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DETERMINATION OF SALT STRESS TOLERANCE LEVELS OF DIFFERENT BEAN GENOTYPES

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DETERMINATION OF SALT STRESS TOLERANCE LEVELS OF DIFFERENT BEAN GENOTYPES

By Mohammed Ghalib Kadhim AL-KHASHMANI

February 2024

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

DETERMİNATION OF SALT STRESS TOLERANCE LEVELS OF DIFFERENT BEAN GENOTYPES

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February 2024

In this study, which was carried out to determine the salt tolerance levels of different bean genotypes, 20 different bean genotypes were used as materials. Seeds of the genotypes were planted in 12 L pots containing peat: perlite mixture in a ratio of 2:1. Stress treatments were started 36 days after seed planting. For this purpose, starting from a dose of 50 mM NaCl, the salt concentration was gradually increased and a final concentration of 150 mM NaCl was reached. Plants exposed to salt stress for sixteen days were harvested and measurements and analyzes were carried out. Plants were examined in terms of morphological and physiological parameters. In terms of scale evaluation, GT-1 and GT-16 genotypes showed the closest development to control plants with a scale value of "1,33", while GT-8, GT-14, GT-18 and GT-19 genptypes showed the most development against salt stress with a scale value of "5". In other words, the genotypes that suffered the most were determined to be affected. Salt stress (150 mM NaCl) caused a decrease at varying rates in terms of shoot fresh and dry weights, stem length and diameter, leaf area, leaf relative water content (RWC) and SPAD value. Accordingly, the genotypes with lower losses compared to control plants are GT-6, GT-7, GT-11 and GT-16, it was observed that the genotypes with the highest losses were GT-8, GT-14, GT-18, GT-19 and GT-20. Membrane damage index (MDI) increased by 42%, Na and Cl ion contents increased by 724% and 845% on average compared to control plants. Among the genotypes that act selectively in terms of toxic ion content and accumulate less Na and Cl ions, GT-6, GT-7, GT11 and GT-16 ranked first, GT-2, GT-4, GT-8 and GT-9. Were the genotypes containing the most Na and Cl ions. K (48,16% decrease) and Ca (43,53% decrease) ion contents decreased with the application of 150 mM NaCl. This decrease was at a lower rate in GT-6, GT-7, GT-11 and GT-16. Losses in K and Ca ion content increased significantly in GT-8, GT-14, GT-19 and GT-20 bean genotypes. In line with all these results, in the study carried out at the early development stage, bean genotypes GT-6, GT-7, GT-11 and GT-16 were found to be tolerant, Genotypes GT-8, GT-14, GT-18 and GT-19 were determined to be sensitive.

2024, 46 pages

Keywords: *Phaseolus vulgaris*, MDI, RWC, Sodium chloride, Salt stress

ÖZET

FARKLI FASULYE GENOTİPLERİNİN TUZ STRESİNE TOLERANS DÜZEYLERİNİN BELİRLENMESİ

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Tarım ve Yaşam Bilimleri Yüksek Lisans
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Şubat 2024

Farklı fasulye genotiplerinin tuza tolerans düzeylerini belirlemek amacıyla gerçekelştirilen bu çalışmada 20 farklı fasulye genotipi materyal olarak kullanılmıştır. Genotiplere ait tohumlar 2:1 oranında torf: perlit karışımı içeren 12 L hacmindeki saksılara ekilmiştir. Tohum ekiminden 36 gün sonra stres uygulamlarına başlanmıştır. Bu amaçla 50 mM NaCl dozundan başlanarak tuz konsantrasyonu kademeli olarak artırılmış ve final konsantrasyon olarak 150 mM NaCl dozuna ulaşılmıştır. On altı gün tuz stresine maruz bırakılan bitkiler hasat edilerek ölçüm ve analizler gerçekleştirilmiştir. Bitkiler morfolojik ve fizyolojik parametreler bakımından incelenmiştir. Skala değerlendirmesi bakımından GT-1 ve GT-16 genotipleri "1,33" skala değeri ile kontrol bitkilerine en yakın gelişme gösteren genotipler olurken; GT-8, GT-14, GT-18 ve GT-19 genptipleri ise "5" skala değeri ile tuz stresinden en fazla etkilenen başka bir ifade ile zararlanmanın en fazla genotipler olarak belirlenmiştir. Tuz stresi (150 mM NaCl) yeşil aksam yaş ve kuru ağırlıkları, gövde boyu ve çapı, yaprak alanı, yaprak oransal su içeriği (YOSİ) ve SPAD değeri bakımından değişen oranlarda azalmaya neden olmuştur. Buna göre kontrol bitkilerine oranla kayıpların daha düşük oranda meydana geldiği genotiplerin GT-6, GT-7, GT-11 ve GT-16, kayıpların en fazla olduğu genotiplerin ise GT-8, GT-14, GT-18, GT-19 ve GT-20 olduğu görülmüştür. Membran zararlanma indekis (MZİ) tuz stresi ile birlikte ortalama olarak %42, Na ve Cl iyon içeriği ise kontrol bitkilerine oranla ortalama olarak %724 ve %845 oranında artış göstermiştir. Toksik iyon içeriği bakımından seçici davranarak bünyesinde daha az oranda Na ve Cl iyonu biriktiren genotipler arasında GT-6, GT-7, GT-11 ve GT-16 ilk sırada yer almış, GT-2, GT-4, GT-8 ve GT-9 en fazla Na ve Cl iyonu içeren genotipler olmuştur. K (%48,16 azalma) ve Ca (%43,53 azalma) iyon içerikleri ise 150 mM NaCl uygulaması ile birlikte azalma göstermiştir. Bu azalma GT-6, GT-7, GT-11 ve GT-16 daha düşük oranda olmuştur. GT-8, GT-14, GT-19 ve GT-20 fasulye genotiplerinde K ve Ca iyon içeriğindeki kayıplar belirgin olarak artmıştır. Tüm bu sonuçlar doğrultusunda erken gelişim aşamasında gerçekleştirilen çalışmada, GT-6, GT-7, GT-11 ve GT-16 nolu fasulye genotiplerinin tolerant, GT-8, GT-14, GT-18 ve GT-19 nolu genotiplerin hassas olduğu belirlenmiştir.

2024, 46 sayfa

Anahtar Kelimeler: *Phaseolus vulgaris*, MZİ, Sodyum klorür, Tuz stresi, YOSİ

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Mohammed Ghalib Kadhim AL-KHASHMANI
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LIST OF SYMBOLS

Ca	Calcium
cm	Centimeter
Cl	Chlorine
EC	Electrical conductivity
g	Gram
L	Liter
mg	Milligram
mM	Millimolar
%	Percentage
K	Potassium
Na	Sodium
NaCl	Sodium chloride
cm^2	Square centimeter

LIST OF ABBREVIATIONS

SPAD	Chlorophyll Meter for agricultural products
RWC	Relative water content
MDI	Membrane damage index
POD	Peroxidase
PPO	Polyphenol oxidase
ROS	Reactive oxygen species
LSD	Slightly significant difference test

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1. INTRODUCTION

With the increasing world population, the need for food is also increasing. It is estimated that the population will reach 10 billion by 2050, and as a result, the food demand is expected to increase by 50% (Mora *et al.* 2020). Considering the negative impact of climate change and the increasing demand for food, current agricultural practices are insufficient (Lowry *et al.* 2019). This situation has led to a focus on agricultural productivity and efforts to obtain the highest yield from existing production areas. Abiotic stress factors such as salinity, drought, and high temperatures have restrictive effects on global food security, food quality, and plant productivity. This problem has been exacerbated by excessive fertilizer use, the use of unsuitable irrigation water, insufficient irrigation, industrial pollution, natural environmental conditions, and global climate change (Zhao *et al.* 2021).

Losses in crop productivity associated with salt stress are approximately 50% worldwide (Zhao *et al.* 2021). Salinity directly affects agriculture and food security. Therefore, ensuring tolerance to salt stress is of great importance for the establishment of global food security and the sustainable implementation of modern agriculture.

Salinity, one of the most important limiting factors affecting plant growth and development, and therefore yield, can occur in different forms. Salinity can arise due to neutral soluble salts in the soil, depending on the calcium, magnesium, chloride, and sulfate content, as well as due to sodium carbonate salts that produce alkaline hydrolysis, especially sodium salts (Parihar *et al.* 2015).

Salt stress negatively affects germination, growth and development, flowering, and fruit set in plants. High sodium concentrations in saline soil limit water uptake and nutrient absorption in plants. Water deficiency and nutrient imbalance highlight primary stresses, including osmotic stress and ionic stress. These primary stresses lead to oxidative stress and can also cause secondary stress. Salt stress leads to various physiological and molecular changes, limiting photosynthesis and causing problems in plant growth and development (Van Zelm *et al.* 2020, Gong 2021).

Salt stress, which leads to negative effects on plant growth and development, occurs parallel to the increasing NaCl and other soluble salt concentrations in the soil. The increase in salt concentration in the soil solution and the decrease in water potential reduce the osmotic potential of plant cells. As a result, depending on the intensity and duration of salt stress, many biological processes in plants such as growth, development, germination, cell division, and photosynthesis are affected (Bressan 2008), which limits crop productivity and product quality in agricultural fields. Therefore, salinity is one of the most important stress factors limiting agricultural production (Fahad *et al.* 2015). Generally, yield losses occur in many crops when the electrical conductivity (EC) exceeds 4 dS m^{-1} and the osmotic potential reaches 0.2 MPa. In this case, especially morphological, physiological, and biochemical changes occur due to Na ion toxicity (Acosta-Motos *et al.* 2017).

Salt stress affects cell division and elongation, leading to a decrease in the number of cells, mitotic activity, and cell division rate in the roots and shoots of plants. Consequently, a decrease in stem and root length and weight, leaf shrinkage and thinning, a decrease in leaf numbers, thinning of the waxy layer and cuticle layer on the leaf surface, and a decrease in the differentiation and development of vascular tissue occur. In addition, the root system is directly exposed to salinity, and leaf growth is more sensitive to salt stress than root growth in plants, resulting in an increase in the root/shoot ratio under salt stress. Although the mechanism of this increase has not been fully explained, different changes in the cell walls of roots and leaves in response to salinity are considered to be the cause (Çulha and Çakırlar 2016).

The most important reason for the damage caused by salt stress is the negative effects on plant ion balance. Ion toxicity occurs as a result of the disruption of ion balance and leads to nutritional disorders in plants. The salt present in the plant growth medium increases the amount of Na^+ in competition with K^+ uptake. As the amount of Na^+ increases in the cells, K^+ efflux occurs, leading to a deficiency of K^+ in the cytosol (Chokshi *et al.* 2017).

The increased amount of Na^+ inside the cells can replace the Ca^{2+} in the cell membrane. As a result, the amount of Na^+ changes the cellular ion balance, especially the K^+/Na^+ ratio, during salt stress (Rahman *et al.* 2016).

In soil or any plant growth environment, high salinity directly reduces water uptake by lowering the osmotic potential, leading to water exit from the cell and stomatal closure (Shabala and Cuin, 2008, Chokshi *et al.* 2017, Rajput *et al.* 2017). Both osmotic stress and ion toxicity hinder plant photosystem, leading to excessive production of reactive oxygen species (ROS) ($^1O_2$, $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$) (Hasanüzzaman *et al.* 2013).

Beans, a herbaceous plant of the Phaseolus genus in the Fabaceae family, originated from Central America and are one of the most widely cultivated crops worldwide for both fresh pods and dry beans. With approximately 76 species, beans constitute 50% of the consumed legumes worldwide. Five different types of beans are cultivated globally (Celmeli *et al.* 2018), including two types, *P. vulgaris* L. and *P. coccineous* L., grown in Turkey (Smýkal *et al.* 2015). In addition to being a rich source of vitamins (A, B9, and C) and minerals (iron and magnesium), beans play an important role in human nutrition as a primary protein source (Hernandez *et al.* 2007). Formerly a significant exporter of beans, Turkey has been striving to become more self-sufficient in recent years (Faostat, 2018). Due to its geographical structure, Turkey has a wide diversity of bean genotypes. Dry beans can be cultivated in every region of Turkey. According to the Turkish Statistical Institute (TUİK), beans rank third in terms of both cultivation area and production quantity among grain legumes, following chickpeas and lentils. In 2020, the total production quantity of dry beans on approximately 103 thousand hectares in Turkey was around 280 thousand tons, with a yield of approximately 2.71 tons/ha (TUİK, 2021).

Compared to other species, legumes are among the most sensitive groups to salinity, and beans are known to be one of the most salt-sensitive species (Ashraf, 1994). Genetic diversity within a species provides a valuable opportunity for salinity tolerance studies. Beans (*Phaseolus vulgaris, L.*) are one of the major crops cultivated worldwide and are highly sensitive to salinity (Marchner, 1995). Despite not being its primary homeland, Turkey is one of the important countries in terms of genetic richness in beans and cowpeas

(Daşgan *et al.* 2006). Observations in areas where beans are cultivated indicate significant differences in plant vegetative growth and pod and grain yield among different bean genotypes under saline and water-restricted conditions. This variation within the same species is associated with differences in genotypes' adaptation mechanisms to salinity and drought (Costa França *et al.* 2000).

In line with this, the aim of this study is to determine the tolerance levels of different bean genotypes to salt stress.

2. LITERATURE REVIEW

2.1 Salt Stress in Plants

Salinity is a major environmental stress factor that negatively affects plant growth, development, and the quality and quantity of the yield. In crop production, about 50% of the yield losses are due to abiotic stress factors. Currently, around 6% of the total irrigable land, accounting for 30% of irrigated areas, is affected by soil salinity. On the other hand, mistakes in cultural practices such as fertilization and irrigation are contributing to the increasing problem (Hasanuzzaman *et al.* 2013).

Plants are classified as glycophytes or halophytes based on their responses to salt stress. Halophytes have the ability to tolerate high levels of sodium chloride (NaCl) and survive in soils twice as salty as seawater. Elevated NaCl levels lead to imbalances in nutrient uptake in plants, such as reduced intake of calcium and nitrate ions. Salinity primarily hinders plant growth through three main mechanisms: ion toxicity, osmotic stress, and nutrient imbalance (Patel *et al.* 2010).

The salts present in irrigation water or soil can create salt stress levels that limit the growth and development of plants. These compounds include chloride, sulfate, carbonate, bicarbonate, and borate. However, sodium chloride (NaCl) is the most commonly encountered form of salt in nature. An increase in salt levels in the soil due to stress leads to an increase in osmotic pressure, a decrease in water potential, and a restriction of water uptake by the roots, resulting in osmotic stress. Another effect of the stress is defined as a toxic effect. When salt ions, especially high concentrations of Na^+ ions, are present, a toxic effect occurs, leading to negative impacts on plant growth and development (Stavridou *et al.* 2017).

Salt stress, characterized by low osmotic potential in the growth medium, imbalance of plant nutrients, ion toxicity, or a combination of these factors, has harmful effects on plants. The impact varies depending on the salt level, duration of stress, plant age, species, and variety. Under stress conditions, there is a decrease in osmotic potential and water potential, leading to disruption of cell membrane permeability and integrity (Acosta Matos *et al.* 2017, Shahzad *et al.* 2022). Cell division and elongation are affected, resulting in decreased stem and root length and weight, as well as a reduction in leaf number and area.

Salt stress causes damage to plants mainly due to changes in ion regulation. The increasing accumulation of salt in the root zone restricts the uptake of essential elements and minerals such as K^+, Ca^{+2}, and NO_3^-, while inducing the uptake of Na^+ and Cl^-, negatively affecting the cellular ion balance. The toxic accumulation of harmful ions affects photosynthesis and protein synthesis, deactivates enzymes, and damages chloroplasts and other organelles. When sodium (Na^+) reaches toxic levels, it also affects the uptake and regulation of K^+, Ca^{+2}, and Mg^{+2} cations in the plant, while when chloride (Cl^-) reaches toxic levels, it affects the uptake and regulation of nitrate (NO_3^-) anions (Atalan and Gökçe 2021).

2.2 The Effects of Salt Stress in Beans

Ndakidemi and Makoi (2009) exposed four different bean varieties (Lyamungo 90, Jesca, Flora de Mayo, and CAB 19) to different levels of salinity (0, 2.5, 5.0, 10.0 mM) at 10 and 20-day intervals after the seedling stage to determine their responses to NaCl induced salt stress. They found that high NaCl concentrations reduced plant height and dry matter yield, changed leaf color, promoted leaf injuries, and that compared to the control, Lyamungo 90 and CAB 19 varieties were more affected by >5 mM NaCl, while some varieties showed more tolerance to high salinity than others.

Kaya (2011) investigated the tolerance levels of eighty-one different bean genotypes to drought and salinity at the early stage of plant development. To reveal the bean genotypes' responses to salt stress, 200 mM NaCl was used, while drought stress was induced by gradually with holding water. In the study, bean plants were grown under salt and drought stress as well as non-stress control conditions. The trial focused on bean plants at the early 28-day development stage, without reaching the yield. A series of morphological and physiological measurements and analyses were conducted to determine which bean genotypes were tolerant to salt and drought. These included scoring symptomatic damage in genotypes on a 0-5 scale, fresh and dry weights of green parts, fresh and dry weights of roots, leaf number, plant height, leaf area, membrane damage index, SPAD-chlorophyll meter readings, leaf relative water content, leaf water potential, leaf osmotic potential, stomatal conductance, leaf temperature, and analysis of Na, K, Ca, and Cl in green parts and roots. Percentage changes in all examined parameters in stressed plants compared to control plants were calculated. Additionally, the relationships between the parameters were investigated. The study concluded that there was a wide variation in the responses of the examined bean genotypes to salt and drought stress. Eighty-one different bean genotypes were classified as tolerant, moderately tolerant, and sensitive to salt and drought.

In bean plants, as the salt concentration increases in the root region, the leaf water potential and leaf osmotic potential decrease (Gama *et al.* 2007), and there are decreases in transpiration rate due to a signal being sent from the root to the shoot (Kaymakanova and Stoeva, 2008). Kouam *et al.* (2017) stated that the osmotic effect, which limits the uptake of water necessary for the germination of bean seeds under salt stress, causes germination to be delayed. Bayram *et al.* (2014) reported that the application of 300 mM NaCl decreased the relative water content in bean plants by 31% and 59%, respectively, in soil and soilless cultures. The decrease in water content was caused by the increase in the osmotic pressure of the salts in the nutrient solution and the direct reduction of the water availability to the roots. Kaymakanova and Stoeva (2008) reported that the decrease in water potential in some bean varieties under sodium chloride and sodium sulfate applications was due to salt increasing cellular water loss. In plants treated with 100 mM NaCl, the water potential decreased by about 200%, and this decrease caused a decrease in transpiration rate, and consequently a decrease in biomass.

Cokkizgin (2012) found that salt stress can reduce seed germination in beans due to osmotic effects or ion toxicity. Additionally, accumulation of sodium and chloride can cause an imbalance in nutrient uptake. High sodium concentration and low K/Na ratios in the tissue, inhibition of root elongation, and calcium deficiency in the shoots are among the characteristic symptoms of sodium toxicity (Tejera *et al.* 2005). Metwali *et al.* (2015) found that the decrease in plant growth and chlorophyll content under salt conditions in beans could be attributed to the toxic effect of sodium and chloride. Ndakidemi and Makoi (2009) reported that plants exposed to high NaCl induced salt stress showed more severe leaf damage symptoms than plants exposed to lower salt levels.

Many researchers have reported that increasing salinity can significantly reduce seed germination percentage, germination rate, root and shoot lengths, and root and shoot weights of plants (Lotfi *et al.* 2018). In a 6-week experiment conducted by Kouam *et al.* (2017), it was found that all growth parameters, except root length, decreased in bean plants with increasing salinity. Leaf sodium accumulation significantly increased, while potassium accumulation decreased. The same researchers also reported that plant height was significantly affected by only 200 mM NaCl salinity level.

Assimakopoulou *et al.* (2015) reported a decrease of 47%, 30%, and 36% in total biomass for the "Romano," "Corallo," and "Starazagorski" fresh bean varieties, respectively, when grown under saline conditions. Additionally, the harvested marketable pod yields showed a reduction of 35%, 45%, and 46%, respectively. Similarly, it was noted that bean plants were affected by different salinity levels, influencing pod length and nutrient content. At salinity levels of 0,5, 2, and 3 dS/m, pod lengths of 8,52, 10,02, and 11,84 cm were obtained, as reported by Abdel-Mawgoud *et al.* (2010).

Farhangi-Abriz and Torabian (2018) stated that in soybeans, an increase in salt concentration resulted in a decrease in plant growth and development and a reduction in K^+ ion uptake. However, they also observed an increase in antioxidative enzyme activities, MDA content, phenolic compound content, hydrogen peroxide content, and Na^+ ion.

In a study examining the tolerance levels of forty-seven different bean genotypes to salt and drought, Arteaga *et al.* (2020) found that salt led to a reduction in plant growth and development. The research investigated the effects of drought and salt stress on plant growth in 47 bean (*P. vulgaris* L.) genotypes. The study reported a decrease in the growth of genotypes under both salt and drought conditions.

Abdel Latef *et al.* (2021) stated that under salt stress, the disruption of mineral homeostasis occurs, leading to a slowdown in plant development. In beans, it was emphasized that excessive accumulation of Na in organs such as shoots, roots, and stems results in a decrease in Ca, K, and Mg content. The rapid absorption of Na and Cl ions is highlighted to cause a significant decrease in other ions (Abdel Latef *et al.* 2021).

In their study on beans, Kul *et al.* (2021) reported a significant reduction in growth, chlorophyll content, and leaf water content with the application of 100 mM NaCl. They also noted a significant increase in H2O2, MDA, proline, and sucrose content.

In a study on beans, Al-huraby and Bafeel (2022) reported a decrease in germination rate with increasing NaCl concentrations (0, 50, 100, 150, and 200 mM). In the same study, they also reported that high salt concentration limits photosynthetic activity and results in a decrease in photosynthetic pigments.

Al-Shammari *et al.* (2023) reported that under salt stress conditions in peas, there was a decrease in plant growth and development (both in terms of fresh and dry weight, plant height, and flower number per plant), yield and yield parameters, chlorophyll a and b, and leaf relative water content. However, they also noted an increase in MDA, H_2O_2, and electrical conductivity levels, as well as antioxidative enzyme activities (SOD, CAT, POX).

Desouky *et al.* (2023), highlighting salt stress as a factor affecting yield in fava beans (*Vicia faba* L.), examined the salt tolerance characteristics among genotypes from both physiological and biochemical perspectives. The study, conducted under in vitro conditions, characterized the response to different seawater concentrations (1000, 3000, 5000, and 7000 ppm) for two fava bean genotypes (Sakha 3 and Nubaria 2). The results showed a significant decrease in shoot and root length, leaf and branch numbers, and dry matter gain for both genotypes with increasing salinity. Additionally, there was a notable reduction in photosynthetic pigments (chlorophyll a, b) with the rise in salinity. Changes in tissue ion levels, peroxidase (POD), and polyphenol oxidase (PPO) activities varied based on genotype, tissue type, and salinity level. The adverse impact of salt stress on the growth performance of the Nubaria 2 genotype was found to be less severe compared to the Sakha 3 genotype (Desouky *et al.* 2023).

3. MATERIALS AND METHODS

3.1 Material

In the study, a total of 20 different bean genotypes were used as the material.

3.2 Method

The study was conducted at the Faculty of Food and Agriculture Vocational School in Uluyazı and Ballıca campuses, in greenhouses and laboratories. Seeds of bean genotypes were sown in plastic pots containing a mixture of peat and perlite in a 2:1 ratio, with three plants per pot. The plants were irrigated with a standard nutrient solution until they reached the stage of having three true leaves. The "drainage solution/applied solution" ratio was used as the basis for irrigation. Daily drainage levels were determined, and this ratio was maintained at around 30% based on the growth of the plants throughout the experiment. Stress and control applications were organized with three replications in the study.

3.2.1 Stress treatments

Stress treatments began 36 days after seed sowing. For this purpose, starting with a dose of 50 mM NaCl, the salt concentration was gradually increased, reaching a final concentration of 150 mM NaCl. Plants exposed to salt stress for sixteen days were then harvested, and measurements and analyses were conducted.

Figure 3.1 Planting of bean seeds and establishment of the experiment

Figure 3.2 Plant development in beans

Figure 3.3 The responses of different bean genotypes to salt stress

3.2.2 Measurements and analyses

Scale evaluation (0-5)

A scale was established to assess the degree of morphological damage in plants. For this purpose, plants were assigned scores ranging from 0 to 5 based on the degree of damage.

In the salt stress experiment with bean plants, scores from 0 to 5 were given according to the symptoms outlined below (Kusvuran 2010).

- 0: No impact of salt stress on plants (control plants)
- 1: Localized yellowing and curling of leaves
- 2: Yellowing of leaves and a 25% necrotic spot
- 3: Presence of necrotic spots between 25-50% on leaves with the initiation of leaf shedding
- 4: Necrosis covering 50-75% of leaves and occurrence of plant deaths
- 5: Severe necrosis covering 75-100% of leaves and/or complete plant death

Determining shoot fresh and dry weights

As a result of stress applications, three randomly selected plants from the harvested ones will be weighed on a sensitive scale to determine their fresh weights in grams. Subsequently, the same samples will be dried in an oven at 65 ºC for 48 hours, and their dry weights will also be recorded.

Determination of stem height and diameter

The region from the root collar to the growth tip in the plant will be measured in centimeters (± 0.5) and recorded in meters. The stem diameter will be determined using a digital caliper and recorded in millimeters (± 0.1).

Determining the leaf area

The leaf area was determined using the CI BIO Science CI 202 model leaf area meter device and expressed as cm^2/plant.

Determination of relative water content

In salt stress tolerance trials for beans, relative water content (RWC) (%) was determined using the methods employed by different researchers for various plants. The RWC was determined following the procedure outlined by Sanchez *et al.* (2004) and Türkan *et al.* (2005). Sixteen days after the application of stress, plants were harvested, and the fresh weights of leaf samples were measured to determine their relative water content. After determining their weights, the leaf samples were dried in an oven at 65ºC for 48 hours, and their dry weights were recorded in grams.

Determination of membrane damage in leaf cells (membrane injury index)

The membrane damage index (MDI) was calculated by measuring the electrolyte leakage from the cells (Fan and Blake, 1994). Discs of 17 mm in diameter were taken from the third leaf from the bottom of both stress and control plants. After soaking these discs in deionized water for 5 hours, the electrical conductivity (EC) was measured. The same discs were then kept at 100°C for 10 minutes, and the EC value of the solution was recorded again. Membrane damage (%) in leaf cells was determined through this process.

Determination of chlorophyll ratio

The chlorophyll content in plants was measured using a Minolta brand chlorophyll meter.

Determination of ion contents (Na, Cl, K, and Ca)

The method outlined by Dasgan and Koc (2009) was followed for the determination of ion contents (Na, K, and Ca). The determination of plant green tissue chlorine concentration was conducted according to Nielsen (2017) and using the Mohr method.

3.3 Statistical Analysis

The study was conducted using a completely randomized block design with three replications, each consisting of three plants per replication. JMP (version 8.0) software was employed for the statistical analysis of the data. Differences between means were grouped according to the LSD test ($p \leq 0.05$).

4. RESULTS AND DISCUSSION

The obtained findings on the examination of salt stress tolerance statuses of different bean genotypes are provided below.

4.1 Scale Evaluation

In the study examining the tolerance status of different bean genotypes to salt stress, plants were evaluated based on a visual scale assessment (Figure 4.1). Ratings ranging from 1 to 5 were assigned depending on the degree of damage in the assessment, considering "0" as the control. Accordingly, genotypes GT-1 and GT-16 exhibited the closest development to control plants with a scale value of "1,33". Following them were genotypes GT-6 and GT-7 with a scale value of "1,67". Genotypes GT-8, GT-14, GT-18, and GT-19 were identified as the most affected by salt stress, or in other words, the genotypes with the highest damage, with a scale value of "5". The scale values for other genotypes ranged between 2 and 4,67.

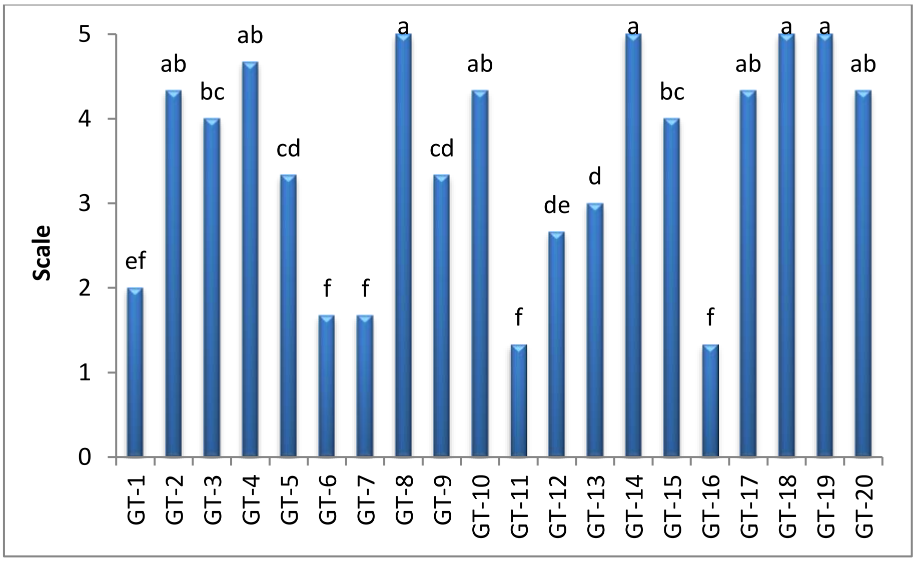

Figure 4.1 Scale values under salt stress in bean genotypes

4.2 Shoot Fresh and Dry Weights

Salt stress (150 mM NaCl) has led to a decrease in shoot fresh weights in varying proportions (Table 4.1). This decrease exhibited variations among genotypes. Under salt stress conditions, the highest green shoot fresh weight values were observed in genotypes GT-6 (42,67 g/plant) and GT-16 (41 g/plant), while the lowest values were determined in genotypes GT-8 (7 g/plant) and GT-20 (6,67 g/plant). To highlight the losses imposed by salt stress on plants, percentage changes were determined. Accordingly, genotypes with the least percentage change and, therefore, closest to control plants, were identified as GT-11 (7.85% decrease), GT-16 (15.76% decrease), and GT-7 (17,51% decrease). However, genotypes with the highest percentage changes were listed as GT-19 (80.01% decrease), GT-8 (80,19% decrease), and GT-20 (81.67% decrease).

Shoot dry weights also showed a decrease depending on salt stress, revealing differences among genotypes (Table 4.2). In general, with the application of 150 mM NaCl, there was a reduction in green biomass dry weights ranging from 17% to 94%. When considering the percentage changes, the decrease in genotypes GT-6, GT-11, and GT-7 ranged from 17% to 30% compared to control plants, while the highest reduction was observed in genotypes GT-17, GT-14, GT-4, and GT-8, with a decrease of 91-93% compared to control plants.

Table 4.1 The effect of salt stress on the shoot fresh weight of bean genotypes

Shoot Fresh Weight (g plant^{-1})			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	40,00 a-d	32,67 b	-18,33
GT-2	47,67 ab	16,67 e-h	-65,03
GT-3	44,67 a-c	13,33 f-i	-70,16
GT-4	39,00 a-d	11,00 g-i	-71,79
GT-5	33,33 b-d	18,00 e-g	-45,99
GT-6	52,00 a	42,67 a	-17,94
GT-7	26,67 d	22,00 c-e	-17,51
GT-8	35,33 b-d	7,00 i	-80,19
GT-9	41,33 a-d	26,67 b-d	-35,47
GT-10	36,33 a-d	10,67 g-i	-70,63
GT-11	34,00 b-d	31,33 b	-7,85
GT-12	39,67 a-d	29,33 bc	-26,07
GT-13	30,00 c-d	20,33 d-f	-32,23
GT-14	38,67 a-d	9,67 hi	-74,99
GT-15	42,67 a-c	9,33 hi	-78,13
GT-16	48,67 ab	41,00 a	-15,76
GT-17	38,00 a-d	10,00 hi	-73,68
GT-18	33,00 b-d	8,67 i	-73,73
GT-19	46,67 ab	9,33 hi	-80,01
GT-20	36,33 a-d	6,67 i	-81,64
LSD ($_{0.05}$)	15,80	7,82	

Table 4.2 The effect of salt stress on shoot dry weight in bean genotypes

Shoot Dry Weight (g $plant^{-1}$)			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	4,53 a-c	2,61 ab	-42,38
GT-2	5,54 ab	0,83 e-g	-85,02
GT-3	5,81 a	0,67 e-h	-88,47
GT-4	4,68 a-c	0,33 h	-92,95
GT-5	2,97 e	1,08 c-e	-63,64
GT-6	3,64 c-e	2,99 a	-17,86
GT-7	3,34 c-e	2,32 b	-30,54
GT-8	4,59 a-c	0,28 h	-93,90
GT-9	3,88 c-e	1,55 c	-60,05
GT-10	4,36 b-d	0,55 f-h	-87,39
GT-11	3,06 de	2,19 b	-28,43
GT-12	3,60 c-e	1,47 cd	-59,17
GT-13	3,60 c-e	1,03 d-f	-71,33
GT-14	5,41 ab	0,39 gh	-92,79
GT-15	4,64 a-c	0,51 gh	-89,01
GT-16	4,38 a-d	2,87 a	-34,47
GT-17	4,56 a-c	0,40 gh	-91,23
GT-18	3,63 c-e	0,43 gh	-88,15
GT-19	4,67 a-c	0,47 gh	-89,94
GT-20	3,27 c-e	0,33 h	-89,91
LSD ($_{0.05}$)	1,44	0,49	

4.3 Stem Length and Diameter

Under salt stress conditions, the tallest stem height was determined in genotypes GT-16 (70 cm/plant), GT-6 (60,67 cm/plant), and GT-11 (59 cm/plant) (Table 4.3). The lowest stem height was observed in bean genotypes GT-18 (27,33 cm/plant), GT-8 (26,67 cm/plant), and GT-10 (23 cm/plant). Salt application led to a decrease in stem height compared to the control plants. Some genotypes were found to result in less loss compared to other genotypes when compared with control plants, with GT-11 (16%), GT-16 (25%), GT-7 (28%), and GT-6 (28%) taking the top positions. Among the genotypes, GT-2, GT-10, GT-18, and GT-8 experienced the highest losses, ranging from 63% to 66% compared to control plants under stress conditions.

In all genotypes exposed to salt stress, a varying degree of decrease in stem diameter compared to control plants was determined (Table 4.4). Genotypes that were less affected by salt stress and formed values close to control plants experienced losses ranging from 3,24% to 8,77%. Among the genotypes with the least loss in stem diameter, GT-11 (3,24%), GT-6 (7,99%), and GT-7 (8,77%) ranked at the top. Genotypes most affected by salt stress, with high losses in stem diameter compared to control plants, included GT-15 (70,85%), GT-14 (71,90%), and GT-18 (72,27%).

Table 4.3 The effect of salt stress on stem length in bean genotypes

Stem Length (cm plant^{-1})			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	77,67 c-e	47,33 c	-39,06
GT-2	108,67a	39,67 c-f	-63,49
GT-3	75,00 de	41,00 c-f	-45,33
GT-4	81,33 c-e	37,00 d-g	-54,51
GT-5	66,00 ef	42,00 c-e	-36,36
GT-6	85,00 b-d	60,67 b	-28,62
GT-7	78,00 c-e	56,00 b	-28,21
GT-8	79,00 c-e	26,67 hi	-66,24
GT-9	57,67 f	40,00 c-f	-30,64
GT-10	66,50 f	23,00 i	-65,41
GT-11	70,67 d-f	59,00 b	-16,51
GT-12	64,33 ef	38,33 d-g	-40,42
GT-13	81,33 c-e	43,67 cd	-46,31
GT-14	79,00	30,00 g-i	-62,03
GT-15	76,00 de	34,67 e-h	-54,38
GT-16	94,33 a-c	70,00 a	-25,79
GT-17	68,00 d-f	33,00 f-h	-51,47
GT-18	80,67 c-e	27,33 hi	-66,12
GT-19	76,67 de	42,33 c-e	-44,79
GT-20	101,33 ab	37,67 d-g	-62,82
LSD $_{(0.05)}$	17,15	8.61	

Table 4.4 The effect of salt stress on stem diameter in bean genotypes

Stem Diameter (mm plant^{-1})			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	6,10 a-d	3,19 ef	-47,70
GT-2	5,57 c-f	2,10 g	-62,30
GT-3	6,13 a-d	2,95 f	-51,88
GT-4	6,23a-d	2,02 gh	-67,58
GT-5	6,20 h	3,18 ef	-48,71
GT-6	6,63 a-d	6,10 a	-7,99
GT-7	5,70 c-f	5,20 b	-8,77
GT-8	4,30 e-g	1,93 gh	-55,12
GT-9	5,30 d-f	3,67 d	-30,75
GT-10	5,00 gh	2,19 g	-56,20
GT-11	6,17 a-d	5,97 a	-3,24
GT-12	7,37 ab	4,73 c	-35,82
GT-13	5,83 b-e	3,53 de	-39,45
GT-14	4,27 f-h	1,20 i	-71,90
GT-15	7,10 a-c	2,07 g	-70,85
GT-16	7,40 a	5,33 b	-27,97
GT-17	6,27 a-d	2,83 f	-54,86
GT-18	6,13 a-d	1,70 h	-72,27
GT-19	6,23 a-d	1,93 gh	-69,02
GT-20	5,95 a-d	2,22 g	-62,69
LSD (0.05)	1,56	0,37	

4.4 Leaf Area

Changes in terms of leaf area are shown in Table 4.5. The highest leaf area values under the application of 150 mM NaCl occurred in genotypes GT-6 (1273,15 cm^2/plant), GT-16 (122,.77 cm^2/plant), GT-12 (855,78 cm^2/plant), GT-1 (833.90 cm^2/plant), and GT-11 (827.27 cm^2/plant). The lowest leaf area values were determined in genotypes GT-8 (199,89 cm^2/plant) and GT-20 (187,99 cm^2/plant). When compared to the control plants, the least losses in terms of leaf area were observed in genotypes GT-7 (20,35%), GT-16 (23,91%), and GT-11 (24,02%). Among the genotypes with the highest changes, GT-19 (81,62%), GT-8 (82,41%), and GT-20 (83,96%) took the top positions. Changes in other genotypes ranged between 26% and 79%.

Table 4.5 The impact of salt stress on the leaf area of bean genotypes

Leaf Area (cm^2 $plant^{-1}$)			
Genotypes	Control	Salt	% Change
GT-1	1302,95 a-d	833,9 b	-36,00
GT-2	1576,64 ab	544,98 de	-65,43
GT-3	1469,55 a-c	425,98 ef	-71,01
GT-4	1267,25 a-d	342,69 fg	-72,96
GT-5	1064,96 b-d	592,58 d	-44,36
GT-6	1731,34 a	1273,15 a	-26,46
GT-7	826,97 d	658,71 cd	-20,35
GT-8	1136,36 b-d	199,89 g	-82,41
GT-9	1350,55 a-d	775,94 bc	-42,55
GT-10	1172,06 a-d	330,79 fg	-71,78
GT-11	1088,76 b-d	827,27 b	-24,02
GT-12	1291,05 a-d	855,78 b	-33,71
GT-13	945,96 cd	575,88 de	-39,12
GT-14	1255,35 a-d	295,09 fg	-76,49
GT-15	1398,15 a-c	283,19 fg	-79,75
GT-16	1612,34 ab	1226,77 a	-23,91
GT-17	1231,55 a-d	306,99 fg	-75,07
GT-18	1053,06 b-d	259,39 g	-75,37
GT-19	1540,94 ab	283,19 fg	-81,62
GT-20	1172,06 a-d	187,99 g	-83,96
LSD ($_{0.05}$)	564,02	161,81	

4.5 Leaf Relative Water Content (RWC)

Changes in leaf relative water content (RWC) are shown in Table 4.6. The highest RWC values were observed in genotypes GT-11 (71,02%), GT-16 (70,59%), and GT-12 (68,49%) in response to salt stress application. The lowest RWC values were determined in genotypes GT-18 (21,16%) and GT-14 (20,91%). When compared to the control plants, the least losses in LRWC were found in genotypes GT-11 (13,83%), GT-16 (14,20%), and GT-12 (18,44%). Among the genotypes with the most significant changes, GT-18 (73,52%) and GT-14 (74,36%) took the lead. Changes in other genotypes ranged from 20% to 64%.

Table 4.6 The effect of salt stress on leaf relative water content (RWC) in bean genotypes

	RWC (%)		
Genotypes	**Control**	**Salt**	**% Change**
GT-1	81,52	58,97 ed	-27,66
GT-2	85,71	35,69 f	-58,36
GT-3	81,69	37,39 f	-54,23
GT-4	82,55	30,30 g	-63,29
GT-5	79,38	51,18 e	-35,53
GT-6	80,67	64,40 bc	-20,17
GT-7	84,28	61,47 cd	-27,06
GT-8	81,36	28,61 g	-64,84
GT-9	82,87	50,70 e	-38,82
GT-10	81,61	39,08 f	-52,11
GT-11	82,42	71,02 a	-13,83
GT-12	83,98	68,49 ab	-18,44
GT-13	87,00	52,99 e	-39,09
GT-14	81,56	20,91 h	-74,36
GT-15	82,00	39,22 f	-52,17
GT-16	82,27	70,59 a	-14,20
GT-17	81,72	29,07 g	-64,43
GT-18	79,92	21,16 h	-73,52
GT-19	83,64	29,50 g	-64,73
GT-20	83,65	30,44 g	-63,61
LSD $_{(0.05)}$	ns	4,38	

4.6 Membrane Damage Index (MDI)

Changes in terms of membrane damage index (MDI) are illustrated in Figure 4.2. Due to stress application, MDI values increased in all genotypes, ranging between 13% and 62%. As the level of damage increased, MDI values also exhibited an increase. Among the genotypes subjected to salt stress, the ones with the lowest and hence least damage were ranked as GT-11 (13,62%), GT-16 (14,61%), and GT-6 (18,84%). The genotypes with the highest damage included GT-19 (61,16%), GT-14 (61,44%), and GT-18 (62,61%), ranking at the top. MDI in other genotypes ranged between 20% and 57%.

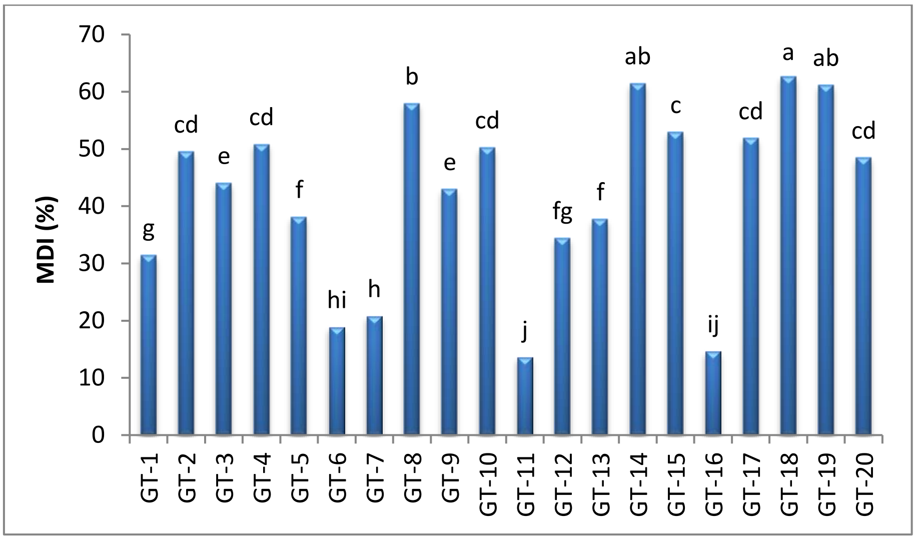

Figure 4.2 The effect of salt stress on membrane damage ındex (MDI) in bean genotypes

4.7 SPAD Value

The changes in leaf SPAD values are shown in Table 4.7. A decrease in SPAD values compared to control plants occurred under stress conditions. The highest SPAD values under salt stress were observed in genotypes GT-16 (51,10) and GT-11 (40,50). The lowest SPAD values were determined in genotypes GT-19 (16,13) and GT-20 (12,73). When compared to control plants, the least losses in terms of SPAD values were found in genotypes GT-11 (16,62), GT-16 (17,02), GT-5 (18,28), and GT-6 (19,99). Among the genotypes with the most significant changes, GT-19 (71,52) and GT-20 (76,56) ranked first. The changes in other genotypes ranged between 24% and 54%.

Table 4.7 The effect of salt stress on SPAD value in bean genotypes

SPAD			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	46,80 b-e	34,73 b-d	-25,79
GT-2	48,27 a-e	25,13 d-g	-47,94
GT-3	47,03 b-e	27,40 c-f	-41,74
GT-4	42,70 c-e	20,00 f-h	-53,16
GT-5	40,87 e	33,40 b-e	-18,28
GT-6	45,37 b-e	36,30 bc	-19,99
GT-7	42,30 de	29,87 c-e	-29,39
GT-8	55,00 a-d	26,43 d-f	-51,95
GT-9	42,47 de	31,47 b-e	-25,90
GT-10	46,73 b-e	25,30 d-g	-45,86
GT-11	48,57 a-e	40,50 ab	-16,62
GT-12	41,80 de	26,60 c-f	-36,36
GT-13	43,63 c-e	32,73 b-e	-24,98
GT-14	61,63 a	28,13 c-f	-54,36
GT-15	59,27 ab	31,03 b-e	-47,65
GT-16	61,57 a	51,10 a	-17,01
GT-17	48,97 a-e	24,90 e-g	-49,15
GT-18	45,37 b-e	25,90 d-g	-42,91
GT-19	56,63 a-c	16,13 gh	-71,52
GT-20	54,33 a-e	12,73 h	-76,57
LSD (0.05)	14,04	0,74	

4.8 Sodium Ion Content

The study examined the tolerance levels to salt stress in twenty different bean genotypes by analyzing the sodium ion contents in green tissues, and the findings are presented in Table 4.8. Sodium (Na) ion content was determined to be the lowest in control plants, showing a variation of 0.59-0.73%. Under salt stress conditions, there was an increase in Na ion content, and this increase varied among genotypes. The highest Na values under salt stress were observed in genotypes GT-14 (7,08%), GT-19 (7,10%), and GT-18 (7,14%). The lowest Na content was determined in genotypes GT-11 (2,56%) and GT-16 (3,00%). When compared to control plants, genotypes GT-11 (255,56%) and GT-16 (310,96%) ranked first in selectively incorporating lower levels of toxic ions. In contrast, genotypes GT-8 (951,56%) and GT-19 (959,70%) were identified as the ones incorporating the highest amounts of Na ions. In other genotypes, the increase in Na ion content compared to control plants ranged from 382% to 933%.

Table 4.8 The effect of salt stress on sodium (Na) ion content in bean genotypes

Na (%)			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	0,64 c-e	4,42 f	590,63
GT-2	0,60 de	6,08 cd	913,33
GT-3	0,71 a-c	5,26 e	640,85
GT-4	0,59 e	6,10 cd	933,90
GT-5	0,69 a-c	5,23 e	657,97
GT-6	0,65 b-e	3,19 g	390,77
GT-7	0,69 a-c	3,33 g	382,61
GT-8	0,64 c-e	6,73 ab	951,56
GT-9	0,73 ab	5,70 d	680,82
GT-10	0,69 a-c	6,64 b	862,32
GT-11	0,72 a-c	2,56 h	255,56
GT-12	0,68 a-d	4,41 f	548,53
GT-13	0,60 de	5,88 d	880,00
GT-14	0,69 a-c	7,08 a	926,09
GT-15	0,66 a-e	6,73 ab	919,70
GT-16	0,73 a	3,00 g	310,96
GT-17	0,66 a-e	6,58 b	896,97
GT-18	0,72 a-c	7,14 a	891,67
GT-19	0,67 a-d	7,10 a	959,70
GT-20	0,65 b-e	6,46 bc	893,85
LSD $_{(0.05)}$	0,08	0,41	

4.9 Chloride Ion Content

In a study investigating the tolerance levels of twenty different bean genotypes to salt stress, the chlor ion contents of green leaf tissues were examined, and the findings are presented in Table 4.9. The determination of chlor (Cl) ion content showed the lowest values in control plants, ranging from 0,38 to 0,51. Under salt stress conditions, there was an increase in Cl ion content, and this increase varied among genotypes. The highest Cl values under salt stress were observed in genotypes GT-14 (5,20%), GT-18 (5,21%), GT-8 (5,23%), and GT-19 (5,29%). The lowest Cl content, on the other hand, was determined in genotypes GT-16 (2,16%), GT-11 (2,19%), and GT-6 (2,27%). When compared to control plants, genotypes GT-16 (323,55%) and GT-11 (338,00%) ranked first in terms of being selective in absorbing lower levels of toxic ions. In contrast, genotypes GT-2 (1152,63%), GT-4 (1291,89%), and GT-8 (1703,45%) were identified as genotypes absorbing the highest amounts of Cl ions. In other genotypes, the increase in Cl ion content compared to control plants ranged from 427% to 1069%.

Table 4.9 The effect of salt stress on chloride (Cl) ion content in bean genotypes

Cl (%)			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	0,42 a-c	2,84 i	576,19
GT-2	0,38 b-d	4,76 d	1152,63
GT-3	0,49 a	4,31 e	779,59
GT-4	0,37 cd	5,15 a-c	1291,89
GT-5	0,47 ab	3,64 gh	674,47
GT-6	0,43 a-c	2,27 jk	427,91
GT-7	0,39 b-d	2,36 j	505,13
GT-8	0,29 d	5,23 ab	1703,45
GT-9	0,51 a	3,72 g	629,41
GT-10	0,47 ab	4,17 e	787,23
GT-11	0,50 a	2,19 k	338,00
GT-12	0,46 a-c	3,94 f	756,52
GT-13	0,38 b-d	3,54 h	831,58
GT-14	0,47 ab	5,20 ab	1006,38
GT-15	0,44 a-c	4,74 d	977,27
GT-16	0,51 a	2,16 k	323,53
GT-17	0,44 a-c	5,12 bc	1063,64
GT-18	0,50 a	5,21 ab	942,00
GT-19	0,45 a-c	5,29 a	1075,56
GT-20	0,43 a-c	5,03 c	1069,77
LSD (0.05)	0,10	0,15	

4.10 Potassium Ion Content

In the study examining the tolerance levels to salt stress of twenty different bean genotypes, the potassium ion contents of the green parts were investigated, and the findings are presented in Table 4.10.

Potassium (K) ion content was determined in the highest control plants and ranged from 3,85% to 5,32% (Table 4.10). Under salt stress conditions, a decrease in K ion content was observed, and this decrease varied among genotypes. The highest K values under salt stress were observed in genotypes GT-16 (3,63%) and GT-6 (3,58%). The lowest K content was determined in genotype GT-19 (1,16%). Among genotypes that preserved their K content compared to control plants, GT-7 (15,63%), GT-11 (17,49%), and GT-6 (19,37%) ranked highest. In contrast, genotypes GT-18 (71,00%), GT-14 (72,03%), GT-20 (72,34%), and GT-19 (72,83%) showed the highest K loss compared to control plants under salt stress conditions. In other genotypes, the decrease in K ion content compared to control plants occurred in the range of 20-70%.

Table 4.10 The effect of salt stress on potassium (K) ion content in bean genotypes

K (%)			
Genotypes	**Control**	**Salt**	**% Change**
GT-1	3,85 ef	2,32 de	-39,74
GT-2	4,29 bc	1,88 g	-56,18
GT-3	4,08 c-f	2,05 fg	-49,75
GT-4	4,22 b-e	1,60 hi	-62,09
GT-5	3,90 d-f	2,34 de	-40,00
GT-6	4,44 bc	3,58 a	-19,37
GT-7	3,84 ef	3,24 b	-15,63
GT-8	5,32 a	1,57 hi	-70,49
GT-9	4,39 bc	2,84 c	-35,31
GT-10	4,97 a	2,18 ef	-56,14
GT-11	3,83 f	3,16 b	-17,49
GT-12	4,37 bc	3,24 b	-25,86
GT-13	4,16 c-f	2,52 d	-39,42
GT-14	5,22 a	1,46 hi	-72,03
GT-15	4,27 b-d	1,58 hi	-63,00
GT-16	4,58 b	3,63 a	-20,74
GT-17	4,55 b	1,65 h	-63,74
GT-18	5,00 a	1,45 hi	-71,00
GT-19	4,27 b-d	1,16 j	-72,83
GT-20	5,17 a	1,43 i	-72,34
LSD (0.05)	0,38	0,23	

4.11 Calcium Ion Content

The study investigated the levels of tolerance to salt stress in twenty different bean genotypes, and the calcium ion contents in green tissues were examined. The findings are presented in Table 4.11. Calcium (Ca) ion content was determined in the highest control plants, ranging between 2,24% and 3,33%. Under salt stress conditions, a decrease in Ca ion content was observed, and this decrease varied among genotypes. The highest Ca values in response to salt stress were observed in genotypes GT-6 (2,57%) and GT-7 (2,46%). The lowest Ca content was determined in genotypes GT-8 (1,09%) and GT-19 (1,01%). When compared to control plants, genotypes GT-16 (10,04%) and GT-11 (11,28%) ranked highest in maintaining Ca content. In contrast, genotypes GT-8 (65,94%) and GT-19 (68,04%) exhibited the highest Ca loss under salt stress conditions compared to control plants. In other genotypes, the decrease in Ca ion content ranged from 16% to 62% compared to control plants.

Table 4.11 The effect of salt stress on calcium (Ca) ion content in bean genotypes

	Ca (%)		
Genotypes	**Control**	**Salt**	**% Change**
GT-1	2,24 g	1,37 gh	-38,84
GT-2	3,08 a-c	1,24 hi	-59,74
GT-3	2,55 f	1,44 fg	-43,53
GT-4	3,10 a-c	1,23 ij	-60,32
GT-5	3,33 a	2,22 cd	-33,33
GT-6	3,08 a-c	2,57 a	-16,56
GT-7	3,04 bc	2,46 ab	-19,08
GT-8	3,20 a-c	1,09 jk	-65,94
GT-9	2,98 cd	1,98 e	-33,56
GT-10	3,20 a-c	1,26 hi	-60,63
GT-11	2,66 ef	2,36 bc	-11,28
GT-12	3,17 a-c	2,18 d	-31,23
GT-13	3,28 ab	2,34 bc	-28,66
GT-14	2,93 c-e	1,16 ij	-60,41
GT-15	3,09 a-c	1,55 f	-49,84
GT-16	2,59 f	2,33 bc	-10,04
GT-17	2,76 d-f	1,19 ij	-56,88
GT-18	3,06 a-c	1,16 ij	-62,09
GT-19	3,16 a-c	1,01 k	-68,04
GT-20	3,18 a-c	1,25 hi	-60,69
LSD ($_{0.05}$)	0,28	0,14	

The necessity to meet the nutritional needs of the increasing world population has heightened the interest in enhancing plant production and productivity. Consequently, improving yield and quality in plant production has become an urgent global agricultural issue. Salt stress is a significant environmental problem threatening agriculture worldwide. Approximately 20% of the world's irrigated agricultural areas are adversely affected by soil salinization. Factors such as environmental degradation, poor irrigation practices, fertilization, and climate change contribute to the escalating nature of this issue. In this context, efforts to effectively enhance yield are of great importance (Zhao *et al.* 2021).

Salinity negatively impacts morphological and biochemical functions in plants, affecting seed germination, plant growth, development, and yield adversely (Zhang and Dai 2019). It reduces the content of chlorophyll and carotenoids, disrupts chloroplast structure, and limits the efficiency of photosynthetic systems, including the PSII system (Pan *et al.* 2020). Soil salinity leads to a decrease in soil water potential and leaf water potential, causing imbalances in plant water relations and ultimately resulting in osmotic stress (Navada *et al.* 2020). Salinity induces osmotic stress due to an increase in sodium and chloride ion contents. Under stress conditions, there is a reduction in plant growth and development, an increase in reactive oxygen species (ROS) levels, leading to oxidative stress (Arif *et al.* 2020, Abdel Latef *et al.* 2021).

In a study examining the effects of 150 mM NaCl application on beans, salt stress resulted in a decrease in morphological parameters such as fresh and dry weight, stem length and diameter, and leaf area. Overall, compared to control plants, there was a 52% reduction in fresh weight, 70% in dry weight, 46% in stem length, 47% in stem diameter, and 56% in leaf area. This variation revealed differences among genotypes, with lower-scale genotypes showing less reduction. Salt stress is one of the most significant factors adversely affecting plant growth and development, particularly due to osmotic effects, ion toxicity, reduced photosynthetic activity, and nutrient deficiency under stress conditions (Almodares *et al.* 2008). In salt stress conditions, a decrease in soil water potential leads to a slowdown in plant development. In the ionic phase, toxic ions that cannot be retained in vacuoles for an extended period rapidly accumulate in the cell wall or cytoplasm, causing damage to the cells (Acosta-Matos *et al.* 2017).

In their study examining the tolerance levels of 81 bean genotypes to salt stress, Kaya (2011) reported a decrease in morphological parameters such as fresh and dry weight, stem height, and leaf area due to salt stress. They highlighted that this change revealed differences among genotypes. Garrido *et al.* (2014), investigating the physiological and biochemical changes under salt stress, expressed a decrease in physiological parameters such as fresh weight, leaf area, and leaf relative water content under stress conditions. Similar results were emphasized in a study by Ahmed *et al.* (2019), where researchers indicated a decrease in morphological and physiological parameters 21 days after stress application.

In a study determining the tolerance levels of different tomato varieties to salt stress, parameters such as stem and root length, fresh and dry weight showed a decrease with increasing salt concentration. The negative relationship between these parameters and salt stress was attributed to ion toxicity, a decrease in vitamins, osmotic consequences of salinity, and imbalance in water absorption (Sivakumar *et al.* 2020). Sardar *et al.* (2023) stated that salt stress is one of the most significant abiotic stress conditions negatively affecting plant growth, development, and yield. They reported a decrease in plant growth and development under salt stress conditions applied at different concentrations (25, 50, 75, and 100 mM NaCl) in lettuce.

The presence of high salt concentration increases soil osmotic pressure, reducing or even completely stopping the plant's water absorption capability. Ion toxicity, especially with Na and Cl ions, causes damage to cells involved in water transport, leading to disturbances in the plant-water relationship. The relative water content (RWC) in plant tissues and cells supports the continuation of metabolic activities by facilitating adaptive osmotic changes and adaptation to stress conditions. Sitohy *et al.* (2020) indicated a decrease in RWC values under salt stress in their bean study and highlighted the importance of high RWC presence in plant tissues as a crucial element supporting metabolic activities under stress conditions.

In the bean study, a 46% decrease in leaf relative water content was observed under salt stress. This decrease ranged from 13% to 18% for genotypes GT-11, GT-16, and GT-12, while it averaged 73% for genotypes GT-18 and GT-14. Hand *et al.* (2021) suggested that the decrease in leaf relative water content could be associated with Na and Cl ion toxicity. Studies conducted on various crops such as okra, corn, melon, and beans have reported a decrease in YOSI values along with salt stress. This decrease was lower in genotypes tolerant to salt stress and higher in sensitive genotypes (Kuşvuran 2010, Kaya 2011).

Salt stress leads to the breakdown of chloroplasts, instability of pigment-protein complexes, disappearance of chlorophylls, and changes in the quantity and composition of carotenoids (Bayram *et al.* 2021). Indeed, in beans, a 40% decrease in average SPAD values occurred in conjunction with salt stress. Inhibition of chlorophyll biosynthesis, accelerated degradation, and oxidative damage caused by salinity are considered reasons for the decrease in chlorophyll content. Osuna-Rodríguez *et al.* (2023), in their studies on peppers, expressed that chlorophyll content decreased with salt stress, emphasizing the fundamental role of chlorophyll in regulating the photosynthetic capacity of plants and effective carbon assimilation for plant growth and development. They reported that tolerant genotypes had higher chlorophyll content. Studies conducted on eggplant, spinach, and tomatoes also reported a decrease in chlorophyll content under salt stress (Sanwal *et al.* 2022, El-Nakhel *et al.* 2022, Tao *et al.* 2023).

Free radicals formed as a result of stress are responsible for irreversible damage to membrane lipids and proteins. Reactive oxygen species easily affect membrane lipids, leading to the formation of unsaturated aldehydes. The most important mechanism in the formation of tissue damage due to free radicals resulting from stress is the peroxidation of lipids in the cell membrane. Free oxygen radicals react with polyunsaturated fatty acids, initiating lipid peroxidation. The end product of lipid peroxidation, malondialdehyde (MDA), is ethene and pentane. The generated MDA affects ion exchange from cell membranes, leading to cross-linking of compounds in the membrane and causing negative outcomes such as changes in ion permeability and enzyme activity. Lipid peroxidation leads to the loss of membrane integrity, increased permeability of the cell to electrolytes, and membrane damage index (MDI) is used as a significant indicator in determining salt tolerance in plants (Stefanov *et al.* 2023). Salt stress has led to an increase in MDI in all bean genotypes to varying extents. This increase ranged from 13% to 18% in genotypes GT-6, GT-11, and GT-16, while it reached 61%-62% in genotypes GT-14, GT-18, and GT-19. Güneş *et al.* (2023) reported an increase in MDI levels in peppers due to salt stress, showing an increase in MDI values with an increase in salt concentration.

The accumulation of salt in the root zone, in addition to osmotic stress, limits the content of macro-nutrient elements such as potassium and calcium, negatively affecting ion balance in the cell by increasing sodium and chlorine intake. Ion toxicity impacts photosynthesis and protein synthesis, inactivates enzymes, and causes damage to chloroplasts and other organelles. When sodium reaches toxic levels, it affects the uptake and regulation of potassium and calcium ions in the plant, while toxic levels of chlorine affect the uptake and regulation of NO_3 anions (Atalan and Gökçe). Potassium, absorbed through active absorption, plays a role in providing osmotic potential and is effective in cellular water entry. Salinity also brings about drought, a secondary stress factor. During drought stress, water uptake by roots is restricted, leading to decreased nutrient flow to tissues and organs, resulting in nutrient deficiencies. A decrease in calcium content in tissues also occurs over the process. In eggplant, salt stress has led to an increase in Na and Cl ion content in green tissues, causing variations among genotypes (Kıran *et al.* 2015). Studies on beans, okra, melon, corn, and pepper indicate an increase in Na and Cl ion content in plant tissues under salt stress conditions induced by NaCl applications. The reason for growth reduction under stress is the closure of stomata, limiting photosynthate production due to toxic accumulation of Na and Cl in leaves. In this study, the application of 150 mM NaCl resulted in an average increase of 724,29% in Na and 845,61% in Cl ion content in all genotypes. This change varied among genotypes, with genotypes GT-6, GT-7, GT-11, and GT-16 showing an increase in Na and Cl ion content ranging from 255,56% to 505,13%, while genotypes GT-4, GT-8, and GT-19 showed an increase ranging from 933,90% to 1703,45%. Studies on beans, okra, and wheat have reported lower Na and Cl ion content in genotypes with lower scale values and higher increases in Na and Cl content in genotypes with higher scale values (Kaya 2011, Kuşvuran 2012, Tao *et al.* 2021). Potassium is a fundamental component of plant structure and organic molecules. It plays a role in many physiological and biochemical reactions affecting plant development, yield, and quality. Potassium controls turgor pressure, the functioning of stomata during photosynthesis and respiration, and photophosphorylation. Potassium is essential in physiological processes such as the transport of photosynthetic products, osmoregulation maintenance, and the activation of enzymes under stress conditions. Potassium deficiency leads to a significant decrease in photosynthetic CO_2 fixation and disruptions in the use of photo-assimilates. Calcium is a vital nutrient element playing a crucial role in cellular and molecular physiological processes that influence plant growth and responses to abiotic stress (Waraich *et al.* 2011, García-Martí *et al.* 2019). Calcium is involved in controlling stomatal closure. The effect of calcium on stomatal closure is an adaptation mechanism observed in tolerant plants under stress conditions such as high temperature, drought, and salinity (Akhoundnejad *et al.* 2021). In this study involving different genotypes, salt stress led to a decrease in K and Ca ions. This decrease occurred by an average of 48,16% in K ion content and 43,53% in Ca ion content. The change in K and Ca ions varied among genotypes. Genotypes GT-6, GT-7, GT-11, and GT-16 stood out with a 10-19% decrease in K and Ca ions, emphasizing their ability to preserve K and Ca ions. However, genotypes GT-8, GT-14, GT18, GT-19, and GT-20 experienced a decrease in the range of 62-73%. The accumulation of salt in the root zone, in addition to osmotic stress, limits the uptake of essential elements and minerals such as K, Ca, and NO_3, while inducing Na and Cl intake, adversely affecting cellular ion homeostasis. The accumulation of harmful ions at toxic levels affects photosynthesis and protein synthesis, inactivates enzymes, and damages chloroplasts and other organelles. When the Na cation reaches toxic levels, it also affects the uptake and regulation of K, Ca, and Mg cations in the plant (Atalan and Gökçe 2021).

5. CONCLUSIONS AND RECOMMENDATION

One of the most significant stress factors negatively affecting plant production and yield is salt stress. In a study involving twenty different genotypes, differences in salt stress tolerance were determined based on the parameters examined. A visual assessment scale was created to evaluate the effects of salt stress, and as a result, genotypes GT-6, GT-7, GT-11, and GT-16 showed the closest development to control plants. The scale values ranged between "1,33-1,67," and it was observed that as salt damage increased, the scale value increased. Genotypes GT-8, GT-14, GT-18, and GT-19 were identified as the genotypes most affected by stress with a "5" scale value.

Salt stress led to a decrease in plant fresh and dry weights, stem height and diameter, and leaf area compared to control plants. This decrease varied among genotypes. Regarding the changes compared to control plants, genotypes with lower scale values exhibited a lower rate of decrease, while an increase in scale value corresponded to an increase in losses in terms of morphological parameters.

When morphological parameters were examined, genotypes GT-6, GT-7, GT-11, and GT-16 experienced lower losses compared to control plants, whereas genotypes GT-8, GT-14, GT-18, GT-19, and GT-20 incurred the highest losses. Leaf relative water content (RWC) was determined highest in control plants and decreased in all genotypes under salt stress. Genotypes GT-11, GT-12, and GT-16 had the closest LRWC values to control plants, while GT-8, GT-14, and GT-18 stood out as genotypes with the highest losses.

The Membrane Damage Index (MDI) has increased due to salt stress. This increase has shown similarity through a scale evaluation. While the genotypes with the least damage were GT-6, GT-11, and GT-16, the genotypes with the most significant damage were listed as GT-14, GT-18, and GT-19.

SPAD values were determined to be highest in the control plants, and a decrease in SPAD values occurred with the application of 150 mM NaCl. This decrease was minimal in GT-

5, GT-11, and GT-16 genotypes. The most pronounced decrease in SPAD values was observed in GT-14, GT-19, and GT-20 genotypes.

In the study examining the effects of salt stress on different bean genotypes, an increase in Na and Cl ion contents was detected as a result of salt application. Among genotypes exhibiting selective behavior in terms of toxic ion content, GT-6, GT-7, GT-11, and GT-16 ranked highest, while GT-2, GT-4, GT-8, and GT-9 contained the highest amounts of Na and Cl ions. K and Ca ion concentrations showed a decrease with salt stress. This decrease was lower in GT-6, GT-7, GT-11, and GT-16. GT-8, GT-14, GT-19, and GT-20 bean genotypes exhibited significant increases in losses in K and Ca ion content.

In light of all these results from the study conducted in the early development stage, it was determined that bean genotypes GT-6, GT-7, GT-11, and GT-16 are tolerant, while GT-8, GT-14, GT-18, and GT-19 genotypes are sensitive. In future studies, it is recommended to conduct more detailed physiological studies with tolerant and sensitive genotypes, as well as field studies, to evaluate productivity and yield parameters.

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