T.R.
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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF HORTICULTURE

ASSESSMENT OF THE RESPONSE OF SOME MELON GENOTYPES (*CUCUMIS MELO. L*) TO DIFFERENTS DOSES OF SODIUM CHLORIDE (NACI)

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Supervisor
Assoc. Prof. Dr. Hasan PINAR

M.Sc. Thesis

October 2022
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SCIENTIFIC ETHICS CONFORMITY

I hereby declare that all information in this thesis has been composed and presented in accordance with the academic rules and ethical guidelines. I also declare that, as required by these rules and conduct, I have fully and accurately cited and referenced all materials and results that are not original to this work.

Ulrich Gaetan FungaTCHOUNKWE

SUITABILITY FOR INSTRUCTION GUIDE

The master thesis entitled**"Assessment of the Response of Some Melon Genotypes (*Cucumis Melo. L*) to Different Doses of Sodium Chloride (NACL)"** has been composed in accordance with the Postgraduate Thesis Proposal and Thesis Writing Directions of Erciyes University.

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ABSTRACT

The present study, aimed at the determination of salt (NaCI) tolerance level of some melon genotypes and their progenies. Three cross pollinated genotypes (Semame x Ananas, Ananas x Semame and Midyat x Ananas) and two standard melon (Cucumis melo) genotypes (Semame and Ozbek) were tested under in vitro and pot experiment using different NaCI doses(control, 100, 150 and 200 mMol). The results have shown that selection for salt tolerance at high salt concentration (150mM) has a negative significant effect on melon performance. Among the five tested cultivars, Midyat x Ananas and Özbek showed better tolerance compared to other genotypes. In the greenhouse (pot experiment), plant height, stem diameter and leaf length of Midyat x Ananas had their highest value at 100, 150 and 200 mMol respectively. Meanwhile, the best performances of Özbek for plant height, root dry matter content, root length, leaf length and diameter were obtained at 100 mMol. Except for Midyat x Ananas, an increase in salt concentration had a negative impact on plant growth. There was more correlation among morphological and physiological variables in greenhouse experiment compared to in-vitro experiment, therefore it is advised to select melon plants once they start flowering or later as it has been demonstrated in this experiment.

Key Words: melon, salt tolerance, genotype

BAZI KAVUN GDENOTIPLERİNİN (Cucumıs Melo.L) FARKLI DOZLARDA SODYUM TEPKILERININ DEĞERLENDIRMESI

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Yüksek Lisans Tezi, Ekim 2022
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ÖZET

Bu çalışmada bazı kavun genotipleri ve onların melezlerinden elde edilen bireylerin farklı tuz dozlarına(NaCI) tölerans seviyelerinin belirlenmesi amaçlanmıştır. Çalışmada 2 adet standard kavun genotipi(Şemam ve Özbek) ve üç adet melezlemeyle elde edilmiş kavun(Cucumis melo) genotipi(Semame x Ananas, Ananas x Semame and Midyat x Ananas) materyal olarak kullanılarak in vitro ve saksı koşullarında farklı tuz(NaCI) dozları(control, 100, 150 and 200 mMol) test edilmiştir. Elde edilen bulgulara göre 150 mMol NaCI dozu bütün genotipşlerde negative etki oluştururken testlenen 5 adet kavun genotipleri arasında MidyatXAnanas ve Özbek genotipleri diğer genotiplere göre daha yüksek performans göstermişlerdir. Sera koşullarında yapılan saksı denemesinde bitki boyu, gövde çapı ve yaprak uzunluğu bakımından 100, 150 and 200 mMol NaCI dozlarında en yüksek değere MidyatXAnanas genotipi sahip olurken bitki boyu, kök kuru ağırlığı kök uzunluğu yaprak uzunluğu ve yaprak çapı bakımından 100 mMol seviyesinde en yüksek performansı Özbek genotipi göstermiştir. MidyatXAnanas dışında artan NaCI dozu bütün genotiplerde bitki büyümesini olumsuz etkilemiştir. Aynı zamanda çalışmada belirlenen morfolojik ve fizyolojik parametreler arasında in vitro ve saksı denemelerinde önemli korelasyonlar belirlenmiştir. Elde edilen bulgulara göre kavunda çiçeklenmeden sonra yapılacak tuz testlesi ilede seleksiyon yapılması tavsiye edilebilir.

Anahtar Kelimeler: melon, tuz toleransı, genotip

CONTENTS

ASSESSMENT OF THE RESPONSE OF SOME MELON GENOTYPES (*CUCUMIS MELO. L*) TO DIFFERENTS DOSES OF SODIUM CHLORIDE (NACI)

ABBREVIATION

µs : Micro second

APX : Ascorbate Peroxidase

CAT : Catalase

Cm : Centimeter

DMC : Dry Matter Content

Gr : Gram

K^+ : Potassium ion

MDI : Membrane damage index

Mm : Millimeter

mMol : Millimol

Na^+ : Sodium ion

POX : Peroxidase

RWC : Relative Water Content

SOD : Superoxide Dismutase

SPAD : Soil Plant Analysis Development

TSS : Total Soluble Solid

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INTRODUCTION

Soil salinity is one of the major problems in agriculture, most saline soils are found in arid and semi-arid regions. In fact, 20% of arable lands are facing salinity issues and 50% of irrigated lands are affected by soil salinity (Ulas et al., 2020). Low precipitation, high surface evaporation, decomposition of bedrocks, irrigation processes with salty water can be considered as the main causes of soil salinity. Every year, the proportion of salt affected areas increases by 10%. For this reason, it is estimated that 50% of arable lands will suffer of salinity by 2050 (Shrivastava & Kumar, 2015). In Turkey for instance, Barren land accounts for 1518722 hectares among which 41% are slightly saline, 33% saline, 0.5% alkaline, 8% slightly saline-alkaline, and 17.5% saline-alkaline (Karaoğlu & Yalgın, 2018). In the world, activities such as irrigation also contribute in the accumulation of salt in the soil; especially when salty water is used, data of soil salinity caused by the use of saline water has been recorded in several countries.

Table1. Countries Using Salty Water For Irrigation.

Country	**Amount (millionhectares)**
Pakistan	7
China	6.7
USA	4.4
India	3,3
Uzbekistan	2.14
Iran	2.1
Irak	1.75
Türkiye	1.52

Soil salinity is detrimental for plant growth; plants grown under saline conditions develop poorly and may die because of low water potential, which prevents plants from absorbing sufficient waternecessary for nutrients production, and its transportation within plant tissues.

In turkey, melon (*Cucumis melo* L.) is a crop with high economic value, after China the country is the second largest producers in the world, its production is estimated at around 1.7 million for a surface area of 110,000 hectares (Özbahçe, 2014). Melon production is mostly produced in Aegean, Marmara, Central Anatolia, Eastern Anatolia, Southeast Anatolia and Mediterranean regions (Özey &caliskan, 2019). It is a crop which tolerates salt and water stress conditions, since it can also be grown in area where irrigation is not practiced and soil salinity is an issue (Demir, S. ve ark., 2012). However, crop yield remains impacted, less yield isobtained per surface area and producers keep getting low incomes. Therefore, there is need to identify those genotypes which offer more resistance especially to salt stress condition, they can be adopted by farmers or used in plant breeding programs to develop more resistant varieties which will ensure a more sustainable production. The aim of this study is to identify salt tolerant genotypes among existing local varieties (2) and breeding combinations(3) developed in the Department of Horticulture atErciyes University. Cultivars had been grown in greenhouse and in vitro condition for long and short term period respectively. Growth and physiological parameters in greenhouse and in vitro condition had been analyzed in order to evaluate plant responses to different salt concentration. Then correlation between both growth and physiological parameters had been evaluated in order to determine cultivars which are more tolerant to salinity stress.

CHAPTER I

LITERATURE REVIEW

1.1.Origin and Distribution

Muskmelon with chromosome number 2n=24 is a plant which belongs to the curcubitaceae family, which encompasses other major commercial crops such as squash and pumpkin (*Cucurbita* spp.), watermelon (*Citrullus lanatus*), and cucumber (*Cucumis sativus* L.).There has been some confusion on the origin of melon (*Cucumis melo*); while some people believed it originated from Asia others assumed it originated from Africa, other evidences recently revealed pointed Australia as a possible source of origin of melon. Wild melon types were observed in both continents; African wild relatives were characterized by their small fruit size which was similar to a plum and smelled like a fresh green melon, meanwhile wild species from Asia (India) had a size ranging from plum to lemon. Bases on those morphological characters melon was classified into two sub-specie; Agrestis from Africa and Melo from Asia. However, it was noticed that ssp. *Agrrestis* was more widespread than ssp. *Melo* which was only restricted to southwest Asia. Thus, it was concluded that early trade that took 3000 years ago led to the spread of some wild specimens from Africa to Asia where domestication was more intensive (Kerje and Grum, 2000). Million years ago before the big bang, Africa and India which were very close seemed to be the center of the earth, the dislocation of the continent was accompanied by a share of vegetation, which might explain the presence of melon in Asia through India (Mallick & Masui, 1986). A recent study carried out by Endl et al. (2018) supports the fact that modern melon cultivars have two lineages; *Cucumis melo* subsp. Melo and *Cucumis melo* subsp. Meloides, and that the Asian type is the source of widely commercialized cultivar groups. According to the author, melon was domesticated twice; in India and in Africa, though closest wild relative of melon was also found in Australia, there are no evidence of its domestication. *Cucumis picrocarpus* is the closest sister of

C.melo group, but it has been synonymized under this *C.melo* meanwhile morphologically and genetically they are completely different (Sebastian et al., 2010).

In Asia, it is assumed that *C.melo* was independently domesticated in Southeast Asia, India and East Asia. But today, Southwest and Central Asia because of their extremely polymorphic species are considered as primary center of diversity, countries of those regions include mainly Turkey, Syria, Iran, Afghanistan, North and Central India and Transcaucasia, Turkmenistan, Tajikistan, and Uzbekistan; other regions which are considered as secondary center of origin include China, Republic of Korea and the Iberian peninsula. Its presence in the rest of the world might have been caused by birds, animals and man, for instance this crop was introduced in central America in 1516 and in New York in 1629 (Swamy, 2017). There are several varieties of muskmelon distributed throughout the world; so far forty varieties including wild species have been identified(Mallick & Masui, 1986).

Table2.Botanical Varieties of *Cucumis melo*.

No	Botanical variety	Common name
1	*C.melo* var. acidulus	Sour melon
2	*C.melo* var. aestivales	Summer tough m.
3	*C.melo* var. agrestis	Weed m.
4	*C.melo* var. albida	New m.
5	*C.melo* var. autumnales	C.Asia fall m.
6	*C.melo* var. bucharici	Summer soft m.
7	*C.melo* var. cantalupa	Cantaloupe m.
8	*C.melo* var. cantalupensi	Rock m.
9	*C.melo* var. chandalack	Chandalak m.
10	*C.melo* var. chate	Ajjuol m.
11	*C.melo* var. chito	Lemon m.
12	*C.melo* var. conomon	Pickling m.
13	*C.melo* var. dudaim	Pocket m.
14	*C.melo*var.duripulposus	Europe summer m.
15	*C.melo* var. erythraeus	
16	*C.melo* var. flava	Ogon m.
17	*C.melo* var. flexuosus	Snake m.
18	*C.melo* var. ginmakuwa	Yellow sweet m.

Table 2. Botanical Varieties of *Cucumis melo*. (more)

No	Botanical variety	Common name
19	*C.melo* var. grubek	Grubek m
20	*C.melo* var. hassanbey	Winter casaba m.
21	*C.melo* var. hibernus	C. Asiawinter m.
22	*C.melo* var. hime	Orange m.
23	*C.melo* var. hiemalis	Gimofuka m.
24	*C.melo* var. hinodorus	Minter m.
25	*C.melo* var. kikumelon	Yuki m.
26	*C.melo* var. makuwa	Orientalsweet m.
27	*C.melo* var. melitensis	Glatte m.
28	*C.melo* var. microcarpus	Afghanistan m.
29	*C.melo* var. microspermus	Seikan m.
30	*C.melo* var. momordica	
31	*c.melo* var. monoclinus	
32	*C.melo* var. neo-makuma	
33	*c.melo* var. praecox	Russian early m.
34	*C.melo* var. reticulatus	Netted m.
35	*C.melo* var. rokkiford	Rokkiford m.
36	*C.melo* var. saccharinus	Pineapple m.
37	*C.melo* var. tamago	Egg m.
38	*C.melo* var. tarra	Tarra m.
39	*C.melo* var. utilisssimus	
40	*C.melo* var. zhukoski	Summer casaba m.

1.2.Importance of Melon

Melon is a warm climate crop, which can be grown in greenhouses as well as in colder region. It is a crop which thrives well in well drained, rich and fertile soils. There exist two categories of melon; summer melon (mostly cantaloupe melon), which is usually harvested at the end of summer, that is three month after planting, and winter or late melon (casaba, honeydew and creenshaw varieties), which is harvested in late autumn or early winter). In 2018, global production was estimated at thirty three million tons, with China (46.5%), Turkey (6.4%), Iran (6.3%), India (4.5%), and Kazakhstan (3.3%) respectively identified as the major producers (Team, 2020).

Melon (*Cucumis melo*) exists in two different forms; climacteric and non-climacteric. Those considered as climacteric usually have orange flesh, high aromatic content and short shelf-life due to quick softening and environmental conditions. On the contrary,

non-climacteric often slowly, are poor in aromatic compound and have a long shelf-life (Acs, 2020). Non climacteric melon is often consumed as dessert, and climacteric in culinary forms; it can be eaten raw, boiled, fried or used to make soup. Beyond its use as food, melon is also known for its therapeutic properties; in India, melon juice made from seeds is used to fight against indigestion (Swamy, 2017a). Ganji et al.(2019) analyzed phenolic content and antioxidative activities in peels of some commercial cultivars; high antioxidative activity was observed in peels of Hondura Galia variety. Cantaloupe melon seeds were also used to produce flour for bread and cake manufacturing, analyses of seed flour revealed the following nutritional values: 18% proteins, 3% moisture, 4% ash, 30% lipids, and 35% dietary fibre. Melon flour also has a significantly high content of minerals, mainly phosphorus (1507.62 mg/100 g), potassium (957.35mg/100 g), and magnesium (504.03 mg/100 g) (Rolim et al., 2020).

1.3.Melon Biology

1.3.1.Plant Morphology

Pozner et al.(2003) describes *Cucurbitaceae* as annual and perennial herb with relatively thin root and shoot. Perennial species are characterized by their tuberous roots and herbaceous shoots, which die and re-grow in annual cycle, also their root length can go over one meter. Plants in this family have spiral leaves, but only the first leaves following the cotyledon are opposite. Cotyledons are usually large, fleshy, ovate to elongate, green, and long-lived. Most *Cucurbitaceae* have tendrils, they are modified shoots which results from plant evolution. Tendrils that coil below the tendril branching point are found in the more basal clade and are referred as zanonioid. Flowers are usually born in few to many flowered racemes, thyres, panicles, and rarely in spikes or umbels. Upon the existing 960 species, 50% are monoecious, and inflorescence can be cosexual or unisexual, but most of the time flowers are unisexual, bisexual flowers are exceedingly rare. The calyx and corolla in *Cucurbitaceae*are pentamerous, sepal aestivation is valvate or open, exceptional overlapping. Meanwhile the corolla consists of ± connate or distinct petals highly variable in size, shape, and consistence, despite being uniform in color (usually white, yellow, or orange). According to Naudin, melon can be classified into 7 groups (Wehner et al., 2018; Swamy, 2017).

Table 3. Simplified Version of Naudin's Taxonomy of *Cucumis Melo.*

Variety	Nature	Fruit size	Color	Flower	Taste	Regions	Type
C. melo* var.*agrestis	wild	Very small (<5 cm)	skin green with dark green patches	Monoecious	Sweet	Africa, Asia	
C. melo* var. *cantalupensis	Domesticated	Large	Variable netted ring colour	Andromonoecious	Sweet		Includes dessert melon types
C. melo* var. *inodorus	Domesticated	Large	whiteflesh	Andromonoecious	Sweet	Asia and Spain	Includes sweet dessert melons (Honeydew and Casaba)
C. melo* var. *flexuosus	Domesticated	Veryelongated		Monoecious	Non sweet	Middle East and Asia	
C. melo* var. *conomon	Domesticated		White flesh	Andromonoecious	Sweet or bland		
C. melo* var. *chito*and *dudaim	Domesticated	Small(peach size	White flesh	Monoecious	Bland	China	
C. melo* var. *momordica	Domesticated		Lightpinkishflesh	Monoecious	Bland	India	

Figure1. Cucumis melo species

1.3.2.Muskmelon General Characteristics

Cucumis melo is an annual crop which in some varieties may exhibit a determinate or indeterminate growth habit; determinate growth habit is characterized by the formation of cluster flower and leaves at the ends of branches thereby terminating vine growth during the growing season, meanwhile varieties exhibiting indeterminate growth habit have vines that keep elongating during the growing season. Stems are usually hairy

andcan be yellow or green. Leaf shape are found in various forms; entire, trilobate, pentalobate, palmate and cordate. Leaf surface sometimes are sparsely hispid, hispid or lanate with tendrils which can be described as short, medium or long (International Plant Genetic Resources Instit., (IPGRI), 2003).

Muskmelon is a *monoecious* plant which produces two different types of flowers; unisexual and hermaphrodite (perfect) flowers, there is a large disproportion between the number of male and female flowers produced, more male flowers are produced than females. Fruits only originates from pistillate flowers which differentiate themselves from the bulb like structure below the level of sepals which develops into fruit after pollination (Pilgrim & Petersen, 2011).

1.3.3.Flowering

Flowering in muskmelon melon usually occurs forty days after planting, the ratio between staminate flowers (S flowers) and hermaphrodite flower (H flowers) ranges from 6 – 19:1. Anthesis occurs mainly early in the morning, but sometime difference between both flowers is at least 15 to 60 minutes. After anthesis, flowers remain open during the day and close overnight, however it was estimated that lifetime of flowers is maximum 12 hours. Pollen released during anthesis remains viable throughout the day for a maximum of 12 hours, but it was also reported that pollen viability could last only for few hours and few minute when temperature is high (Revanasidda & Belavadi, 2019).Flowering period in melon was estimated to be 8-10 weeks for male inflorescence and 6 weeks for female.During this time, flower opening occurs before dawn mostly between 03:00 and 04:00 with anthers opening before the male flower, because of the bright color of petals and their campanulate shape could be attracted thereby favoring pollination (Agbagwa et al., 2007). The process is facilitated by different insects species though they can only fly over short distances (<11m); most of those pollinators belong to the *Apidae* family (Hypotrigona and Trigona stingless bees as well as*A. mellifera*). *Cucumis melo* ssp.agrestis has a very high self-compatibility potential; the execution of self and cross pollination did not show any difference in reproductive parameters (Kouonon et al., 2009).

1.3.4.Fruits Characteristics

In turkey, cultivated muskmelon falls in the “cassaba” and “Ameri” group. The cassaba group comprises sub-groups such as Kirkagac, Hasanbey and Kuscular;their fruitsare generally more or less wrinkled, rib, vein tracks or netting patterns are absent, the skin is usually thick with light and green flesh, the horizontal cross section presents five white placenta, the mesocarp is thick, green and possess high sugar content, these fruits are also characterized by their late maturity and long shelf live. Cultivars found in the “Ameri” group have a medium to long shelf-life; they are either oval or cylindrical in shape. Fruits of this groupsometimes exhibit netting patterns; flesh color varies from white, light-green to light-orange, some fruits are crispy, however the sugar content in most varieties is high. Cultivars in this category are: Ananas, Maculati, Bargi and Mashhadi. Compared to cultivars in the cassaba group, they possess dehiscent peduncle with three independent white or orange lobes (Grumet& al., 2017)

1.4.Breeding Progresses

Major works to improve breeding in muskmelon have focused on the identification of gene responsible for sex expression, male sterility, QTL for fruit quality improvement, the development of transgenic plants and disease resistant cultivars.

1.4.1.Male and Female Sex Expression in *Cucurbitaceae*

It is assumed that in some *Cucurbitacea* plants as it is the case of cucumber, all floral buds are hermaphrodites; they possess both staminate and pistillate primordia, the development of one of those primordia in the detriment of the other leads to the development of either male or female flower. However, simultaneous development of primordial will result in the formation of bisexual flowers (Li et al., 2019). In *Cucumis sativum*, male flower development was described as followed: initiation of floral meristem in the axils of leaf primordial (stage), development of floral bud through initiation of sepals, petals, stamen and carpel primordia (stage 2-5), further development of stamen through enlargement of stamen primordia while carpel primordial remain unchanged (stage 6), development of floral bud and enlargement anther (stage 7), vascular bundle and filament differentiation (stage 8), microsporocyte initiation (stage 9), meiosis (stage 10), uninuclear pollen formation (stage 11), mature pollen formation (stage 12).

By contrast, in morphogenesis of female flowers, stage 6 is marked by the elongation and development of carpel primordia while stamen primordia remains unchanged, differentiation of female primordial into ovule (stage 7), elongation of primordial stigma (stage 8), formation of integuments (stage 9), meiosis (stage 10) embryo sacs formation (stage 11), breaking of nectary tissues (stage 12) (Peng & Gu, 2004).

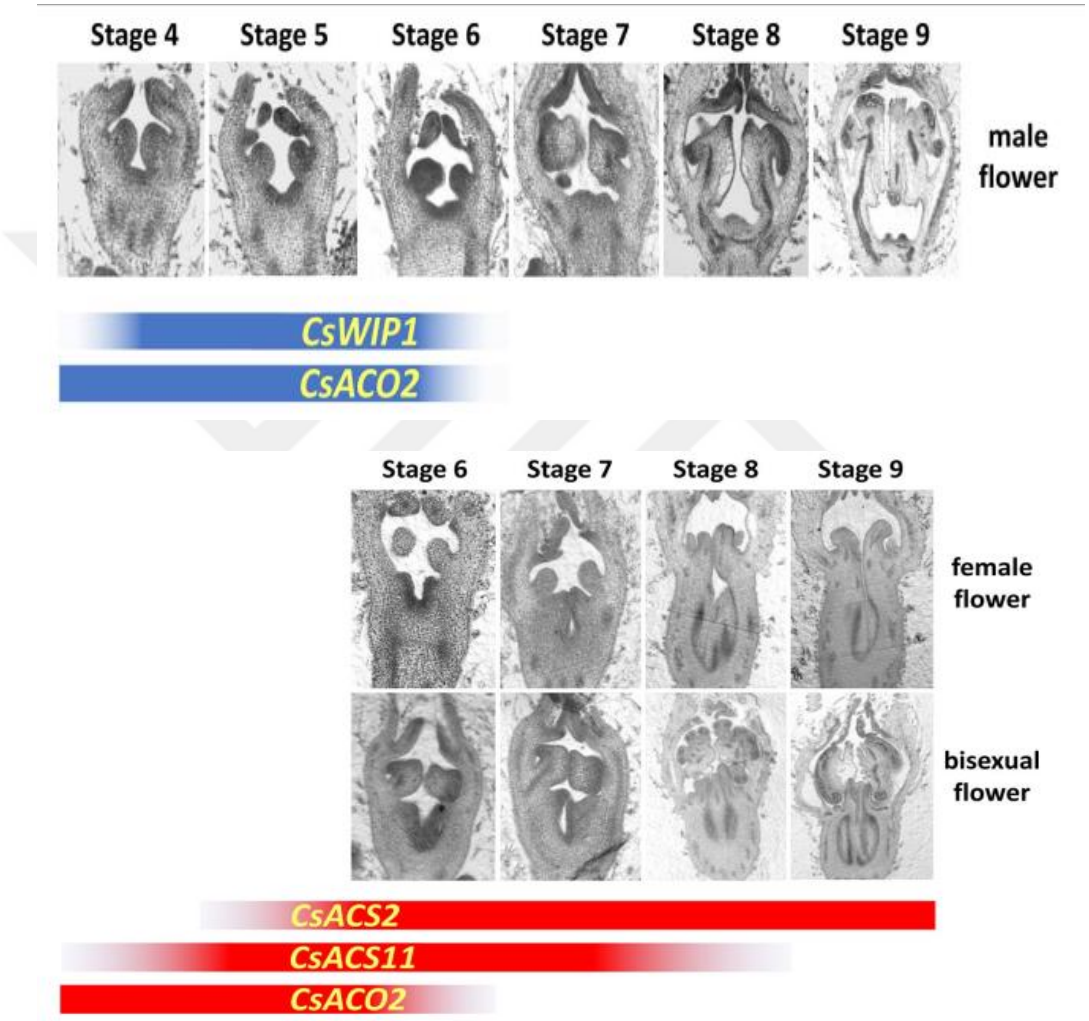

*Figure 2.*Flower Development in Cucumber (Li et al., 2019)

1.4.2.Gynoecious Line

The gynoecy controlling locus in cucurbit is expressed in two forms; G and g. G form is a dominant gene and is responsible for the development of female flowers in species such as *Cucumis sativum*, however the g is recessive, leads to the development of male flowers in other cucurbits such as melon and watermelon (Li et al., 2019). G gene is identified as CsACSI and g gene is known as CmWIP1, it is the gene responsible of carpel abortion in

melon. On the other hand, CmACS7 restricts male flower initiation, though it is required for carpel development, the gene also encodes a 1-aminocyclopropane-1- carboxylic acid synthase like CsACS1G, and the mutation severely loses the enzymatic activity (Boualem et al., 2008). A study which aimed to evaluate whether components of monoecy pathway between Cucumis and Citrullus genus concluded that active ClACS7 enzyme leads to the development of female flowers in monoecious lines, whereas a reduction of enzymatic activity yields hermaphrodite flowers. ClACS7, like CmACS-7/CsACS2 in melon and cucumber, is highly expressed in carpel primordia of buds determined to develop carpels and not in male flowers (Boualem et al., 2020).

Gynoecious lines can be inducedby the use of silver thiosulphate, Ethral and Alar to obtain perfect flowers, thecombination between AAgg genotypes and the recessive mm is the means through gynoecy can still be obtained. Some of the developed gynoecious line named Gylan was characterized by its oval shape, orange shape and its weight 0.9 - 1.1 kg. Also, crosses involving a gynoceous line resulted in the development a variety two times more productive than the local variety(Kesh & Kaushik, 2021). Ethylene when applied at early stage (10 leaves old) was able to turn bisexual flowers into female flowers at a proportion superior to 95% for lines such as GY7075 and GY7111 (Manzano et al., 2008). The use of ethylene increases the transcription of genes responsible of the development of female flowers *CsACS1*, *CsACS2*, *CsACS11*, *CmACS11*, and *CmACS7* and down regulates male genes *CsWIP1* and *CmWIP1*(Wang et al., 2018; Li et al., 2019).

1.4.3.QTL Breeding

Quantitative trait loci refer to a gene which is associated with a quantitative trait and expressed at phenotypic level, some quantitative traits are influenced by several genes which are influenced by environmental factors. The principle behind QTL analysis lies in the co-segregation of maker locus and QTL together (Chandra & Pandey, 2017). In fact, heritable phenotypic variation is the key of evolution and is responsible of the adaptation and diversification of organisms. Therefore, quantitative genetics which is the major factor behind phenotypic variations assumes that, complex phenotypes of a trait is caused by simultaneous segregation of a very large number of genes that interact among themselves, and possess additive abilities (Mauricio, 2001).

The use of quantitative trait loci has always been of significant important in plant breeding; genes responsible of traits expression in muskmelon have been identified using molecular markers such as SNP, AFLP, RFLP, and RAPD. Recombinant inbred lines and near-isogenic lines have been used separately to identify traits in melon related to texture, flesh color, extractable juice, sugar and carotenoid content, fruit and seed morphology, and color of immature fruits (Obando et al., 2008, Pereira et al., 2018).

1.4.4.Biotic Stress

Plant diseases mostly found in melon are: powdery mildew, fusarium wilt, downy mildew and viruses (Thakur et al., 2019). Resistance to powdery mildew(p.xanthii) has been reported from more than 30 sources for the 22 existing races, however only four resistant genes have been identified for the 22 available races(Mccreight, 2015).

Downy mildew (oomycete pseudoperonospora) is a foliar disease in melon which occurred between 1979 – 2000, fortunately in Israel, a variety PI 124111 was identified as resistant to 6 pathotypes of downy mildew (Cohen et al., 2003).

Two resistant genes to *fusarium oxysporum fom* 1, 2 (Baudracco-arnas, 2002) have already been identified. These genes respectively possess resistance to races 0, 2 and 0, 1 (Chikh-rouhou, 2013).Melon plant worldwide is affected by 59 well identified viruses (Lecoq and Desbiez, 2012).

Cucumber mosaic virus (CMV), *zucchini yellow mosaic virus* (ZYCMV), *papaya ringspot virus* (PRSV), *water melon mosaic virus* (WMV), *cucumber green mosaic mottled virus* (CGMMV) and *melon necrotic spot virus* (MNSV) are the common widespread viruses transmitted by aphids, and some of them have been identified in countries such as America, Brazil, Spain and South Korea(Park et al., 2007). Resistance to virus transmission by aphids was observed in C-105, meanwhile resistance to PRSV, WMV and ZYMV was identified in accession C-885 and C-769, also resistance to CMV was found in C-189 (Díaz et al., 2003).

1.5.Causes of Soil Salinity

Saline soils are soils which water soluble salt exceed 4ds/m (Shokat & Großkinsky, 2019). So far, two types of soil salinity have been identified; dry soil salinity and secondary soil

salinity. Dry soil salinity usually happens following the rise and subsequent evaporation of the water table. Rising of water table might be caused by a restricted drainage due to an impermeable layer, and the replacement of deep-rooted trees with shallow rooted crop. Consequently, water dissolved salts embedded in rocks as well as in soil reaching the soil surface evaporates to cause salinity. Secondary soil salinity is a consequence of Man's irrigation practices when periods of water scarcity, saline water is used. Shahid et al. (2018) enumerated general factors responsible of soil salinity, there are the following:

- Inherent soil salinity (weathering of rocks, parent material)
- Brackish and saline irrigation water
- Sea water intrusion into coastal lands as well as into the aquifer due to over extraction and overuse of fresh water
- Restricted drainage and a rising water-table
- Surface evaporation and plant transpiration
- Sea water sprays, condensed vapors which fall onto the soil as rainfall
- Wind borne salts yielding saline fields
- Overuse of fertilizers (chemical and farm manures)
- Use of soil amendments (lime and gypsum)
- Use of sewage sludge and/or treated sewage effluent
- Dumping of industrial brine onto the soil
- Physiological effects of soil salinity in plants

High salt content in the root zone often leads to ion toxicity in plants; this toxicity is due to the substitution of K+ and Ca+ by Na+ during different biochemical reactions (Hussain et al., 2013). Consequently, affected soils will exhibit high Na+/Ca and K+/Na ratio because of excess sodium in soils. However, low uptake of Ca+ and K+ by plants results in the improper functioning of cells, instability of cell membrane and hindrance of enzymatic activities (Quintero et al., 2007). Salt stress in plants occurs in two phases; the ionic and osmotic phase. Ionic stress is due to the high concentration of Na+ in shoots, which inhibits photosynthesis and induces premature leaf senescence of older leaves

(Seemann & Critchley, 1985). On the other hand, osmotic stress occurs following the accumulation of salt in the root zone, thereby decreasing the ability of plant roots to take up water. Plant in turn close their stomata to reduce water loss by transpiration,which leads to decrease in plant growth with the rate of leave expansion and new leave emergence is reduced (Al-shareef & Tester, 2019).

Plants suffering of ionic toxicity produce excessive reactive oxygen species (ROS) in cytosol, chloroplast and mitochondria (Munns & Tester, 2008). Reactive oxygen species include O^2, O^{2-}, H_2O_2 and OH^-. It is considered as stress indicator secondary messenger at the level ofcells, which occupies an important function in biological activities of plants ranging from gene expression and translocation to enzymatic activities (Foyer & Noctor, 2003). Production of ROS may eventually cause alteration of lipids, protein and nucleic acids, leading to an interruption of plant metabolism (Adly, 2010).

1.6.Salt Tolerance Mechanisms

Salt tolerant crops are those with the ability to grow on high salt content area without showing any sign related to salt stress such as loss in vigor, chlorosis or excess decrease in yield. Three type of salt tolerance mechanism have been developed by plants.

Osmotic tolerance is the capacity plants have to maintain their normal growth after they have been exposed to salt. Though little is known is known about this process, it is assumed that long distance signaling controlled by reactive oxygen species (ROS) waves or long distance signaling is the major causal factor, therefore the difference in osmotic tolerance could be due to a difference in the initial electric signal in the long distance signaling (Al-shareef & Tester, 2019).

Sodium exclusion from shoot is another mechanism used by plant to overcome salt tolerance. In factsodium accumulates when it is deposited in the transpiration stream which delivers it to the rather than in the roots. The transpiration stream delivers sodium to the leaf blade, because water that remains in leaves tissues after transpiration is very low and insufficient to move sodium that was deposited in the shoot to the root; most sodium delivered in the shoot will remain in the shoot(Sonon, 2015). Under salt stress, Na+ accumulation is determined by ion exchange activity controlled by Na+ influx and efflux. Na+ Influx involves channels such as high affinity K+ transporter HKT and non-

selective cation channels (NSCC), meanwhile Na efflux is mediated by SOS1, a Na+/H+ antiporter (Wu, 2018).

Sodium ion homeostasis is a process through which plant control and maintains sodium concentration within cells, it is the third mechanism used by plant to overcome salt stress. This process aims to prevent sodium accumulation in the cytoplasm through the inhibition of its influx across plasma membrane and favoring its efflux or sequestration into the vacuole as it is the case in halophytes. However, it should be noted that Na+ toxicity is not only caused by the accumulation of Na+ into the cytosol, but also by the impairment of K+ due to competition of Na+ to K+ binding sites(Hasegawa & Bressan, 2000). Preventing excess Na+ in plant cells allows helps in maintaining ion homeostasis, the sequestration of sodium into vacuole generally facilitated by Na+/H+ antiporters is an alternative (Conde et al., 2011). The distribution of Na^+ between cells to keep sodium concentration in the cytoplasm as low as possible is also an effective method plants use.

The accumulation of sodium ion into the vacuole requires some osmotic adjustments, which can be achieved by increasing cytosolic K+ to sub-toxic level and by synthesizing organic solutes (compatible solutes) that are compatible with metabolism, even at high concentration. These compatible solutes usually include; nitrogen containing solutes such as proline and glycinebetaine; sugars such as sucrose and raffinose; straight –chain polyhydric alcohols such as mannitol and sorbitol; and cyclic polyhydric alcohols (cyclic polyols) (Chen & Murata, 2002).

1.7.Developing Salt Tolerant Varieties: Challenges

1.7.1.Assessment of Tolerance

Salt tolerance is assessed from different farmer's fields, however this evaluation is quite difficult because of the non homogeneity of salinity in various field, which might be worsen by factors such as gaseous pollutants, sol fertility, drainage, temperature, large flux density and water loss due to transpiration. Variation in sensitivity at different growing cycles has been another delicate factor hindering the development of salt tolerant crops; for instance rice has been found more sensitive to salinity during grain development stage than vegetative stage, meanwhile in tomato tolerance at germination was not correlated with resistance to salt stress (Flowers, 2004).

1.7.2.Limited Genetic Sources

There are very few plant species which possess genes for salinity resistance. Like most quantitative traits, it is controlled by poly-genes which depend on gene*environment interaction for their expression. Moreover, these traits are the most difficult to breed using conventional as well as non conventional methods such as marker-assisted selection. A plant is considered salt tolerant when it is capable to germinate under high salinity (50% - 75% the salinity of sea water) and grow to maturity as it is the case of "Kharchia" wheat from India. Salt tolerant genes are mostly found in wild relatives of crops, the introgression of those genes into cultivated species has allowed the development of resistant cultivars in crops as it is the case of the wild tomato species *Lycopersiconcheesmanii*(Schlegel, 2018).

Yamaguchi & Blumwald (2005) reported sources of salt tolerant genes that were identified in some plant species, as it was mentioned very few species have salt resistance abilities. Thanks to biotechnology these genes could be transfer to other crop, they are very useful because they allow vacuolar sequestration of Na+, Na+ extrusion and vacuolar acidification.

Table 4. Salt tolerance in transgenic plants expressing genes involved in ion transporters

Gene	Gene product	Source	Cellular role	Target plant	Parameter studied
AtNHX1	Vacuolar Na+/H+antiporter	Arabidopsis	Na+vacuolar sequestration	Arabidopsis	Biomass
AtNHX1	Vacuolar Na+/H+antiporter	Arabidopsis	Na+vacuolar sequestration	Tomato	Biomass, fruit yield
AtNHX1	Vacuolar Na+/H+antiporter	Arabidopsis	Na+vacuolar sequestration	Brassica napus	Biomass, oil production
AtNHX1	Vacuolar Na+/H+antiporter	Arabidopsis	Na+vacuolar sequestration	Maize	Biomass
AtNHX1	Vacuolar Na+/H+antiporter	Arabidopsis	Na+vacuolar sequestration	Wheat	Biomass, grain yield
GhNHX1	Vacuolar Na+/H+antiporter	Gossypium hirsute	Na+vacuolar sequestration	Tobacco	Biomass
AgNHX1	Vacuolar Na+/H+antiporter	Atriplexgmelini	Na+vacuolar sequestration	Rice	Biomass
OsNHX1	Vacuolar Na+/H+antiporter	Oryza sativa	Na+vacuolar sequestration	Rice	Growth, ion content
BnNHX1	Vacuolar Na+/H+antiporter	Brassica napus	Na+vacuolar sequestration	Tobacco	Growth, seed yield
HbNHX1	Vacuolar Na+/H+antiporter	Hordeum brevisubcula tum	Na+vacuolar sequestration	Tobacco	Biomass
AtSOS1	Plasma membrane Na+/H+ antiporter	Arabidopsis	Na+ extrusion	Arabidopsis	Biomass
SOD2	Plasma membrane Na+/H+ antiporter	Schizosaccharo myces pombe	Na+ extrusion	Arabidopsis	Biomass, photosyn thesis
nhaA	Plasma membrane Na+/H+ antiporter	Escherichia coli	Na+ extrusion	Rice	Biomass, ion content
AVP1	Vacuolar H+-pyro phosphatase	Arabidopsis	Vacuolar acidification	Arabidopsis	Biomass

1.8.Salt Tolerance in Melon (*Cucumis Melo*)

Studies on melon's tolerance to salinity revealed that fruit number is greatly reduces under saline conditions; however this reduction in fruit number did not have any effect on fruit mean weight. F. A. d. L. Pereira et al., (2017) in this experiment where they used irrigation water with different salt concentrations also reported that there was a significant difference in interaction between salt concentrations and tested cultivars.

Akrami & Arzani (2019) studied 55 melon hybrids and 11 parents to evaluate both qualitative and quantitative trait heritability under saline and non saline conditions. He

discovered that both general combining ability (GCA) and specific combining ability (SCA) responsible of additive and dominant effect of genes on fruit yield components was affected by salinity stress. GCA/SCA ratio as well broad and narrow sense heritability of fruit yield parameters of plants grown in saline conditions lower compared to that of plants grown in non saline conditions.

Proline and potassium nitrate proved to be good remedies to solve salinity related problem hindering plant growth. The addition of those chemicals in plant medium regulates osmosis within cells by favoring the uptake of elements such as Ca, N and K^+. Moreover it was observed the addition of proline in the medium increased its content in plant leaves, and 150 mMol NaCl treatment contributed in the increase of electrolyte leakage and decrease of leaf relative water content (Kaya et al., 2007).

In turkey, Kusvuran (2019) studied 36 genotypes that were treated for three consecutive days with 50 mMolNacl, genotypes were 45 days old. At the end of the experiment she concluded that among the studied genotypes, Midyat, Besni and Semame showed more resistance to salt stress compared to Ananas and Yuva which were more sensitive. The experiment revealed a high negative correlation between fresh weight and salt stress damages on leaves(color change necrosis, leaf folding and drying), alsoleaf fresh weight of treated plants was lesser than their control.

In another experiment, it was found that grafting scions of *Cucumis melo* on *cucurbita* root stock did not prevent the negative effect of salt stress. Two melon cultivars; Citirex and Altinbas were grafted on two curcubita (Kardosa and Nun9075) salt tolerant root stocks. In hydroponic condition, 8 ds/m concentrated solution had a negative effect on both grafted plants; shoot and root growth, leaf area, photosynthetic activity, leaf chlorophyll and carotenoid content were significantly reduced (Ulas et al., 2020). However, the use of salt resistant rootstock such as “Shintosa”, “Star” and “Cobalt” provided very satisfactory growth when grown under different salt conditions (150mM, 200mM) compared to non-grafted plants (El-Kafafi et al., 2017). Grafting watermelon on suitable resistant *Curcubita* plants (squash) resulted in high salinity tolerance; neither biomass, neither leaf area norphotosynthesis was affected by salt stress; leaf area and fresh weight of some grafted plants were even greater than that of control plants (Bohm et al., 2017). A reduction in Na and Cl uptake, and the increase of catalase, superoxide

dismutase, ascorbate peroxidase activities was observed when susceptible melon scions were grafted on resistant rootstocks (Kuşvuran et al., 2021).

The effect of salinity on seed emergence revealed that exchangeable sodium percentage above 20% led to poor emergence of melon seeds, around 35% emergence was recorded. Other parameters such as shoot and leaf length, and shoot dry matter of shoots were affected by salinity (Pinheiro et al., 2019).

Salt stress resistant and sensitive melon cultivars were studied in hydroponic conditions to assess ion regulation in different organs. Yield in term of fresh and dry weight in sensitive genotype decreased up to 72%, meanwhile in resistant cultivar maximum loss was 33%. Analysis of ion concentration revealed that there was more potassium accumulation in young leaves than any other organ, meanwhile Na+ was low, but tend to be more concentrated in old leaves (Kuşvuran, 2012).

Response of plants to salt stress in melon varies according to genotypes; in cultivars like "Ananas" increase in salt application resulted in 55% decrease in chlorophyll content, while other tested genotype saw an increase. Similarly, increase in salt content led to an increase of proline, phenolic and flavonoid contents in "Ananas" and a variation in other genotypes (Erdinc et al., 2021).

Though salt stress is detrimental to melon yield, it was found very useful in improving fruit quality. Salinity improved melon nutritional values through an increase of total soluble solids and Na+ concentration in plants (Zong et al., 2011).

1.9.Salt Stress Evaluation in Plants

Bado et al.(2016)numerated parameters that need to be taken into consideration during evaluation for salt stress resistance, they are listed as follow:

Germination: this parameter is quite important in conditions where plants are required to germinate and establish properly in saline condition. However, this do not always mean that the plant is resistant to salt stress.

Plant survival: in case during evaluation a plant was capable of completing its life cycle under moderate or very high salinity levels, it is considered as being tolerant irrespective of its yield.

Leaf damage: it is shown through symptoms such as bleaching and necrosis. Usually plants are unable to restrict toxic salt absorbed by roots and which reaches shoots, they are said to be glycophytes.

Biomass and yield: tolerant plants are characterized by their suitable yield and biomass, related traits used to receive more attention by scientists.

Physiological mechanisms: some physiological mechanisms confer salt tolerant abilities to plants, they must be considered during screening.

Table5.Salt Tolerance Parameters

Crop	Growth stage	Parameters	References
Tomato	Fruit stage	Yield and biomass - Plant height - Fruit yield Physiological and biological factors - SPAD (index) - RWC (%) - TSS - Proline - CAT - APX - SOD - POX - Na^+ and K^+	(Sanwal et al., 2022)
Rice	Seedling stage (30 days)	Survival days Yield and biomass - Shoot height - Root length - Plant and root dry weight	(Th et al., 2019)
Maize	Vegetative, reproductive and grain stage	Physiological and biological factors - RWC - Membrane stability index - Stomatal conductance - Internal CO2 concentration - Chlorophyll level (a and b) - Na+ and K+ - SOD, CAT, POD, PROLINE - Photosynthetic and transpiration rate, - Yield and biomass - Shoot fresh and dry weight	(Abbasi et al., 2015)
Sunflower	20 days	Water uptake Physiological and biological factors - Na^+ and K^+ - Apoplastic water uptake - Rhodamin content Yield and biomass (shoot and root fresh weight)	(Quintero et al., 2007)

CHAPTER II

MATERIAL AND METHODS

This research was carried out in the green house and tissue culture laboratory located at Erciyes University. The experimental design was a 4*5 factorial design with each treatment repeated three times. In this experiment sodium chloride(NaCI) doses 0, 100, 150, 200 mMol NaCl were applied on three different hybrid combinations of melon (Semame x Ananas, Ananas x Semame and Midyat x Ananas) and two local varieties (Ozbek and Semame).

2.1.Seed Planting and Plant Cultivation

Melon seeds were sowedin 20 holes seedling trays containing a mixture of 60% peat moss and 40% perlite, in each hole to seeds were sowed. Watering was done every two days to make sure that germination occurs properly. A month later, when seedlings had reached 3-4 leaves- stage, they were transferred into small size nursery containers filled with a mixture of perlite, peat moss and soil. The medium was supplemented with potassium nitrate (KNO_3) MAP and microelements as plants were growing, the greenhouse temperature varied between 25-30C^0.

*Figure 3.*Greenhouse Soil Components

2.2.Salt Application

The application of NaCl started immediately after plants had been transplanted to nursery pots. For the first week plants were stimulated with a continuous low application of NaCl (50mMol) until the final concentration of each treatment was attained; for 50, 100, 150 and 200mMol dose, 50mMol NaCl was applied daily for one, two, three and four days respectively. From the following week, plants were subjected to direct NaCl treatment 100, 150 and 200mMol, which was done once every week for four weeks. Data were collected after flowering had begun.

Figure 4. Early stage salt application

Figure 5. Late stage salt application

2.3.In Vitro Culture

Seed of individual genotypes were first sterilized and germinated in petri dishes containing hormone free medium (1.2g/L MS + 7g sucrose) , then they were transferred to big jars still containing the same medium for proper development. Sterilization was performed in a laminar flow hood; the seeds firstly were treated with 70% ethanol for 1 minute, 10% hypo-chloride for 4 minutes, and then rinsed with pure water three times. Later, seeds were dried on filter paper before they were put into Petri-dishes. Few days later, germinated seeds corresponding to each genotype were transferred into jars and kept until they developed true leaves; the jars carried 2-3 germinated seeds. Two weeks later, true leaves started appearing and data collection began.

Figure 6. In Vitro Plants

2.4. PhysiologicalFeatures

2.4.1.Membrane Damage Index (MDI)

A disc-shaped section of freshly harvested leaves put into small glass containers half filled with pure water. Five hours later, electrical conductivity of each sample was measured and the tubes were autoclaved at 121ºC, for 30 minutes. The glass containers were allowed to cool down before a second reading was taken using EC meter. The value of electrical conductivity was obtained with EC meter. Membrane damage index (MDI) index was then calculated using the following formula:

$$MDI = (Lt\text{-}Lc/1\text{-}Lc) \times 100$$

Lt: EC reading before autoclaving/EC reading after autoclaving.

Lc: EC reading of control samples before autoclaving/ EC reading of control samples after autoclaving(Deveci et al., 2017).

2.4.2.Relative Water Content Index (RWCI)

The weight of freshly harvested leaves was recorded, and each leaf sample was put into a small glass container also half filled with water, sample leaves were kept in the container for four hours before a second reading could be recorded. The dry matter content was

obtained by drying a leaf of each plant treatment in an oven at 35^{o}C for seven days. The relative water content index was calculated using this equation:

$$RWCI = [YA-KA)/ (TA-KA] \times 100.$$

YA: fresh weight

KA: fresh weight after four hours in water

TA: dry matter content

Plant vigor:

Plant vigor was evaluated on a scale of 0-4 during harvesting.

0= leaves are completely green and have no dry or colored spots.

1= leaves are slightly yellow and possess few dry spots.

2= 20% of plant leaves is affected by dry spots and exhibit yellowish color.

3= 40% of the plant leaves dried up.

4= The plant is dead or more than 50% of the leaves turned yellow or became dry.

Figure 7: Ozbek at Harvesting

Figure 8. Midjat x Ananas at Havesting

Figure 9. Semame at Harvesting

Figure 10. Ananas x Semame at Harvesting

Figure 11: In -vitro root (sem x ana)

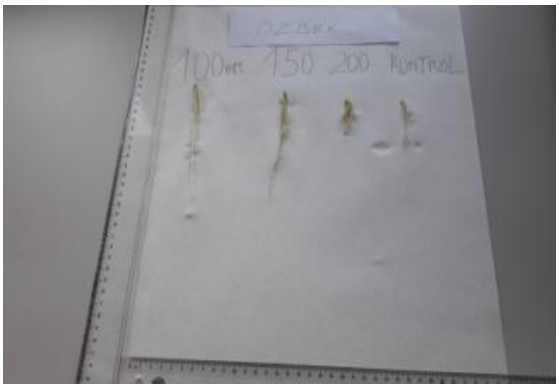

Figure 12: In-vitro root (ozbek)

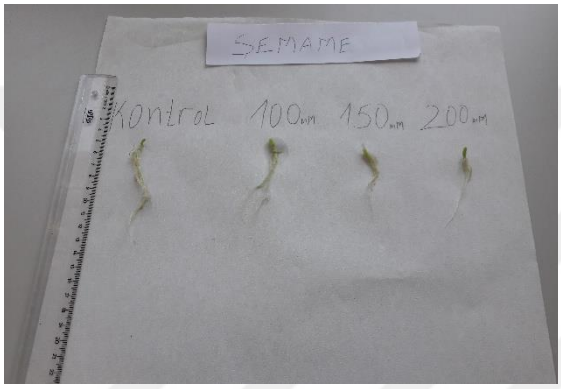

Figure 13. In-vitro Root (Semame)

Figure 14. In-vitro root (midjat x ananas)

Figure 15. Greenhouse Root (Ozbek)

Figure 16. Greenhouse Roots (semame x ana)

Figure 17. Greenhouse Root (Semame)

Figure 18. In-vitro Root (Midjat x Ananas)

2.4.3.Data Analysis

Data that were collected during this experiment were analyzed using online SAS and R software. Both software SAS and R were used to perform different analysis, SAS was used to run ANOVA and LSD test to evaluate the effect of salt concentrations on plant growth. With R software linear regression was ran to determine the relationship between growth and physiological variables.

CHAPTER III

RESULTS AND DISCUSSION

3.1.Result

3.1.1.Effect of Salinity on Plants Grown in Greenhouse

Not all morphological parameters were significantly different for each genotype; while at low NaCl concentration some genotypes exhibited high performance for one or more parameters, others performed well at high concentration.

There was a significant difference in all parameters ($p<0.05$) except in leaf length and leaf number for Özbekvariety. The absence of salt and low dose application (100mMol NaCl) provided the best results in all parameters.

For Şemamex Ananascombination, significant difference ($p<0.05$) was observed in stem diameter, dry matter content in stem and root, leaf diameter, number of leaves and plant vigor. Except for stem diameter which highest performance was recorded at 100mMol sodium chloride, the best performance was revealed in control plants.

There was a significant difference in plant diameter rand leaf number of Ananas x Şemame combination, control plants had the largest diameters and more leaves, meanwhile at 200mMol they were the smallest. Other parameters did not show any significant difference.

Midyat x Ananas combination recorded a significance difference ($p<0.05$) in stem diameter, dry matter content in stem, root length, leaf number, length and plant vigor. The best and poorest performance of stem diameter was respectively observed at 150 and 200 mMol salt concentration. The longest and shortest leaf length occurred when 200 mMol

and 150 mMol sodium respectively were applied. Control plants for other parameters had the highest performance.

The result obtained shows that there was a significant difference in response of semame to treatments for all parameters. The largest stem diameter was obtained when 100mMol sodium was applied meanwhile the smallest was obtained with 150 mMol sodium application. Control plants performance was the best for all other parameters while 200mMol application dose led to poor results.

For all cultivars, plant height and stem diameter arethe two parameterswhich were significantly affected by salt stress. All cultivars developed vigorous stems at low salt concentration. However, the best plant height and most vigorous stem diameter of Midyat x Ananas was obtained at 150mMol.

Table6: Morphological Characteristics of Plants Grown in Greenhouse Condition

Variety	Salt concentration	Plant height (cm)	Stem diameter (mm)	Stem DMC (grs)	Root DMC (grs)	Root lenght (mm)	Leaflenght (mm)	Leafdiameter (mm)	Leafnumber	Plant vigor	Leaf area
Özbek	0	40.5± 0.41a	5.65±0.12a	1.87±0.31a	0.22±0.05ab	56.60±9.79ab	74.85±1.6a	85.55±3.18a	14.5±0.51a	0±0c	62.12±0.05a
	100	40±5.4a	4.5±0.41b	1.10±0.24b	0.23±0.05a	71.20±14.47a	79.43±21.88a	72.46±21.08a	14.25±3.77a	1.75±0.5b	57.56±4.61ab
	150	37±0.82ab	4.85±0.2b	1.02±0.27b	0.15±0.01bc	66.70±10.28a	63.85±10.90ab	82.65±4.44a	11±0.82a	3.5±0.4a	52.77±0.49ab
	200	26.5±12.66b	3.7±0.24c	0.58±0.21c	0.14±0.03c	44.40±16.08b	51.50±11.34b	70.25±23.80a	12±2.45a	1.5±0.4b	36.18±2.70b
Şem x ana	0	64.67±10.78a	3.36±0.24ab	1.6±0.31a	0.25±0.06a	150.60±18.15a	80.03±13.23ab	84.77±12.58a	18.33±3.3a	0±0b	67.84±1.66ab
	100	49±13.39ab	4±0.89a	1.48±0.17a	0.15±0.01b	121.35±62.55a	94.38±12.43a	90.60±9.87a	17.5±3.87a	1±0.81a	85.50±1.23a
	150	51.75±2.75ab	2.28±0.74bc	0.85±0.16b	0.15±0.03bc	135.40±62.55a	74.65±20.27ab	82.03±14.70a	12.75±1.89b	1±0a	61.23±2.98ab
	200	46.25±8.06b	1.9±0.5c	0.63±0.35b	0.08±0.02c	100.48±43a	67.09±14.44b	59.96±8.83b	6.25±1.5c	1±0a	40.23±1.28b
Ana x şem	0	77.75±14.2a	3.15±0.41a	1.56±0.09a	0.082±0.05a	77.92±14.50a	71.35±27.54a	80.45±34.78a	21.5±7.19a	2±0.81a	59.41±11.20a
	100	72.5±22.65ab	2.28±0.19ab	1.03±0.3b	0.07±0.02a	56.47±23.59a	69.67±9.44a	79.42±72a	15.25±2.22ab	1.75±0.95a	55.34±0.92a
	150	68±8.64ab	2.43±0.78ab	0.92±0.04b	0.07±0.01a	63.96±15.68a	70.30±1.55a	80.80±10.78a	16.33±0.47ab	2±0.81a	56.80±0.17a
	200	50.5±12.26b	2.13±0.83b	0.77±0.22b	0.07±0.02a	51.40±22.19a	76.55±19.36a	88.92±12.45a	12.25±6.75b	1.5±0.57a	68.07±2.41a
Mid x ana	0	54±16.31ab	5.47±0.58b	2.03±0.62ab	0.21±0.12a	62.53±11.62b	68.30±14.07ab	92.70±24.97a	18.33±2.62a	0±0d	63.31±3.52a
	100	59.5±1.22a	5.4±0.24b	2.48±0.20a	0.21±0.01a	99.90±1.55a	78.25±9.44a	95.20±24.57a	15±1.63b	0.5±0.4c	74.49±3.96a
	150	52.5±0.41ab	7.95±1.1a	1.80±0.26b	0.14±0.01a	32.90±0.08d	56.10±1.55b	85.80±9.22a	14±1.63b	1±0b	48.13±0.17a
	200	41±2.45b	4.15±0.12c	1.13±0.19c	0.12±0a	50.10±1.3c	79.50±19.36a	87.85±15.79a	10±1.63c	2±0a	69.89±3.04a
Semame	0	50.33±3.86a	3.57±1.45ab	1.52±0.1a	0.20±0.05a	75.53±12.66a	97.30±15.69a	89.20±9.98a	21.67±4.99a	0±0c	86.79±1.57a
	100	40.5±7.9b	4.75±1.53a	1.23±0.48ab	0.21±0.09a	89.12±30.46a	89.10±21.62a	84.65±21.91a	19.25±3.5ab	1.25±0.5b	75.42±4.74a
	150	30.75±4.57c	2.65±1.07c	0.99±52b	0.14±0.01a	80.50±15.16a	75.40±21.30a	77.65±20.29a	16.5±4.51ab	2.5±1.29a	58.55±4.32a
	200	23±2.45c	2.9±0.41ab	0.35±0c	0b	0b	0b	0b	13±0b	0±0c	0±0b

*Data with the same letters are not significantly different

Membrane damage indexof all genotypes was significantly different; it increased with sodium concentration, at 100 and 200mMol, the lowest and highest MDI were respectively recorded. Relative water content was highly influence in most genotype except in Ozbek and Midyat x Ananas, it highly varieswith regard to salt concentration.

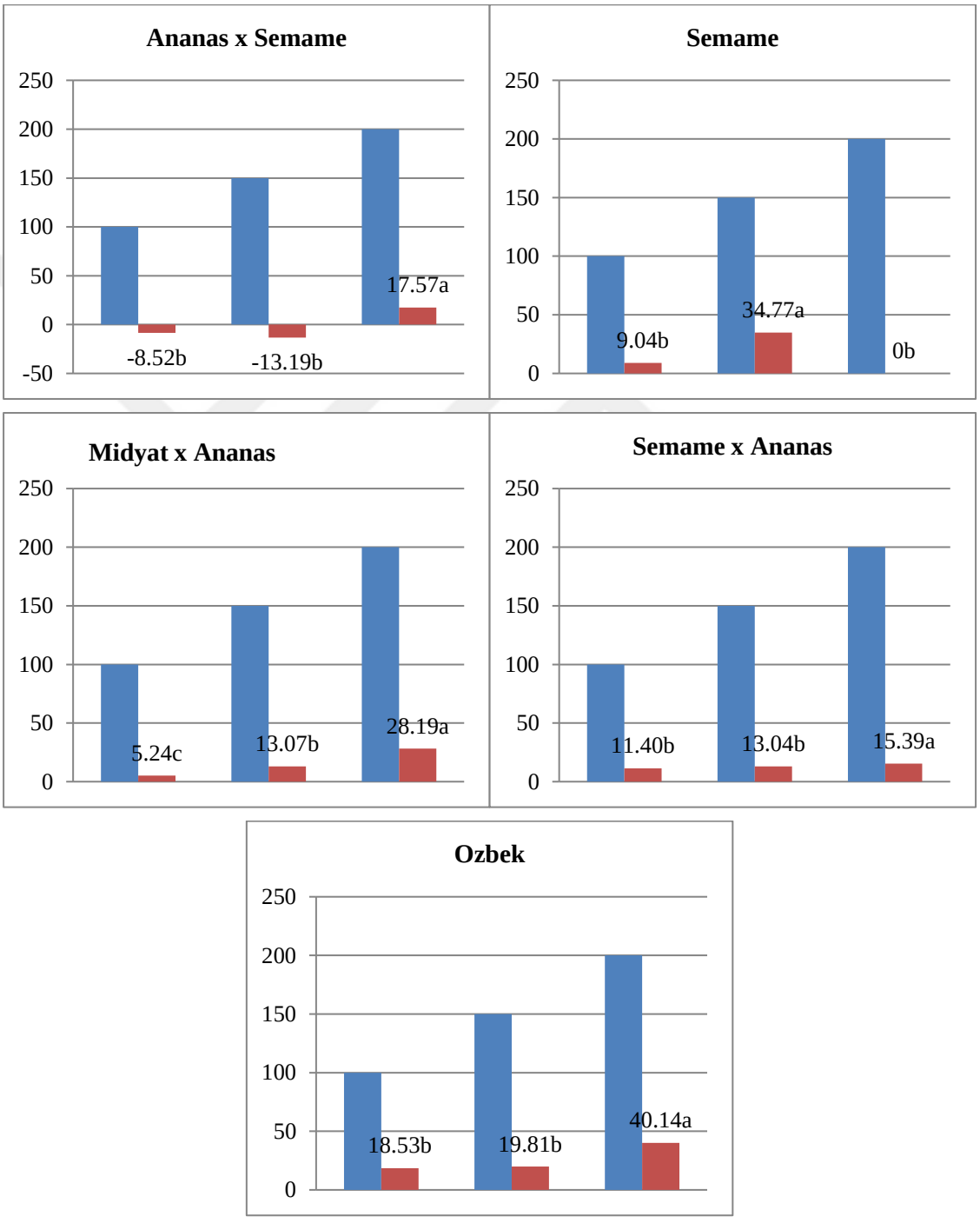

Figure 19. MDI index (µs/cm)

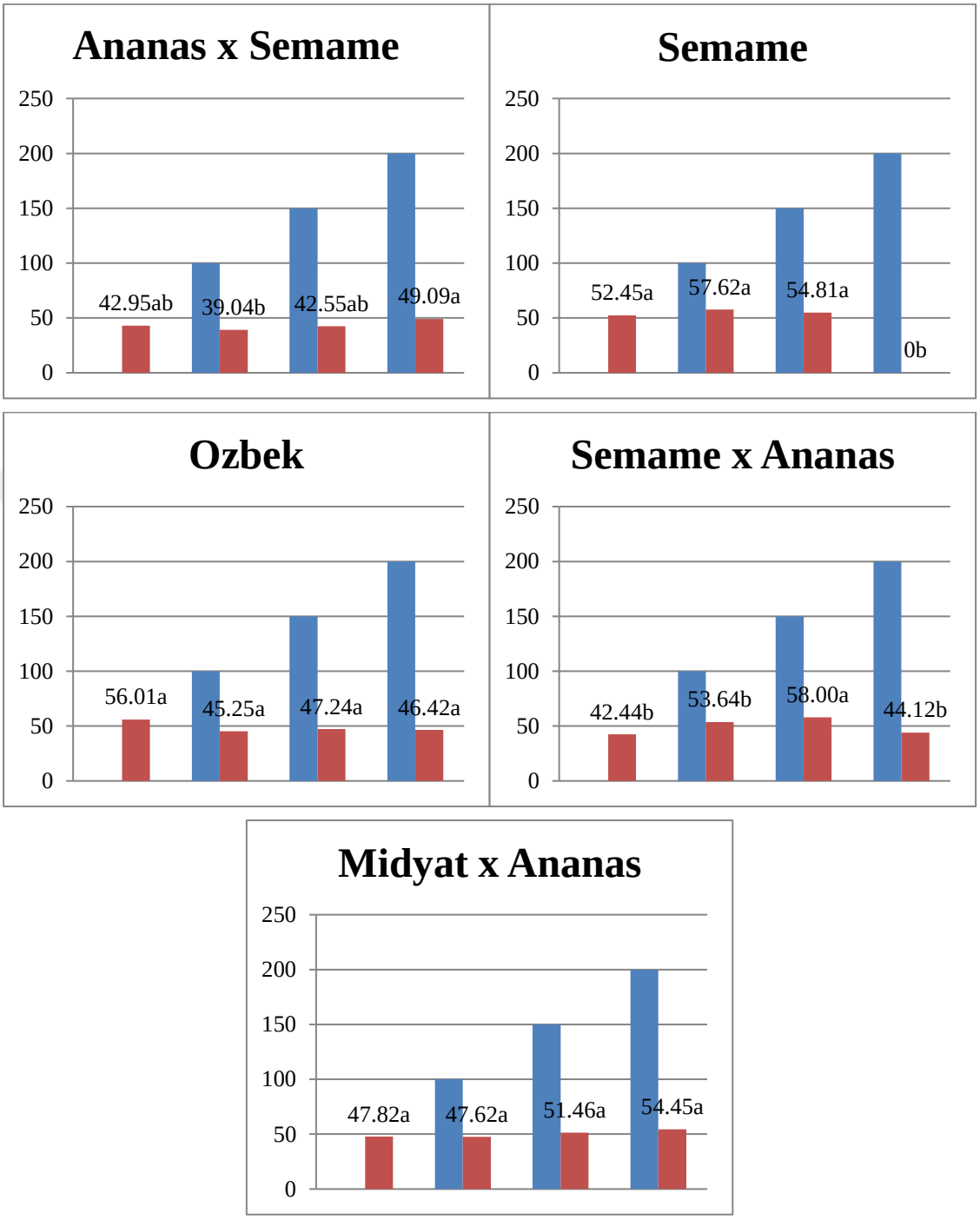

Figure 20. RWCI (%)

3.1.2.Effect of Salt Stress on in-vitro plants

In in-vitro conditions, only leaf diameter of Ozbek cultivar was positively affected by salinity; leaf diameter increased with salt stress. In Şemame x Ananascultivar, significant effect of salt was observed in plant height and leaf length, for both parameters plants response to stress varied.

The genotype Ananas x Şemame showed a significant difference only forplant heightof control plants, 15.40 cm was higher compared to the one obtained when 200mMolsodium was applied.

The combination Midyat x Ananas revealed a significant difference ($p < 0.05$) in almost all parameters, plant height 27.5 cm at 150mMol, stem diameter 2.2 mm at 200mMol, dry matter in stem 0.2gr at 100mMol and leaf length 30.5mm at 200mMol were identified as the best performances.

Salt stress significantly affected dry matter content (stem and root) in Şemame cultivar. Plants performed better in absence of salt or at low concentration (100mMol).

There was no significant effect of salt stress on MDIfor all cultivars except Midyat x Ananas. For this cultivar, the highest MDI was obtained at 100mMol. Midyat x Ananas and Şemame were the only cultivar which relative water content index was significantly affected by saline condition. Both had their highest value when 100Mmol NaCl was applied.

Table 7.Response of Plants to Sodium (in-vitro)

Genotype	Salt concentration	Plant height (cm)	Plant diameter (mm)	Stem DMC (gr)	Root DMC (gr)	Root lenght (mm)	Leaflenght (mm)	Leafdiameter (mm)	MDI index	RWC index
Ozbek	0	11.16a	2.66a	0.01a	0.04a	71.06a	34.60a	19.00ab		72.33a
	100	12.83a	3.13a	0.05a	0.01a	104.83a	31.93a	16.03b	5.77a	57.46a
	150	5.33a	2.63a	0.00a	0.02a	129.86a	29.73a	19.13ab	15.98a	59.06a
	200	8.50a	2.43a	0.00a	0.01a	44.93a	32.40a	21.56a	28.54a	72.33a
Şemame x Ananas	0	10.50ab	2.20a	0.01a	-0.00a	93.06a	24.73ab	12.93a		74.24a
	100	13.86a	2.03a	0.03a	0.00a	90.30a	28.86a	18.36a	1.470a	73.27a
	150	5.40ab	2.83a	0.01a	0.00a	106.10a	25.46ab	19.16a	-0.92a	68.67a
	200	3.70a	2.50a	0.00a	0.04a	58.13a	21.60b	16.36a	1.10a	64.04a
Ananas x Şemame	0	15.40a	1.76a	0.01a	0.00a	84.86a	29.30a	11.93a		58.57a
	100	12.80a	2.56a	0.00a	0.00a	84.16a	26.33a	12.20a	58.55a	51.90a
	150	7.36b	2.73a	-0.00a	0.00a	186.43a	26.86a	16.30a	13.96a	46.60a
	200	4.46b	2.50a	-0.00a	0.00a	118.60a	25.90a	12.90a	-2.68a	54.41a
Midyat x Ananas	0	13.60c	1.60d	0.01b	0.01a	68.00a	18.50c	15.90a		95.95b
	100	18.44b	2.15b	0.02a	0.00a	71.15a	26.25b	18.20a	50.00a	120.29a
	150	27.50a	2.10c	0.01b	0.00b	75.10a	18.80c	15.20a	10.87ab	5.60b
	200	3.70d	2.20a	-0.00c	0.00b	46.00b	30.50a	16.90a	-1.42b	84.36c
Şemame	0	10.50a	2.20a	0.01b	0.01a	57.83a	25.13a	20.50a		84.08ab
	100	13.86a	2.03a	0.05a	0.00b	76.53a	27.56a	22.30a	4.43a	112.07a
	150	5.40a	2.83a	0.00b	0.00b	124.55a	26.46a	23.56a	-22.26a	70.38b
	200	3.70a	2.50a	0.00b	0.00b	71.46a	24.46a	15.46a	-12.64a	63.59b

3.2.Discussion

3.2.1.Plant Growth in Green House Condition

Salt stress had a negative effect on greenhouse grown crops, most plant performed poorly under high salt concentration (150 – 200mMol). Each cultivar except Ananas x Şemame showed significant change in at least five growth parameters. Negative effects of salt stress were observed through leaves yellowing and burning, wilting, stunted growth and plants death. Excess salt in plants also resulted in the restriction of cell division, elongation and stomata closure (Flowers, 2004). This observation corroborates with what was described by Machado and Serralheiro (2017), salt stress in plants usually manifests through wilting, yellowing of leaves and stunted growth.In some cultivars as it is the case of Midyat x Ananas high salt concentration (150mMol) stimulated a positive response like increase in stem diameter and leaf length (200mMol). After evaluation of several local cultivars among which Midyat and Şemame, it was found that these cultivars could tolerate salinity (Kusvuran, 2019), the high performance of Midyat x Ananasis therefore justified. At minimum salt concentration (100mMol), both Şemame and Midyat x Ananas had the most extended root system. This observation is contradictory, at low to moderate concentration roots are also reduced in length and mass but may become thinner (Devi & Arumugam, 2019). Though genetic inheritance from resistant parent could explain this reaction, another reason could also be that low concentration has the potential to activate some mechanisms in plants that leads to root extension.

3.2.2.Membrane Damage Index and Relative Water Content

Membrane damage indexsignificantly increased with salt concentration, it translates salt accumulation in plant tissues, and such accumulation restricts plant growth by hindering chloroplasts functioning and photosynthesis. The presence of salt within plant cells might have affected plants ability to absorb water, thereby reducing relative water content index.Apart from Ozbek and Midyat x Ananas, significant impact of salt stress on relative water content was observed in other cultivars. Relative water content was high at high concentration in all three cultivars Şemame, Şemame x Ananas and Ananas x Şemame. This could be because of the nature of Şemame which is salt resistant. In resistant varieties, relative water content tents to be high at high salt concentration (Abbasi et al., 2015).In a study on coleus plant carried out by Kotagiri and Kolluru (2017), it was

reported that increase in salt concentration led to an increase in electrolyte leakage and decrease of relative water content, salt accumulation in cells caused the dead of primary leaves and the disruption of carbohydrate transportation in plants.

3.2.3.In vitro Screening: Growth And Physiological Parameters

in-vitro environment,increase in salt concentration did not greatly affect plants. Each cultivar encountered significant change most two growth parameters; Leaf diameter for Ozbek (200mMol), plant height and leaf length for Şemame x Ananas (150mMol), plant height for Ananas x Şemame (control) , and dry matter in stem (100mMol) and root (control) for Şemame.Midyatx Ananas seemed to develop better in high salt concentration; plant height, stem diameter and leaf length gave better results than the control. Compared to greenhouse condition, in vitro grown plants appear to be more tolerant to salt stress. Moreover, there was neither significant increase ofMembrane damage nor decrease in relative water content in all genotypes. There is available data that could explain in the resistance of melon plan in in-vitro condition using tissue culture technique. In another controlled environment such as hydroponic crop yield was significantly affected , salt stress led to 33% yield loss in genotypes considered as resistant and 77% in those that were sensitive (Kuşvuran, 2012). In this experiment, in vitro plant were 2-3 weeks old, this stage of development may explain their tolerance level, since plants express salt tolerance at different growing cycle. Variation in sensitivity at different growing cycles remains an obstacle in the development of salt tolerant crops, some crops may exhibit more sensitivity at grain stage than in vegetative stage (Flowers, 2004).

3.2.4.CorrelationAnalysis

The relationship existing among variables was established and it was found that relative water content is significantly correlated to Membrane damage index, correlation coefficient was (r^2 = 0.08). Also, plant height showed a positive significant correlation with ECI (r^2 = 0.19). However, stem and root dry matter was positive and significantly correlated to relative water content, for each variable,r^2 was equal to 0.101 and 0.1127 respectively. Significant correlation was also found for leaf area with r^2= 0.41 with $P<0.001$. Positive and significant correlation was found between Plant vigor and relative water content, correlation coefficient was (r^2 = 0.59, $p<0.05$) meanwhile, r^2 = 0.097 correlation coefficient was recorded for root length and RWCI.

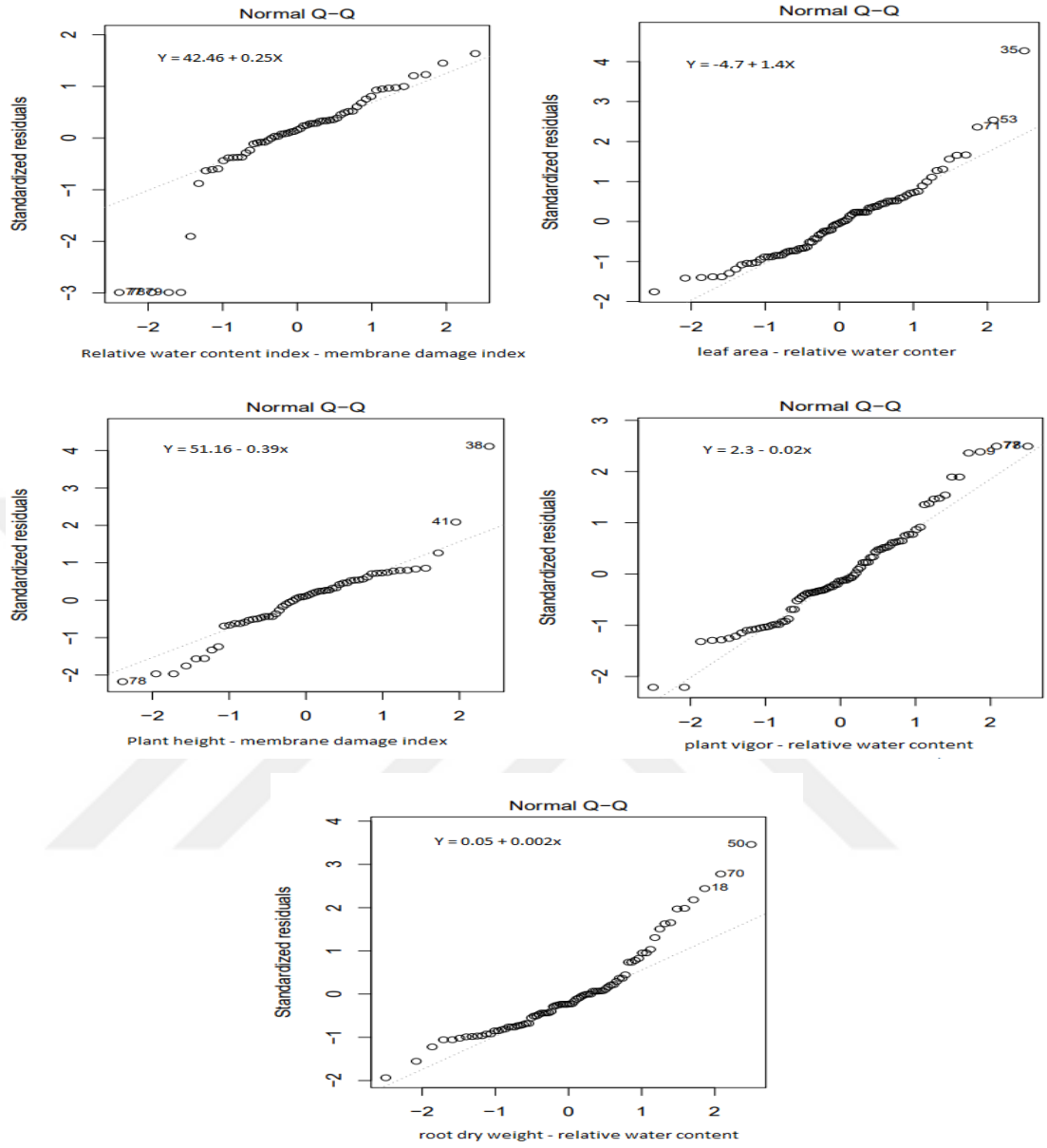

Figure 21: Correlation Between Physiological and Morphological Parameters (Greenhouse)

The relationship between variable in in-vitro condition revealed a positive and strong significant ($p<<0.05$) correlation between stem dry matter and relative water content r^2= 0.921. Correlation between plant height and Membrane damage at $p<0.05$ was found positive and strongly significant r^2= 0.103, also a strong positive correlation was observed between Membrane damage and stem diameter.

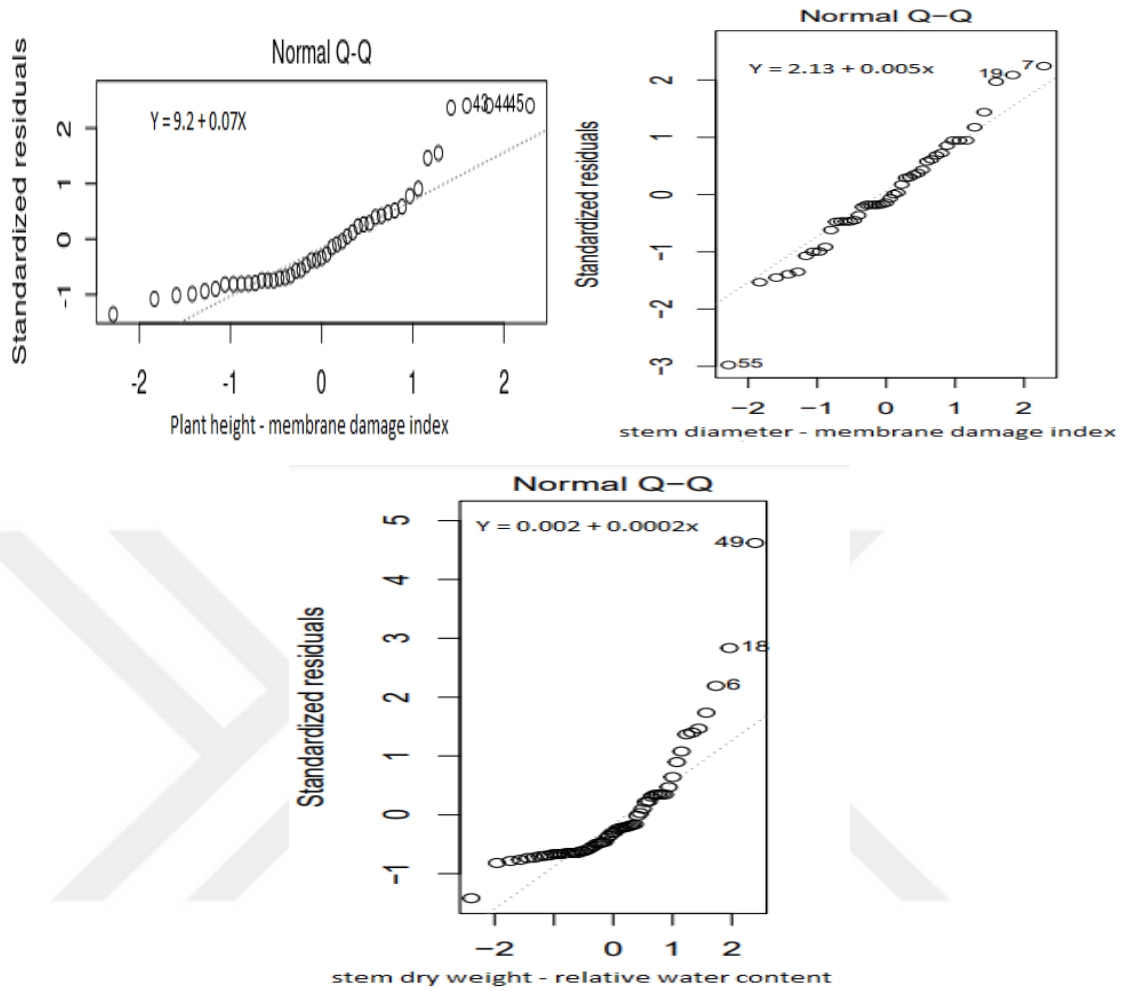

Figure 22. Correlation Between Physiological and Morphological Parameters (In Vitro).

At maturity, growth and physiological growth and physiological factors are more correlated, meanwhile at early stage (in-vitro) there is less correlation. This suggests that as plants develop in salty environment, physiological reactions have more impact on their external expressions. At both developmental circles, membrane damage indexseems to have a positive impact on plant growth, meanwhile relative water content contributes a lot in the building of dry matter content. Relative water content is an important parameter usually used to evaluate plants for resistance to abiotic stress.In our experiment, there was a positive correlation between root length and RWC; similar correlation was also found by Veesar et al.(2021) when he screened 12 cotton cultivars for drought tolerance at seedling stage. In greenhouse condition, a positive relationship between leaf area and RWC suggests that the higher the water content in plant, the larger leaf surface area will be. However, negative and significant correlation between both parameters was reported in lentil plant under drought stress. High relative water content was exhibitedby plants

with small leaf surface area; plants with large surface area lost more water through transpiration, meanwhile water loses in those with small surface area was minimum(Mishra et al., 2018).

CHAPTER IV

CONCLUSION

In this experiment, five melon genotypes (two varieties and three breeding combinations) were evaluated for their tolerance to salt stress. Cultivars were grown in green house as well as in in-vitro condition where they spend respectively three months and two weeks under different salt dose treatments.

Local varieties consisted of ŞemameŞemameand Özbek, and breeding combination were Şemame x Ananas, Ananas x Şemame and Midyat x Ananas. Midyat x Ananas was observed as the most tolerant genotype because of its performance at high concentration.

The experiment revealed that salt stress affects plants growth parameters in various ways; while increase in salt concentration led to the progressive decrease of some growth parameters, the performance of others was improved at low or high salinity level, also some parameters could not be affected.

It was observed that early stage melon plants (in vitro) were not really affected by salt stress; the application of salt concentration did not have a significant effect on most growth parameters in-vitro condition, however as they continue growing, the negative effects of salt stress started appearing, 150 and 200mMol dose had a detrimental effect on most growth factors.

Under salt stress, less correlation between morphological and physiological growth factors was observed in young plants (in-vitro), but at mature stage more correlation between the two types of growth factors was observed.

In-vitro condition, no significant differences for many growth factors were observed under different salt applications.

After thorough evaluation, Midyat x Ananas and Ozbek appeared as the most resistant cultivars; at high salt concentration their relative water content was higher compared to other cultivars, relative water content was positive and significantly correlated to most growth factors.

As a reminder, screening for salt tolerance remain a challenge because plant response to salinity depends on growth cycle, based on the results of this research it can be suggested that, resistance of melon plant (*Cucumis melo*) to salt stress must be evaluated at maturity or flowering stage, because not only significant changes of growth parameters can be detected but also because it becomes possible to perceive relationships between variables.

Limitations

Studies related to salt tolerance need to take into account several important biological and physiological analysis such as: SPAD, proline, CAT, APX, SOD, POX, Na^+, K^+, RWC and others for more relevant results. However, in this experiment only few analyses could be done, therefore the quality of the results is limited.

Soil analysis also was not carried out at the end of the start and end of the experiment. Results that could have been obtained would provide addition evidences behind plant performance.

In in-vitro condition, seeds were first germinated on petridishes before they were transplanted into hormone-free medium to avoid uneven germination. There was a delay in seed transplantation for some genotype because of poor seed germination.

Greenhouse experiment was carried out until early fruit formation for all genotypes.

Recommendations

In other to improve the quality of this experiment, suggestions that can be made are the following:

In addition to biomass and morphological analyses, more biological and physiological analyses must be done. Also, soil analysis results should be done at the beginning and end of the experiment.

Seeds with good germination potential should be used to ensure timely transplantation and maximize even growth of plants.

Because plant tolerance to salt stress depends on plant growth stages, experiment for melon should be carried out till fruit maturity and data should be collected at various growth stages; seedling, flowering and fruit harvesting.

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