameter/Width of Onion Bulbs

An analysis of variance (ANOVA) for the mean/average width of the bulbs produced significant statistical difference for ten of the genotypes (U6, U9, U10, U11, U17, U29, U31, U33, U44, U57) while the remaining ten genotypes, (U2, U12, U16, U23, U24, U47, U49, U55, U63, U65) showed no significant difference among the various stresses for the genotypes.

Table 4.1. ANOVA table for bulb diameter of onion genotypes

Genotypes	F	df1	df2	p*
U2	3.641	3	8	0.064
U6	10.382	3	8	0.004*
U9	11.333	3	8	0.003*
U10	115.778	3	8	< .001*
U11	31.540	3	8	< .001*
U12	0.585	3	8	0.642
U16	1.072	3	8	0.414
U17	13.780	3	8	0.002*
U23	1.235	3	8	0.359
U24	1.395	3	8	0.313
U29	4.062	3	8	0.050*
U31	4.385	3	8	0.042*
U33	6.819	3	8	0.014*
U44	19.316	3	8	< .001*
U47	1.410	3	8	0.309
U49	0.611	3	8	0.627
U55	2.906	3	8	0.101
U57	10.228	3	8	0.004*
U63	2.830	3	8	0.106
U65	2.482	3	8	0.135

Note: *=Statistical significance, p= p-value, F=F value, df1=degree of freedom 1, df2=degree of freedom 2

4.2.1 Comparative Analysis of Bulb Diameter

Using Tukey Post-Hoc test, comparisons of the stressed lines against the control group were made to identify the level and direction of the difference. U6 showed a significant difference under only drought stress. U9 and U33 were found to be susceptible to both SD and drought stresses while U10 and U11 were found to perform very poorly, succumbing to all stress treatments applied for the study. U17 was also found to be susceptible to both SD and drought stresses. Interestingly, U44 and U57 both showed a high level of tolerance to some of the stresses applied. U44 was shown to have bulbs with higher widths than the control for both salt and drought stresses, while U57 was better under salt and SD stresses. Although U29 and U31 produced significant results, that difference was not due to a comparison with the control group.

Table 4.2. Tukey HSD Test for diameter

Genotypes		SS	SD	DS
U6	MD			2.067*
	p-value			0.002
U9	MD		0.533*	0.533*
	p-value		0.007	0.007
U10	MD	0.867*	0.867*	2.13*
	p-value	< .001	< .001	< .001
U11	MD	1.53*	1.667*	2.400*
	p-value	0.001	< .001	< .001
U17	MD	2.07*		1.533*
	p-value	0.002		0.011
U33	MD		1.733*	1.867*
	p-value		0.023	0.016
U44	MD	-2.73*		-1.200*
	p-value	< .001		0.043
U57	MD	-2.07*	-1.600*	
	p-value	0.009	0.034	

Note: Only comparisons showing statistical significance are depicted
*= Statistical significance at p-value ≤ 0.05
MD= mean difference, ST= salt stress, SD=combined salt and drought stress, DT=drought stress

4.2.2 AMMI Biplot Analysis of Bulb Diameter

AMMI biplot analysis of the diameter of the bulbs indicates that the control and salt stress conditions had relatively stronger interactive effects on the genotypes as compared to the drought and combined salt and drought stress conditions, which had similar but weaker interactive effects on the genotypes. U2, U9, U16, U23, U29 and U63 were found to be insensitive to stress conditions, making them broadly tolerant. U6, U10, U12, U24, U47 and U49 were found to moderately tolerant to the stress conditions while U11, U17, U31, U33, U44, U55, U57 and U65 were sensitive to the stress interactions, indicating that they are specifically tolerant.

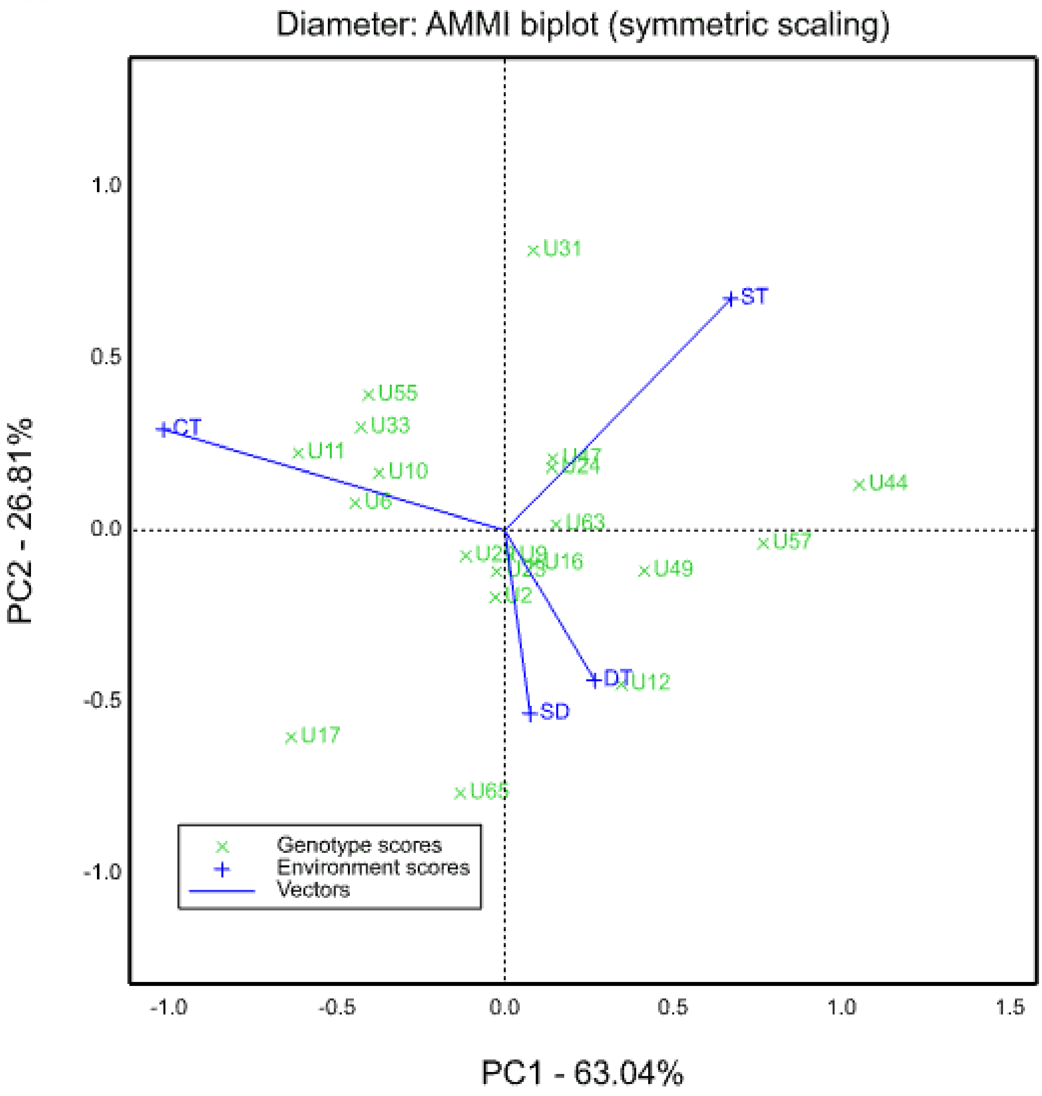


Figure 4.2. AMMI biplot analysis for diameter of onion bulbs
Note: CT= Control Treatment, ST= Salt stress, SD= Combined salt and drought Stress, DT= Drought Stress

4.3 Polar Diameter/height of Onion Bulbs

For the height of onion bulbs, a similar trend as observed in the width analysis was seen. Many of the genotypes (U2, U12, U16, U23, U24, U31, U33, U47, U49, U55, U63, U65) were shown to be quite tolerant to the stresses applied, while the remaining eight genotypes (U6, U9, U10, U11, U17, U29, U44, U57) showed variations in the means of the treatment groups.

Table 4.3. ANOVA table for height of onion bulbs

Genotypes	F	df1	df2	p*
U2	2.009	3	8	0.191
U6	10.365	3	8	0.004*
U9	4.183	3	8	0.047*
U10	71.133	3	8	< .001*
U11	38.431	3	8	< .001*
U12	0.271	3	8	0.845
U16	1.167	3	8	0.381
U17	10.031	3	8	0.004*
U23	1.116	3	8	0.398
U24	1.159	3	8	0.384
U29	4.300	3	8	0.044*
U31	2.490	3	8	0.134
U33	2.937	3	8	0.099
U44	16.122	3	8	< .001*
U47	1.391	3	8	0.314
U49	1.257	3	8	0.352
U55	2.968	3	8	0.097
U57	10.788	3	8	0.003*
U63	3.991	3	8	0.052
U65	2.279	3	8	0.156

Note: *=Statistical significance, p= p-value, F=F value, df1=degree of freedom 1, df2=degree of freedom 2

4.3.1 Comparative Analysis of Bulb Height

Pairwise comparison analysis of the polar diameters/height of the stressed bulbs to the control group showed varying levels of susceptibility and tolerance to the stresses. The results generally followed a similar trend as in the width comparison. U6 showed susceptibility under only drought stress while being tolerant under salt and SD stresses. U10 and U11 were seen to be highly susceptible to all the stresses in terms of onion bulb height. U17 showed susceptibility under both salt and drought stress applications. Under only salt stress conditions, U44 and U57 performed significantly better than the control group, an observation that was not replicated under SD and drought stress conditions. Despite U9 giving significant difference within the group means, that difference was not expressed between comparison of the stressed groups with the control group.

Table 4.4. Tukey HSD Test for height

Genotypes		ST	SD	DT
U6	MD			1.467*
	p-value			0.003
U10	MD	0.867*	0.733*	1.533*
	p-value	< .001	< .001	< .001
U11	MD	1.27*	1.2000*	2.067*
	p-value	< .001	0.001	< .001
U17	MD	1.73*		1.267*
	p-value	0.004		0.025
U44	MD	-1.73*		
	p-value	< .001		
U57	MD	-1.60*		
	p-value	0.007		

Note: Only comparisons showing statistical significance are depicted
*= Statistical significance at p-value ≤ 0.05
MD= mean difference, ST= salt stress, SD=combined salt and drought stress, DT=drought stress

4.3.2 AMMI Biplot Analysis of Bulb Height

For height, AMMI biplot analysis revealed a similar pattern as in the diameter analysis. The control and salt stress conditions had relatively stronger interactive effects on the genotypes as compared to the drought and combined salt and drought stress conditions, which had similar but weaker interactive effects on the genotypes. Bulb heights of U9, U16, U23, U29 and U63 were found to be insensitive to stress conditions, making them broadly tolerant to all the treatments. U2, U6, U10, U12, U24, U33, U47 and U49 were found to moderately tolerant to the stress conditions while U11, U17, U31, U44, U55, U57 and U65 were sensitive to the stress interactions, indicating that they are specifically tolerant in terms of bulb height.

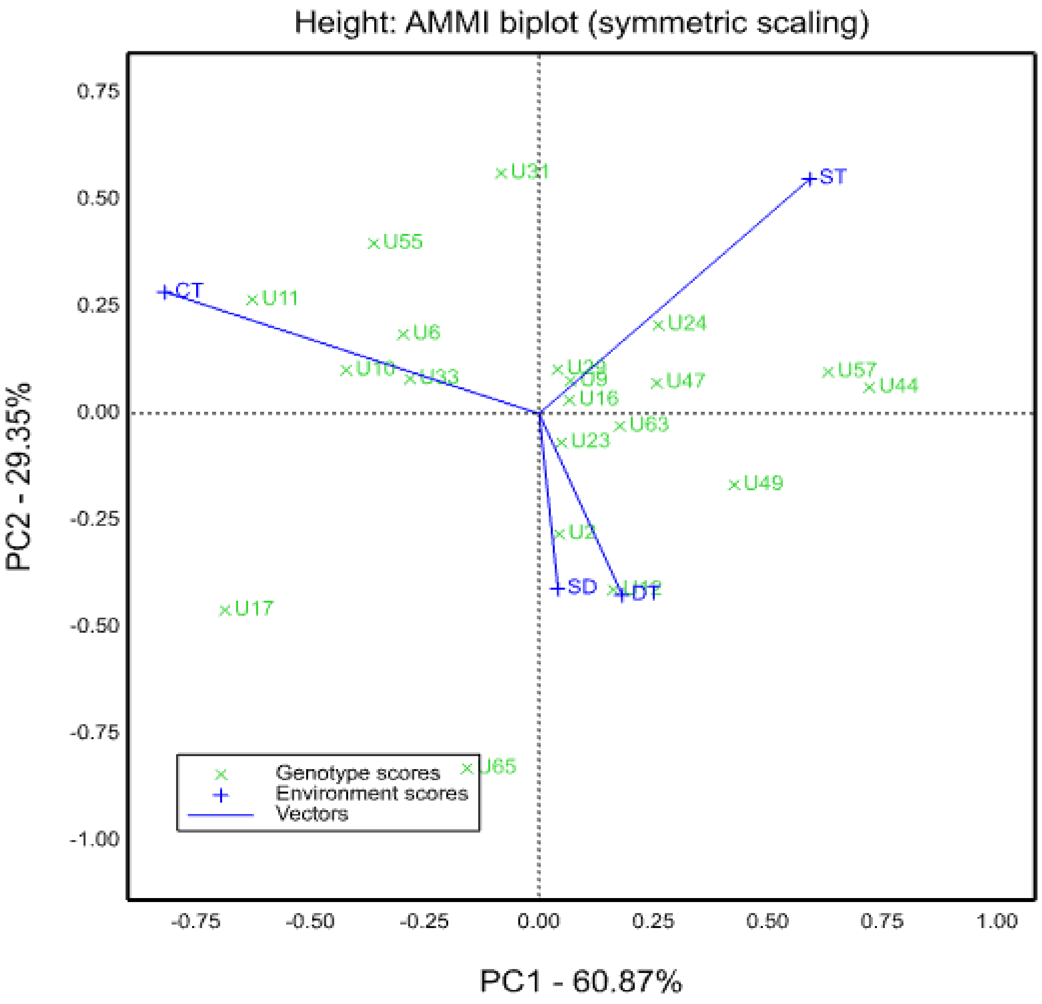


Figure 4.3. AMMI biplot analysis for height of onion bulbs

Note: CT= control treatment, ST= salt stress, SD= combined salt and drought stress, DT= drought stress

4.4 Weight Comparison

Average weights of onion bulbs were found to be significantly affected in nine genotypes (U6, U9, U10, U11, U17, U31, U33, U44, U57) while the eleven remaining genotypes (U2, U12, U16, U23, U24, U29, U47, U49, U55, U63, U65) showed tolerance to the stresses applied, a similar trend observed for both width and height.

Table 4.5. ANOVA table for weight of onion bulbs

Genotypes	F	df1	df2	p*
U2	3.103	3	8	0.089
U6	9.350	3	8	0.005*
U9	5.964	3	8	0.019*
U10	136.378	3	8	< .001*
U11	35.591	3	8	< .001*
U12	1.828	3	8	0.220
U16	0.230	3	8	0.873
U17	10.747	3	8	0.004*
U23	1.148	3	8	0.387
U24	1.607	3	8	0.263
U29	1.797	3	8	0.226
U31	5.177	3	8	0.028*
U33	4.290	3	8	0.044*
U44	17.940	3	8	< .001*
U47	1.355	3	8	0.324
U49	1.203	3	8	0.369
U55	2.793	3	8	0.109
U57	7.611	3	8	0.010*
U63	2.785	3	8	0.110
U65	2.749	3	8	0.112

Note: *=Statistical significance, p= p-value, F=F value, df1=degree of freedom 1, df2=degree of freedom 2

4.4.1 Comparative Analysis of Bulb Weight

Mean weight comparison of stressed bulbs to control groups revealed susceptibility of U6 and U9 to SD and drought stress, U10 and U11 as usual were susceptible to all the stresses. Under salt and drought conditions, U17 and U44 produced contrasting results, with the former being susceptible and the later producing a better yield in terms of weight under these stresses. U57 also produced a better yield under only salt stress conditions while still tolerating SD and drought stress conditions. U31 and U33 expressed no significant difference between the control group and any of the stressed groups.

Table 4.6. Tukey HSD Test for weight

Genotypes		ST	SD	DT
U6	MD		50.80*	75.8*
	p-value		0.034	0.004
U9	MD		15.9*	17.17*
	p-value		0.044	0.031
U10	MD	35.9*	38.83*	71.6*
	p-value	< .001	< .001	< .001
U11	MD	90.9*	98.40*	122.4*
	p-value	< .001	< .001	< .001
U17	MD	84.5*		69.1*
	p-value	0.004		0.013
U44	MD	-69.3*		-32.40*
	p-value	< .001		0.037
U57	MD	-57.8*		
	p-value	0.011		

Note: Only comparisons showing statistical significance are depicted
*= Statistical significance at p-value ≤ 0.05
MD= mean difference, ST= salt stress, SD=combined salt and drought stress, DT=drought stress

4.4.2 AMMI Biplot Analysis of Bulb Weight

Weight analysis of the bulbs indicated that the control and salt stress conditions had relatively stronger interactive effects on the genotypes as compared to the drought and combined salt and drought conditions, which had similar but weaker interactive effects on the bulb weights, replicating the pattern seen in diameter and height. U2, U9, U16, U24 and U29 were found to be insensitive to stress conditions, making them broadly tolerant to all the treatments. U6, U10, U12, U33, U47, U49, U55 and U63 were found to moderately tolerant to the stress conditions while U11, U17, U31, U44, U57 and U65 were sensitive to the stress interactions, indicating that they are specifically tolerant for weight.

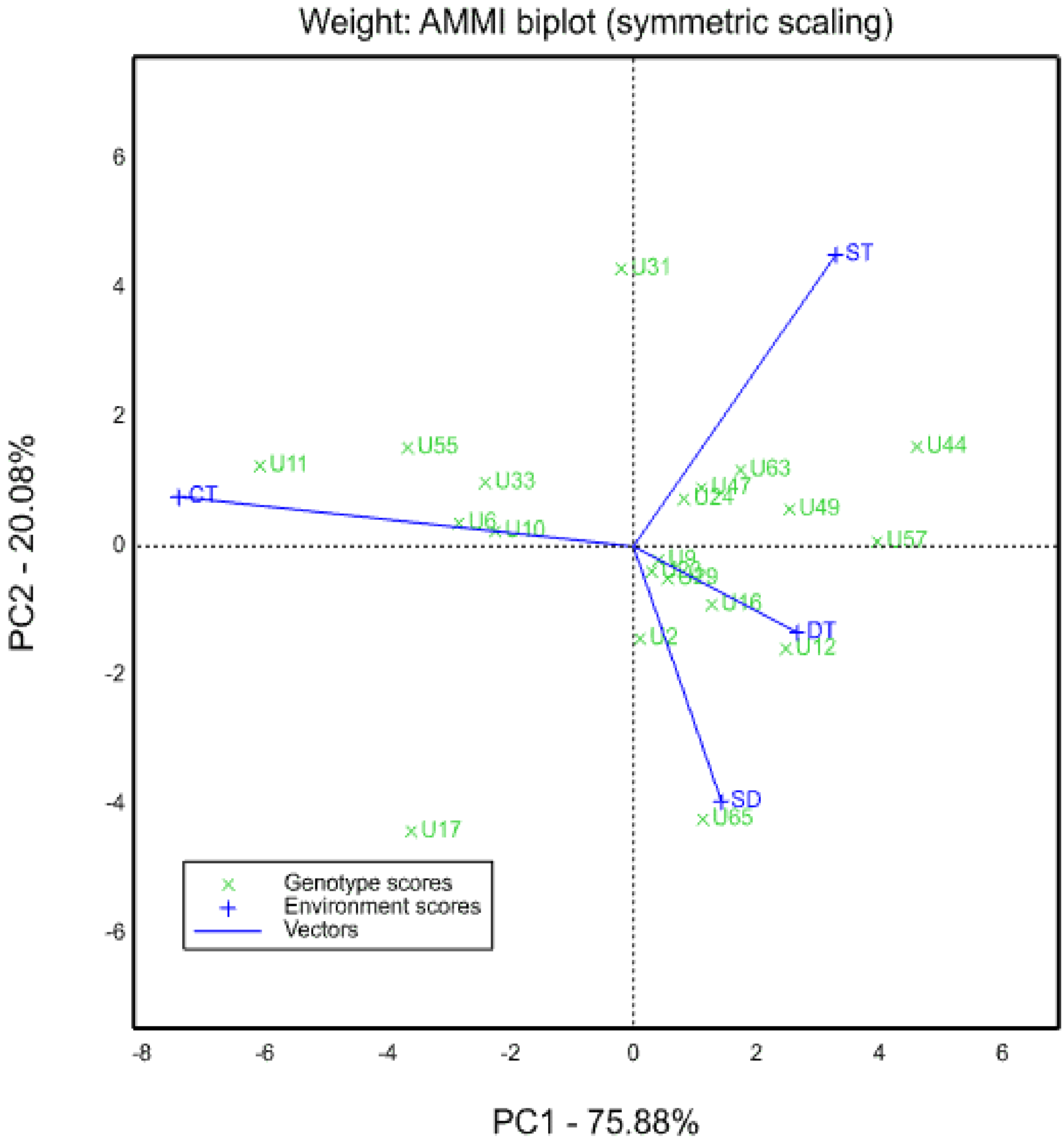


Figure 4.4. AMMI biplot analysis for weight of onion bulbs

Note: CT= control treatment, ST= salt stress, SD= combined salt and drought stress, DT= drought stress

4.5 Bulb Shape

For nine of the genotypes, bulb shape was not affected for any of the stress conditions. U2, U11, U16, U23, U33, U57, U63 and U65 were all prolate shaped while U9 was oblate shaped according to the bulb shape factor. U6, U10, U12, U17, U24, U29, U31. U44, U47, U49 and U55 had differing bulb shapes under different stress conditions.

Table 4.7. Shape factor of onion bulbs under different stress conditions

Genotypes	CT	ST	SD	DT
U2	0.99	0.94	0.91	0.89
U6*	1.06	0.96	1.00	0.96
U9	0.96	0.90	0.91	0.94
U10*	1.00	1.00	0.98	0.88
U11	1.08	1.06	1.02	1.05
U12*	0.97	1.04	1.03	1.01
U16	0.81	0.79	0.77	0.80
U17*	1.04	0.99	1.03	1.00
U23	0.95	0.89	0.89	0.89
U24*	1.03	0.96	0.94	0.95
U29*	1.04	0.94	1.03	0.92
U31*	0.95	1.06	0.91	0.84
U33	0.99	0.90	0.81	0.83
U44*	0.78	1.02	0.87	0.97
U47*	1.04	1.00	0.99	0.89
U49*	1.03	1.02	1.00	0.99
U55*	1.08	1.04	1.03	0.99
U57	0.83	0.96	0.98	0.86
U63	0.93	0.92	0.86	0.86
U65	0.93	0.88	0.94	0.81

Note: spherically shaped= 1, Oblate shaped > 1, Prolate shaped < 1
*=genotypes with varying bulb shapes under different stress conditions
CT= control treatment, ST= salt stress, SD=combined salt and drought stress, DT=drought stress

CHAPTER V

DISCUSSION

The rise in the global population is expected to reach 9.7 billion by 2050 according to UN estimates. To ensure food security, global agricultural production needs to be increased significantly. Despite this, crop production is faced with numerous hurdles ranging from climate change related challenges such as drought, extreme temperatures, salinization of arable lands and population increase associated problems such as accelerated urbanization resulting in decreasing arable land for cultivation (Elferink and Schierhorn, 2016). It is estimated that, over 90% of the global land area is affected by some sort of environmental constraint (Cramer et al., 2011) which have the potential of causing significant yield losses if sustainable agricultural practices in the form of controlled irrigation, weed control and application of farm inputs such as fertilizers are not employed.

Onions are one of the most important vegetable crops worldwide and have seen a gradual increase in production over the years. It is used mainly to add flavor to dishes, although is also used in the production of onion rings (Shock et al., 2005). The existing literature indicates that onion as an herbaceous biennial crop has been found to be sensitive to unfavorable environmental conditions such as drought and salt stress (Sta-Baba et al., 2010). Under these conditions, they have been found to cause a reduction in bulb yield and quality. Therefore, this study was conducted using 20 long day onion breeding lines to identify which of them were tolerant to these abiotic stress conditions in terms of bulb yield and quality, that can be bred into tolerant cultivars to meet the demands of the changing agricultural landscape. This study thus compared bulb yield parameters of each breeding line under induced abiotic stress conditions to unstressed ones to determine whether they were tolerant or not.

Healthy and unblemished bulbs with weight above 20 g are the marketable yield of onions while those with weight of below 20 g and physical deformities such as decays, diseases and multiple centers are unmarketable yield (Demisie and Tolessa 2018). This study determined that all the breeding lines produced bulb weights above the minimum marketable bulb weight, with the least weight of 25.2 g observed in the control of U44. Split and rotten bulbs of less than 25% form another spectrum of the marketable yield.

Split bulbs, also known as multiple centers, is an undesirable trait whereas single center is the desirable trait in onion bulbs. The rate of occurrence of single centeredness has been noted to be genotype dependent although unfavorable environmental conditions such as drought stress are known to elevate the incidence of multiple centers. It was observed that, for all the genotypes, single centeredness fell above the acceptable rate of 75% under all stress conditions except for the drought treatment of U65 which recorded 26.1% occurrence of multiple centers. Although the control also recorded acceptable single center occurrence aside U63 and U12 at 56% and 35% respectively, it was observed that for many of the genotypes, the occurrence of multiple centers in the control was higher than the stressed bulbs. The result of this study is in consonance with Pelter et al. (2004) who observed that, the rate of single centeredness significantly reduced when drought stress was applied during the early growth stages, but at the late growth stages (7-leaf), onions had the potential to produce acceptable rate of single centered bulbs. This study also revealed that salt, drought and salt and drought stress treatments at the bulb initiation stage has the potential to increase marketable yield in terms of single centeredness and reduced bulb rot.

Comparative analysis showed no significant difference between any of the stressed bulbs to the control of U2, U12, U16, U23, U24, U47, U49, U55, U63 and U65, whereas U10 and U11 show highly significant differences between the control and all stressed bulbs for all bulb size parameters (diameter, height and weight) measured. U2, U9, U23 and U29 were found to be broadly adapted to all the stress conditions used in the study (ST, SD and DT) for all bulb size parameters while U11, U17, U44 and U57 were found to be highly unstable across all the stress conditions for all bulb size parameters. Although U11 and U17 were found to be unstable, it was observed that they were usually among the best performing genotypes under all stress conditions for all bulb size parameters, an observation that indicate that they are high yielding genotypes whereas U63 was consistently a low yielding genotype for all bulb parameters. U17 was found to be most sensitive under salt stress for all bulb size parameters, while U44 was found to be highly tolerant under salt stress conditions. Aside from U11 and U17, it was observed that salt stress, drought stress and their combined effect affected the bulb size parameters of each genotype distinctly, as there were fluctuations among the best performing genotypes for diameter, height and weight, an observation which indicates that bulb yield was heavily dependent on the genotype and its interaction with the stress applied. As stated by Dhumal et al. (2007), combined stress factors do not always have a combined effect on crops, and the bulb parameters generally performed better under SD than under DT.

Bulbs were characterized as spherical, oblate or prolate if the shape factor was equal to one, or less than or greater than one, respectively. U2, U9, U11, U16, U23, U33, U57, U63 and U65 did not change shape under any of the stress conditions, meaning that both diameter and height were proportionately affected and can be said to be stable in terms of shape under the stress conditions. All remaining genotypes (U6, U10, U12, U17, U24, U29, U31, U44, U47, U49 and U55) expressed shape variations from the control which may have been due to disproportionate effect of the stresses applied on diameter and height of the bulbs.

In fulfilment of the objective of this study, the effect of salt, drought, and their combined stress effect on bulb yield of 20 long day onions have been quantified in terms of yield potential and tolerance level under those stresses.

CHAPTER VI

CONCLUSION

Several studies concerning the effects of abiotic stress on onion yield have been performed and it has been established that onions are generally sensitive to drought due to its shallow root system and moderately sensitive to salt stress. The effect of these stresses on onion yield has been known to affect bulb quality characteristics such as single centeredness, firmness, size and even pungency, although the severity of the effect is determined by the genotype, type, intensity and duration of stress and stage of stress application. Analysis of bulb parameters for 20 long day onion genotypes under salt, drought and combined salt and drought stress revealed that;

Occurrence of split and rotten bulbs was generally at an acceptable rate under all stresses for all genotypes except for drought stress of U65, although the control also produced generally acceptable rates for marketable yields, the occurrence of split and rotten bulbs was at a higher frequency for most of the genotypes. Average weight of bulbs produced irrespective of genotype or stress were above the minimum marketable yield.

Salt stress and the control treatment were found to have bigger effects on the bulb yield in comparison to drought and combined salt and drought stress.

U2, U9, U23 and U29 exhibited broad adaptability while U11, U17, U44 and U57 were highly unstable under all the stress conditions.

U11 and U17 were found to have the biggest bulb sizes despite their instability, and
U63 was the worst performing genotype.

This study therefore provides further breeding potential of crossing high yielding and tolerant genotypes to produce cultivars that are better suited to the drought, salt and combined salt and drought stresses.

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