TURKISH REPUBLIC
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF FIELD CROPS

THE EFFECT OF GIBBERELLIC ACID APPLICATIONS ON GERMINATION AND SEEDLING DEVELOPMENT OF SOME *FESTULOLIUM* CULTIVARS UNDER SALT STRESS

Prepared by
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Supervisor
Prof. Dr. Satı UZUN

M. Sc. Thesis

July, 2022
KAYSERİ

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COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in this study was obtained in accordance with academic and ethical rules. I also state that, as required by these rules and behaviors, I have fully cited and referenced all materials and results that are not inherent in this study.

Name- Surname : Hilda Farida

Signature :

The Master's thesis entitled "**The Effect of Gibberellic Acid Applications on Germination and Seedling Development of Some *Festulolium* Cultivars Under Salt Stress**" was prepared in accordance Erciyes University Graduate School of Natural and Applied Sciences Institute Thesis Preparation and Writing Guide.

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July, 2022, KAYSERİ

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Hilda FARIDA

Erciyes University, Graduate School of Natural and Applied Sciences
Master Thesis, July 2022
Supervisor: Prof. Dr. Satı UZUN

ABSTRACT

This thesis was carried out to determine the effects of gibberellic acid treatments and salt stress on germination and seedling development stages of different *Festulolium* cultivars. Hostyn and Lofa *Festulolium* cultivars were used as the plant material. Salinity levels were arranged as control, 5, 10, 15 and 20 dS/m. Distilled water, 100 ppm and 200 ppm GA_3 were applied to the seeds for 12 hours at room temperature. Untreated seeds were used as a control. The experiment was carried out with 4 replications. In the experiment, germination percentage, mean germination time, shoot and root length, seedling fresh and dry weight, seedling vigor index and proline contents were investigated. The research results showed that salinity stress caused a decrease in germination percentage, shoot and root length and seedling vigor index of both cultivars, while it caused an increase in mean germination time and proline content of seedlings. Seed germination and seedling growth parameters significantly decreased at 15 and 20 dS/m salinity levels. Seedling vigor index (9.63) of Lofa cultivar was higher than Hostyn cultivar (7.96) at 20 dS/m salt level. At control, 5 and 10 dS/m salinity levels, 200 ppm GA_3 treatment caused an increase in seedling vigor index as compared to both untreated seeds and distilled water treatments. GA_3 treatments at high salinity levels significantly increased proline content of seedlings. In conclusion, 200 ppm GA_3 treatment increased germination percentage, shoot length, seedling vigor index and proline content and decreased mean germination time under salinity stress.

Keywords: *Festulolium*, germination, gibberellic acid, seed treatment, salinity

TUZ STRESİ ALTINDA GİBBERELLİK ASİT UYGULAMALARININ BAZI *FESTULOLİUM* ÇEŞİTLERİNDE ÇİMLENME VE FİDE GELİŞİMİ ÜZERİNE ETKİLERİ

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ÖZET

Bu çalışma farklı *Festulolium* çeşitlerinin çimlenme ve fide gelişim dönemlerinde gibberellik asit uygulamalarının tuz stresine etkilerini belirlemek amacıyla yürütülmüştür. Çalışmada Hostyn ve Lofa *Festulolium* çeşitleri kullanılmıştır. Tuz seviyeleri kontrol, 5, 10, 15 ve 20 dS/m olarak belirlenmiştir. Tohumlara saf su, 100 ppm ve 200 ppm GA_3 12 saat oda sıcaklığında uygulanmıştır. Uygulama yapılmamış tohumlar kontrol olarak kullanılmıştır. Deneme 4 tekerrürlü olarak yürütülmüştür. Denemede çimlenme yüzdesi, ortalama çimlenme süresi, sürgün ve kök boyu, fide yaş ve kuru ağırlığı, fide güç indeksi ve prolin miktarı incelenmiştir. Araştırma sonuçlarına göre tuz stresi her iki çeşitte de çimlenme yüzdesi, sürgün ve kök uzunluğu ve fide güç indeksinde azalmaya neden olurken ortalama çimlenme süresi ve fidede prolin miktarında artışa neden olmuştur. Çimlenme ve fide büyüme özelliklerinde 15 ve 20 dS/m tuz seviyelerinde önemli düşüşler kaydedilmiştir. 20 dS/m tuz seviyesinde Lofa çeşidinin fide güç indeksi (9.63) Hostyn çeşidinden (7.96) daha yüksek bulunmuştur. Her iki çeşitte de kontrol, 5 ve 10 dS/m tuz seviyelerinde tohuma 200 ppm GA_3 uygulaması kontrol ve saf su uygulamasına göre fide güç indeksinin artmasına neden olmuştur. GA_3 uygulamaları yüksek tuz seviyelerinde fidede prolin içeriğini önemli miktarda artırmıştır. Sonuç olarak, tuz stresi altında 200 ppm GA_3 uygulaması çimlenme oranını, sürgün uzunluğunu, fide güç indeksini ve fidede prolin içeriğini artırmış, ortama çimlenme süresini ise düşürmüştür.

Anahtar Kelimeler: *Festulolium,* çimlenme, gibberellik asit, tohum uygulaması, tuzluluk

ABBREVIATIONS and SYMBOLS

GA_3	: Gibberellic Acid
EC	: Electrical Conductivity
NaCl	: Sodium Chloride
SA	: Salicylic acid
μM	: Micro Molar
mM	: Milli Molar
Na/K	: Sodium/Potassium
ppm	: Part Per Million
MGT	: Mean Germination Time
GP	: Germination Percentage
SVI	: Seedling Vigor Index
DF	: Degrees of Freedom
DW	: Dry Weight

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INTRODUCTION

Festuca and *Lolium* are two important genera that include important species as meadow, pasture and fodder plants in the grass family, *Poaceae*. Especially *Festuca arundinacea* has a good drought tolerance and *Festuca pratensis* is known for its winter resistance, *Lolium multiflorum* (Italian ryegrass) and *Lolium perenne* (perennial ryegrass) have high yield, good-feed quality, fast growth, dark leaf color, relatively fine texture, good density and uniformity (Kopecký et al. 2009). However, high-yielding ryegrasses suffer from abiotic stresses (Majka et al., 2020). Researchers successfully crossed *Festuca pratense* or *Festuca arundinacea* and *Lolium perenne* or *Lolium multiflorum*. The cross produced a new hybrid cultivar called *Festulolium* (Kopecky et al., 2006; Kopecky et al., 2009). The genetic character 'high yield' owned by this cultivar was obtained from the parental grass "*Lolium perenne* and *Lolium multiflorum*", while the superior character 'high resistance to biotic and abiotic factors' comes from the parental grass " *Festuca pratensis* and *Festuca arundinacea*" (Borsuk and Fijalkowska, 2019). Intergeneric cross-hybridization between *Festuca* and *Lolium* species resulted in registration of almost one hundred cultivars (Majka et al., 2020).

More than 800 million ha of the world's total land area are affected by salt (Munns and Tester, 2008). Salinity is one of the important problems that reduce agricultural productivity in arid and semi-arid climatic regions of the world, insufficient precipitation and high evaporation, inadequacy of drainage, wrong agricultural practices and soil properties are the leading causes of salinity and affect large areas (Tiryaki, 2018). Salinity affects plant growth and development via osmotic stress, ion toxicity as a result of excessive Cl^- and Na^+ ions and nutritional imbalance (Güneş et al., 2013; Ali et al., 2021). Different strategies have been used to alleviate the impacts of salinity stress. One of these is seed priming. There are several seed priming techniques such as hydropriming, osmopriming, halopriming, hormone priming, hardening, stratification and thermal shock (Ibrahim, 2016). Seed priming regulates several physiochemical and molecular processes

in plants, which can improve crop establishment under salinity stress (Ibrahim, 2016; Johnson and Puthur, 2021). Gibberellins, as one of the major plant hormones, have been reported to be a promoter for plant growth under salinity conditions (Ali et al., 2021). Previous studies have revealed that priming with gibberellic acid (GA_3) could significantly promote seed germination, increase the salt tolerance of seeds, and mitigate the inhibition of seedling growth (Chauhan et al., 2019; Zhu et al., 2019; Jiao et al., 2019). This thesis was conducted to determine the effects of gibberellic acid (GA_3) treatments on germination and seedling development of two *Festulolium* cultivars (Hostyn and Lofa) under salt stress.

CHAPTER 1

LITERATURE REVIEW

Iqbal and Ashraf (2013) investigated the effects of GA_3 priming for induction of salt tolerance in two spring wheat (*Triticum aestivum*) cultivars, namely MH-97 (salt intolerant) and Inqlab-91 (salt tolerant). According to the results, under salt stress, the effect of 150 mg/L GA_3 was much more effective in improving grain yield particularly in the salt intolerant cultivar. The 150 mg/L GA_3 treatment decreased Na^+ concentrations both in the shoots and roots and increased Ca^{2+} and K^+ concentrations in the roots of both wheat cultivars.

Kandil et al. (2014) tested of sugar beet genotypes (Raspoly, Nada, Strube, Almaz, Toro, Oskarpoly) under different NaCl concentrations (distilled water as control, 1500, 3000, 45000, 6000, 7500 and 9000 ppm NaCl) with GA_3 treatments (0, 100 and 200 mg/L GA_3). According to results, the highest germination percentages, coefficient of velocity, seedling vigor index, energy of germination, emergence rate and speed of germination were recorded from 200 ppm GA_3 treatment. Increased salinity levels significantly decreased germination percentages, coefficient of velocity, seedling vigor index, energy of germination, emergence rate and speed of germination.

Sheikh-Mohamadi et al. (2017) examined the response of wheatgrass species and the genotypes to salt stress. Differences were seen between genotypes in terms of salinity tolerance. Some genotypes were more tolerant to salinity stress than the others. For all genotypes, germination percentage decreased and mean germination time increased with increasing salinity levels.

Ma et al. (2018) investigated the effects of seed priming with GA_3 on plant growth and production in *Leymus chinensis*. According to the results, GA_3 treatments significantly increased seed germination rate. The 50 μM GA_3 treatment increased above-ground fresh

and dry weight in both pot-grown conditions and field-grown conditions. It was reported that GA_3 priming led to high germination rates and better seedling growth but appropriate concentrations differed among the plant species.

Based on research by Omidi et al. (2018), salinity stress had harmful effects on growth parameters of *Sesamum indicum* L. Seed priming treatment to alleviate the toxic effects of salt stress. Two different GA_3, (250 ppm GA_3, 500 ppm) and five different salt concentrations (0, 3, 6, 9, 12 dS/m) were applied to the sesame seeds. The results indicated that the highest percentage and rate germination were achieved by the priming seeds with 500 ppm GA_3 with no salt applied to the seeds. Seed priming with GA_3 showed a higher seed germination and seedling growth.

In a research by Oner et al. (2018), gibberellic acid was applied to oat seeds to examine the effects of GA_3 on germination under salinity stress. Five doses of GA_3 (0, 60, 120, 240, and 480 mM NaCl) with 4 doses of salt (0, 75, 150, and 225 mM) were tested in that research. The research showed that salt doses had a significant effect on the germination percentage, radicula and plumula length. The 225 mM salt concentration significantly reduced the development of seedlings and seed germination of oat. There was no significant influence of the GA_3 application on the seedling and germination of oat.

Chauhan et al. (2019) reported that GA_3 may decrease the toxic effect of salinity stress. Three types of oat cultivars (NDO-2, UPO-212 and UPO-94), 2 different concentrations of GA_3 (100 ppm and 150 ppm) and NaCl (25, 50, 75, 100 mM) were tested. Oat seeds were pre-treated (soaked) with GA_3 solution for 24 hours. GA_3 increased the germination percentage, seedling vigor index, shoot and root length, tissue water content, total fresh and dry wight of NDO-2 and UPO-212 cultivars under different salt concentrations. Increasing salinity decreased the germination percentage and growth parameters of oat cultivars. It was concluded that the sensitive oat cultivar was not just sensitive to salinity stress, but also sensitive to high concentration of GA_3.

In a research by Jiao et al. (2019), gibberellic acid was applied to castor bean (*Ricinus communis*) seeds under various salt concentrations to examine the effects of GA_3 on seedling growth. Four doses of GA_3 (0, 200, 250 and 300 μM) with 4 doses of salt (0, 50, and 100 mM) were tested in that research. Plant height and stem diameter, superoxide dismutase and peroxidase activity were significantly higher in the treatment of 250 μM

GA_3 under salinity level of 50 mM NaCl; protein content was the highest when GA_3 concentration was 250 µM under salinity level of 100 mM NaCl. Proline content of castor bean seedlings increased with increasing salinity levels. 300 µM GA_3 treatment had significantly greater proline content under 100 mM NaCl salinity level on 20 and 30 d.

Zhu et al. (2019) examined the effects of GA_3 on the growth of sorghum (*Sorghum bicolor* L. Moench) under salt stress. It was indicated that the salinity level of 50 mM NaCl increased the absorption of cumulative water (cumulative water uptake) and germination index. Even so, salinity level of 100 mM NaCl inhibited the germination of plants. The application of 576 µM GA_3 or 100 mM NaCl in sorghum plants turned out to inhibit the cumulative water uptake, had a negative effect on the length of germ and radicle of sorghum seeds and reduced the germination and cumulative germination. It was recorded that the optimal concentration of GA_3 in reducing the effects of salt stress on sweet sorghum was 288 µM. At this concentration, seed germination still can be increased even under salt stress.

Ibrahim et al. (2019) recommended to soak the wheat seeds in GA_3 solutions to reduce the harmful effect of salinity stress. Four varieties of wheat (Argin, Imam, Xumai 30, Yang 11-10), three concentrations of GA_3 (0, 0.29, 0.58 mM), and four salinity levels (0, 100, 200, 300 mM NaCl) were tested in that research. The result showed that 0.58 mM GA_3 was the most effective hormone on most parameters that measured. The salinity x GA_3 interactions affected the germination rate, germination percentage, dry wight, root length, shoot length, seedling vigor index and salt tolerance index. Moreover, at 300 mM of NaCl and 0.58 mM of GA_3, water uptake increased by 33.9%. The highest germination rate (35.6), dry weight (3.7 mg/plant), shoot length (0.64 cm), salt tolerance index (22.2) and seedling vigor index (0.75) were achieved in Argin variety

According to Korkmaz et al. (2020), plant growth regulators are very important in reducing the negative effects of salt stress. Researchers specifically examined the effects of GA_3 and salicylic acid (SA) treatments on several physiological characters and the growth of canola plants (*Brassica napus* L.) under salt stress in the greenhouse. Factorial experiments were carried out with four levels of NaCl (0, 50, 100, and 150 mM) and three levels of SA (0, 0.5, and 1 mM) and GA_3 (0, 50, and 100 mg/L) with three replications. Observations were carried out on some parameters such as chlorophyll content, leaf area, electrolyte leakage, high plant, and loss of leaf turgor. Compared to the control, salt stress

significantly reduced the value of all the parameters mentioned above. Salt stress also caused a significant reduction in plant height and leaf area up to 63 and 67%, respectively. On the other hand, electrolyte leakage increased along with increasing NaCl levels in plants. The results showed that the application of SA and GA_3 in canola plants under salt stress did not have a significant effect on plant vegetative growth. Even so, GA_3 significantly increased plant height and decreased the loss of leaf turgor but did not have a significant effect on the other parameters. GA_3 can also reduce some partial negative effects of salt stress.

Shiade and Boelt (2020) examined the response of 9 varieties of tall fescue (*Festuca arundinacea*) to salt stress. Tall fescue is known as a grass that is quite tolerant to salinity. According to the result of this study, until the salt concentration reached 15 dS/m, *Armani, Essential, Fatcat,* and *Starlett* varieties showed a germination percentage of 90%. On the other hand, *Eyecandy, Rhizing Star,* and *Thomahawk* varieties showed a decrease in the percentage of germination depending on increasing salt concentrations.

Shihab and Hamza (2020) examined the effect of SA and GA_3 pre-treatments on several sorghum varieties under salt stress. The first factor in that study was cultivar (*Inqath, Rabe* and *Buhoth70*), the second factor was pre-treatment of seeds with 300 mg/L gibberellic acid +70 mg/L salicylic acid, while the third factor was salt doses (6, 9 and 12 dS/m). The results showed that the highest plant height, leaf area, dry seed weight, leaf chlorophyll and proline content, and the lowest percentage of sodium-potassium (Na/K) were sen in Bohoth70 cultivar. It was concluded that the application of SA and GA_3 on sorghum seeds at the beginning (before planting) could improve the performance of the seeds in tolerating salt stress.

According to Ahmad et al. (2021) plants have different responses to salt stress, including in of photosynthetic processes, increasing accumulation of Na^+ ion on shoots and roots and induction of oxidative stress. Researchers applied GA_3 pre-treatments *Pisum sativum L*. seeds to reduce the effects of salt stress, and indicated that seed priming with GA_3 yielded a promising effect on the physiological traits of the plant and alleviated the negative effects of salinity stress by regulating ion homeostasis, inducing the antioxidant system, production of phenol, flavonoids and proline. Plants could adapt to the salt stress by inducing expression of Na^+/H^+ antiporters proteins (SOS1 and NHX1) that increase

Na^+ sequestration. It is concluded that using GA_3 for priming seeds was an effective way to develop the salt-tolerant cultivars.

Ali et al. (2021) confirmed that the interaction between salinity treatment and seed priming with GA_3 significantly affected emergence percentage, seedling length, dry and fresh weight, soluble protein, and chlorophyll content of sorghum seeds. The level of 144.3 and 288.7μM GA_3 were identified as the most effective treatments to eliminate adverse the effects of salinity.

Shahzad et al. (2021) conducted a research about maize seed priming with GA_3 under salinity stress through modulating the morpho-physiological, biochemical and molecular attributes. The results indicated that seed priming with GA_3 made a huge promising impact on the physiological and biochemical activities of the plant. GA_3 treatments could increase the plant growth, K^+ ion concentration and antioxidant genes expression. On the other hand, salinity stress inhibited the growth of shoots and roots, reduced the chlorophyll and carotenoid content, antioxidant enzyme activities, dry and fresh weight, K^+ ion accumulation and increased the accumulation of Na^+ ion and oxidative damage. Compared to control, the seeds that were primed and sprayed with GA_3 showed the highest increase in antioxidant enzyme activities and gene expression, shoot and root length, fresh and dry weight, chlorophyll content, K^+ concentration, and total soluble protein.

Adhikari et al. (2022) treated salt-sensitive ‘Burpee Bibb’ lettuce seeds with 0.05% potassium nitrate, 3 mM GA_3, and distilled water. All seeds (primed or not) germinated at 100 mM sodium chloride (NaCl) or 0 mM NaCl. The results indicated that hydro-priming increased fresh mass and dry mass under salt stress.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Material and Growth Conditions

This research was conducted at Erciyes University Agricultural Faculty under laboratory conditions in 2021. Distilled water, NaCl and gibberellic acid (GA_3) were used as the primary materials of the research. Two types of *Festulolium* cultivars Lofa (*Lolium multiflorum*×*Festuca arundinacea*) and Hostyn (*Lolium multiflorum*×*Festuca pratensis*) were used (Humphreys and Zwierzykowski, 2020). The experimental factors included the type of cultivars, salinity levels (NaCl) and the seed treatment of GA_3. The salinity levels were arranged as 0 (control), 5, 10, 15 and 20 dS/m. The GA_3 levels were 0 ppm (distilled water), 100 ppm, and 200 ppm and non-treated seeds (used as a control). Four replications of 50 seeds for each treatment were used.

All seeds were sterilized first by using a 10% commercial bleach for 10 minutes then flushed with distilled water three times and dried on filter paper. Following to sterilization process seeds were immersed into distilled water and GA_3 solutions (100 ppm and 200 ppm) with continuous shaking (100 rpm) at dark conditions for 12 hours. After 12 hours, the seeds were washed with distilled water for 5 minutes and dried again on filter paper. Apart of sterilized seeds was used directly as a control (untreated seed). Germination tests were conducted in petri dishes with germination paper inside it. The seeds were placed in a petri dish with 5 ml of different salt solutions. All petri dishes were closed and inserted into a Ziplock plastic bag to prevent evaporation. They were incubated at 20 ºC temperature and 16 h light/8 h in the dark photoperiods.

2.2. Measurements

2.2.1. Measurement of seed germination and seedling growth

The number of germinated seeds from the first day of planting until the 14^{th} day after planting was counted (Figure 2.1). The seeds were counted as a germinated seed if it had

2 mm radicles length. The number of germinated seeds were recorded every day and used to calculate the mean germination time and the germination percentage using the following equation (Ellis and Roberts, 1981).

Mean Germination Time (MGT)): $\sum D \times n / \sum n$

D: Days that calculated from the start of the test

n: Number of seeds germinated on day D

Germination Percentage (%) = (n / N total) × 100

n : Total of germinated seeds on the last day of observation (14th day after planting)

N total: Total of whole seeds that were sown in each petri dish

After the incubation period, seedlings were sampled randomly from each petri dish. The shoots and roots were separated from the seed and the length of the shoots and roots were measured with a ruler. Ten selected seedling samples were weighed with sensitive scales to measure the fresh weight of the seedlings (shoot and root). All samples were dried at 65 °C, when the weight of the dried samples was constant or unchanged, all the samples were weighed again to see the dry weight of 10 seedlings/treatment. Then, seedling dry weight was calculated. Seedling vigor index (SVI) was calculated by using the following equation (from Ibrahim et al., 2019 according to Abdul-Baki and Anderson, 1973).

SVI = (Germination (%) × Seedling Length (cm)) /100

2.2.2. Proline Content

Proline content was determined according to Bates et al. (1973). 0.025 g of the dry seedlings were homogenized with 5 ml of 3% w/v sulfosalicylic acid. After centrifugation, 1 ml of the extract was mixed with 1 ml ninhydrin reagent and 1 ml glacial acetic acid and put at 100 °C for 60 min. Afterward, the samples were cooled in ice and then, supplemented with 2 ml toluene. The upper phase was used for measuring proline content by a spectrophotometer at 520 nm, and by using a standard curve.

2.3. Data Analysis

This study consisted of 40 treatments, 4 replications and 3 factors of experiment; (1) **two cultivars:** *Lofa* and *Hostyn*; (2) **four pre-treatments**: *non-treatment, distilled water treatment, 100 ppm* GA_3 and *200 ppm* GA_3 *treatments*; (3) **five salinity levels**: *0, 5, 10,*

15 and *20* dS/m. All data obtained were statistically analyzed in accordance with Completely Randomized Factorial Designs (CRD) with the use of SPSS package. The percentage values were subjected to arcsine transformation. Means were compared by Duncan Multiple Range Test when F values were significant ($p<0.05$) using MSTAT-C statistical software.

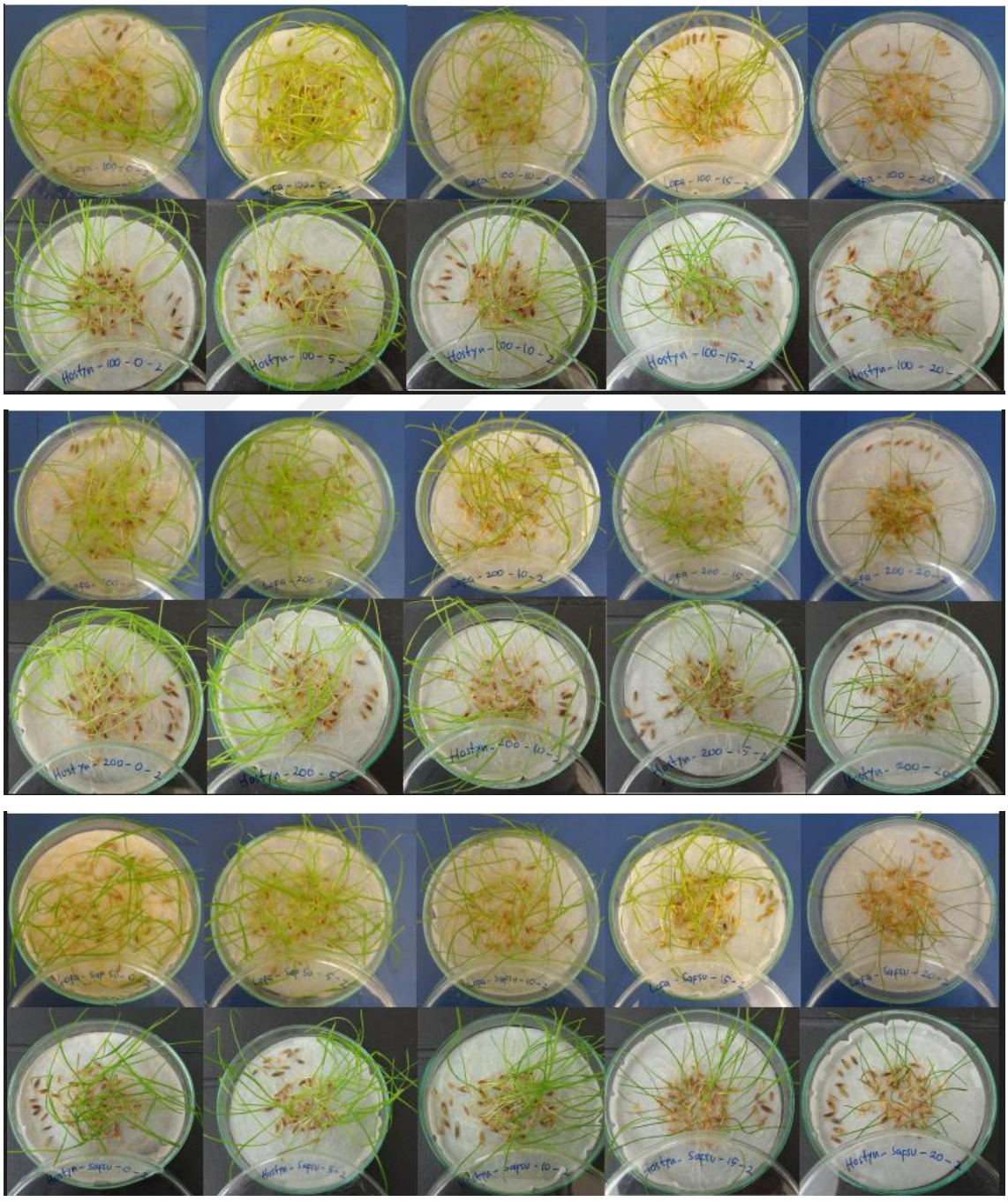

Figure 2.1. Image of the effect of seed treatments and salinity on germination and seedling growth in Hostyn and Lofa cultivars.

CHAPTER III

RESULTS

3.1. Germination Percentage (GP) and Mean Germination Time (MGT)

Variance analysis showed that cultivars, salinity and seed treatments had a significant effect on germination percentage (GP) and mean germination time (MGT; $p<0.01$). However, the interaction among the cultivars, salinity and seed treatments had significant effects only on mean germination time (Table 3.1; $p<0.05$).

Table 3.1. Analysis of variance for the effects of cultivars, salinity and seed treatments and their interactions on germination percentage and mean germination time

Source	DF	Germination Percentage			Mean Germination Time		
		Mean Square	F	Sig.	Mean Square	F	Sig.
Cultivar (A)	1	1420.533	74.571	**0.0004**	0.542	6.770	**0.010**
Salinity (B)	4	160.667	8.434	**0.000**	21.443	267.974	**0.000**
Treatment (C)	3	109.785	5.763	**0.001**	3.529	44.099	**0.000**
A × B	4	15.289	0.803	0.526	0.145	1.807	0.132
A × C	3	9.536	0.501	0.683	0.209	2.615	0.054
B × C	12	21.018	1.103	0.364	0.117	1.460	0.149
A × B × C	12	11.768	0.618	0.824	0.163	2.034	**0.027**
Error	120	19.049			0.080		
Total	159						

Figure 3.1 showed that salinity reduced GP and increased the duration of seeds to germinate in *Festulolium* cultivars. The highest GP was obtained from the control, 5 dS/m and 10 dS/m salinity levels where the GP was above 87.4%. The lowest GP was found in 20 dS/m salinity level as 82.6%. Furthermore, salinity increased MGT of seeds. The highest MGT value was found in 20 dS/m salinity level as 5.83 days. On the other hand, the control seeds showed the lowest MGT values and mean time required for the control seeds to germinate was 3.8 days (Figure 3.1).

Seeds treated with distilled water showed the highest GP with the percentage of seeds that germinated reaching 88.7% (Figure 3.2). The lowest GP was obtained from the control treatment as 84.4%. The highest MGT was observed in the control treatment (5.03 day), followed by 100 ppm GA_3, distilled water and 200 ppm GA_3 treatments (4.58, 4.56 and 4.32 days, respectively).

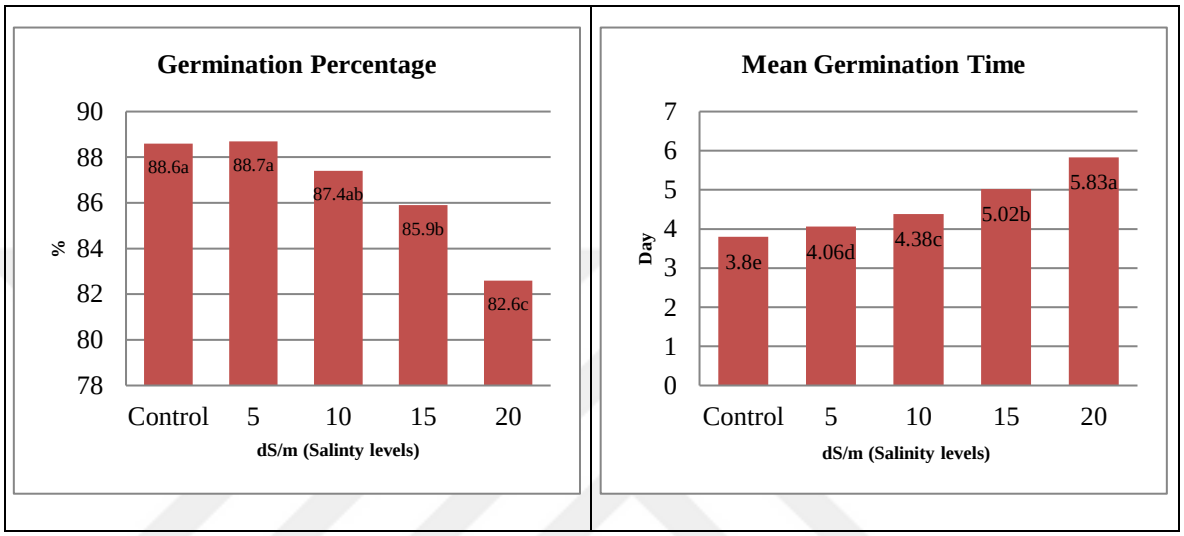

Figure 3.1. The main effect of salinity on germination percentage and mean germination time (bars with different letters are significantly different at $p<0.05$)

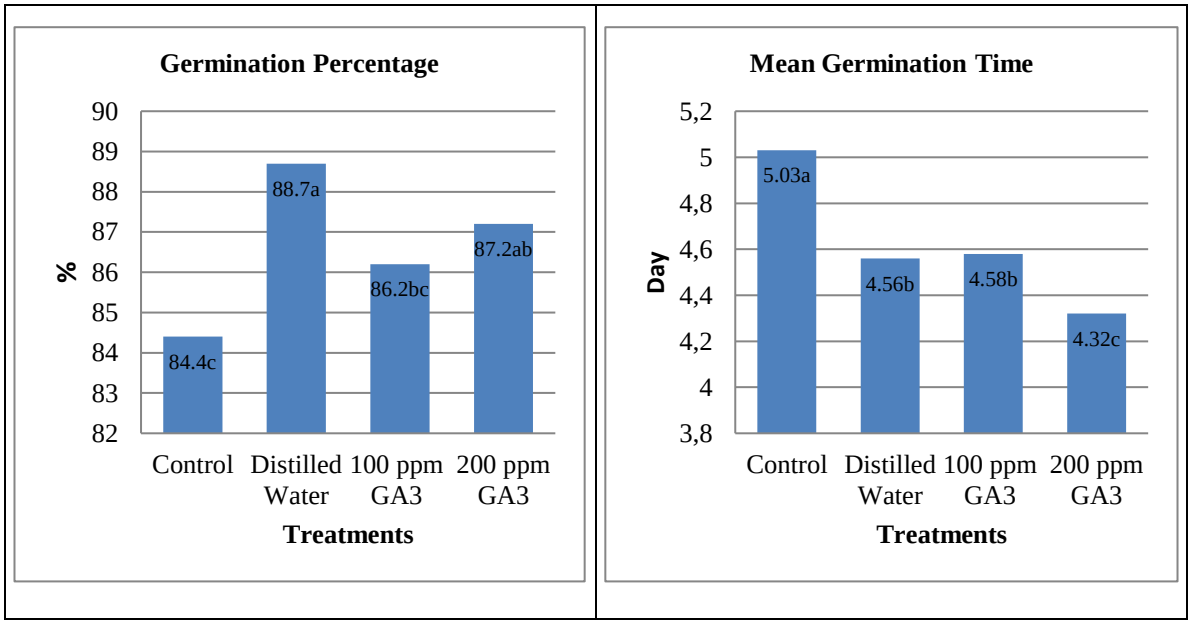

Figure 3.2. The main effect of seed treatments on germination percentage and mean germination time (bars with different letters are significantly different at $p<0.05$)

Figure 3.3 showed the interaction between salinity and cultivars for GP and MGT. GP and MGT were not significantly affected by the interaction between salinity and cultivars. GP changed between 77.6-85.7% in Hostyn and 87.5-91.9% in Lofa cultivar. With increasing salinity level, the GP tended to decrease in both Hostyn and Lofa cultivars. In both Hostyn and Lofa cultivars, it was found that salinity level of 20 dS/m showed the lowest GP. Lofa cultivar could achieve higher GP than Hostyn cultivar (Table 3.2). On the other hand, the highest MGT was found at the salinity level 20 dS/m with the length of time required for Hostyn and Lofa cultivars to germinate as 5.8 days (Figure 3.3). Distilled water and seed treatments speeded up the time it took for seeds to germinate (Figure 3.4). In both Lofa and Hostyn cultivars, the seeds primed with 200 ppm of GA_3 were able to germinate in 4.25 and 4.39 days, respectively (the fastest). Meanwhile, control seeds that did not receive any treatments took 4.98 to 5.07 days (for Lofa and Hostyn) to germinate (the longest).

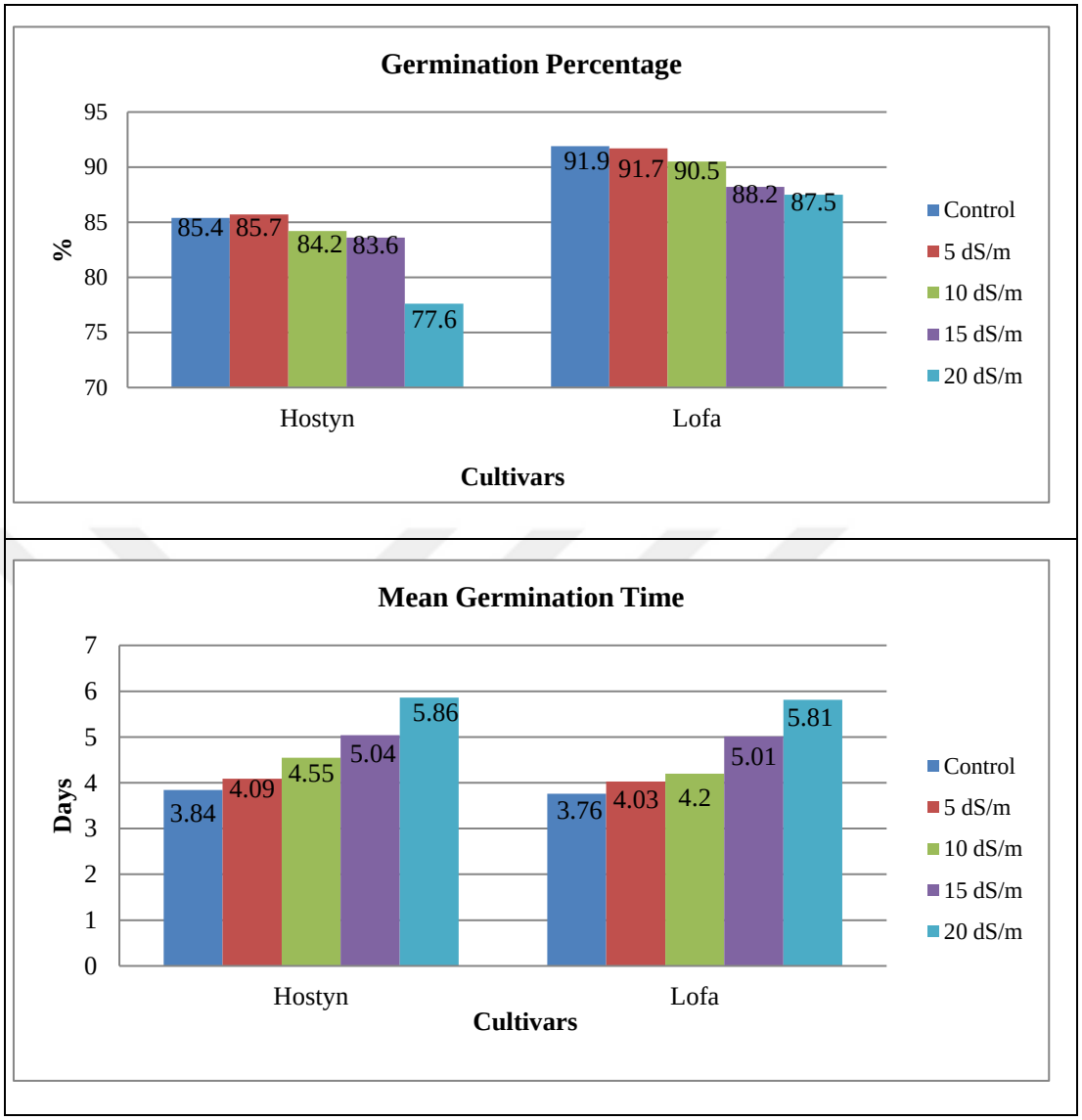

Figure 3.3. Effect of interaction between salinity and cultivars on germination percentage and mean germination time.

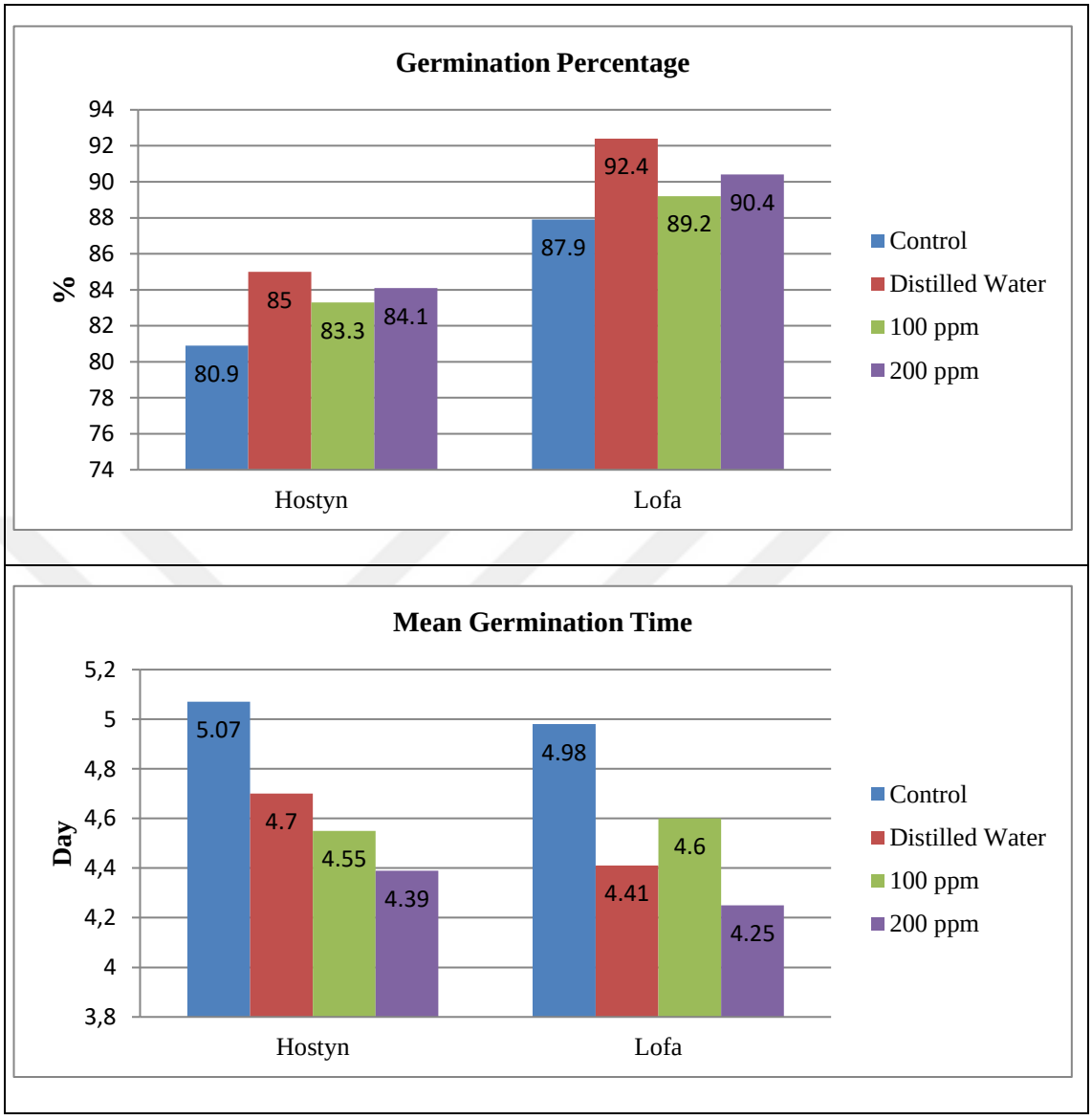

Figure 3.4. Effect of interaction between seed treatments and cultivars on germination percentage and mean germination time

In Table 3.2, effects of interactions among cultivar, salinity and seed treatments on GP and MGT were displayed. According to the results, Hostyn and Lofa cultivars showed the lowest MGT under non-stress conditions in 100 ppm and 200 ppm GA_3 treatments, respectively. In both cultivars, 200 ppm GA_3 treatments for all salinity levels caused a decrease in MGT as compared to the control treatment (non-treated seeds). In Lofa cultivar, on the other hand, at the salinity levels of control, 5, 10 and 15 dS/m, all seed treatments (distilled water, 100 and 200 ppm GA_3) shortened MGT as compared to the control treatment (non-treated seeds).

Table 3.2. Effect of interaction among cultivars, salinity and seed treatments on germination percentage and mean germination time

NaCl levels (dS/m)	Treatments	Germination Percentage (%)			Mean Germinaton Time (day)		
		Hostyn	Lofa	Mean	Hostyn	Lofa	Mean
Control	Control	87.0	94.0	90.5	4.38 j-o*	4.31 k-p	4.34
	Distilled water	84.0	94.0	89.0	3.98 o-t	3.59 stu	3.78
	100 ppm GA_3	86.0	90.0	88.0	3.44 u	3.67 r-u	3.55
	200 ppm GA_3	84.5	89.5	87.0	3.58 tu	3.47 u	3.52
5	Control	82.0	88.0	85.0	4.50 ı-n	4.68 g-k	4.59
	Distilled water	88.5	93.5	91.0	4.06 n-s	3.81 q-u	3.93
	100 ppm GA_3	85.5	92.0	88.7	4.05 n-t	3.88 p-u	3.96
	200 ppm GA_3	87.0	93.5	90.2	3.77 q-u	3.75 q-u	3.76
10	Control	82.0	87.5	84.7	4.92 f-ı	4.62 g-l	4.77
	Distilled water	84.0	95.0	89.5	4.63 g-l	4.04 n-t	4.34
	100 ppm GA_3	85.0	88.5	86.7	4.48 ı-n	4.13 m-r	4.31
	200 ppm GA_3	86.0	91.0	88.5	4.18 l-q	4.01 o-t	4.10
15	Control	80.5	87.0	83.7	5.16 def	5.49 cd	5.33
	Distilled water	84.5	90.0	87.2	5.05 d-g	4.84 f-j	4.94
	100 ppm GA_3	84.0	87.5	85.7	4.97 e-h	5.16 def	5.07
	200 ppm GA_3	85.5	88.5	87.0	4.99 e-h	4.54 h-m	4.76
20	Control	73.0	83.0	78.0	6.41 a	5.81 bc	6.11
	Distilled water	84.0	89.5	86.8	5.81 bc	5.79 bc	5.80
	100 ppm GA_3	76.0	88.0	82.0	5.79 bc	6.19 ab	5.99
	200 ppm GA_3	77.5	89.5	83.5	5.41 cde	5.46 cd	5.44
Mean		**83.3**	**90.0**		**4.67**	**4.67**	

*Within the same column and row, means followed by different letters are significantly different at $p<0.05$

3.2. Shoot and Root Length

The analysis of variance showed that all the factors of this experiment and the interaction between cultivars, salinity and seed treatments had significant effects on both shoot length and root length, except for cultivars for root length (Table 3.3; $p<0.05$).

Table 3.3. Analysis of variance for the effects of cultivars, salinity and seed treatments and their interactions on shoot and root length

Source	DF	Shoot Length			Root Length		
		Mean Square	F	Sig.	Mean Square	F	Sig.
Cultivar (A)	1	11.492	32.079	**0.000**	1.382	2.680	0.104
Salinity (B)	4	125.048	349.070	**0.000**	193.424	375.097	**0.000**
Treatment (C)	3	51.649	144.177	**0.000**	16.214	31.443	**0.000**
A × B	4	2.555	7.133	**0.000**	2.657	5.152	**0.001**
A × C	3	9.207	25.702	**0.000**	1.748	3.390	**0.020**
B × C	12	1.125	3.139	**0.001**	2.181	4.229	**0.000**
A × B × C	12	0.781	2.180	**0.017**	1.317	2.554	**0.005**
Error	120	0.358			0.516		
Total	159						

The results showed that increase in salinity inhibited the growth of shoot and root (Figure 3.5). The highest shoot length was found in 5 dS/m (11.05 cm) and the lowest shoot length was found in 20 dS/m (5.95 cm) treatments, the highest root length was found in the control (11.03 cm) and the lowest was found in 20 dS/m (4.64 cm; Figure 3.5) treatments. Seed treatments had positive effects on shoot and root length. The highest shoot length was achieved from 200 ppm GA_3 treatment (10.30 cm) while the lowest shoot length was from the control (untreated seeds) treatments. The seeds primed with 100 and 200 ppm GA_3 produced the longest root length. The shortest root length was also obtained from the seeds that had not been treated (Figure 3.6).

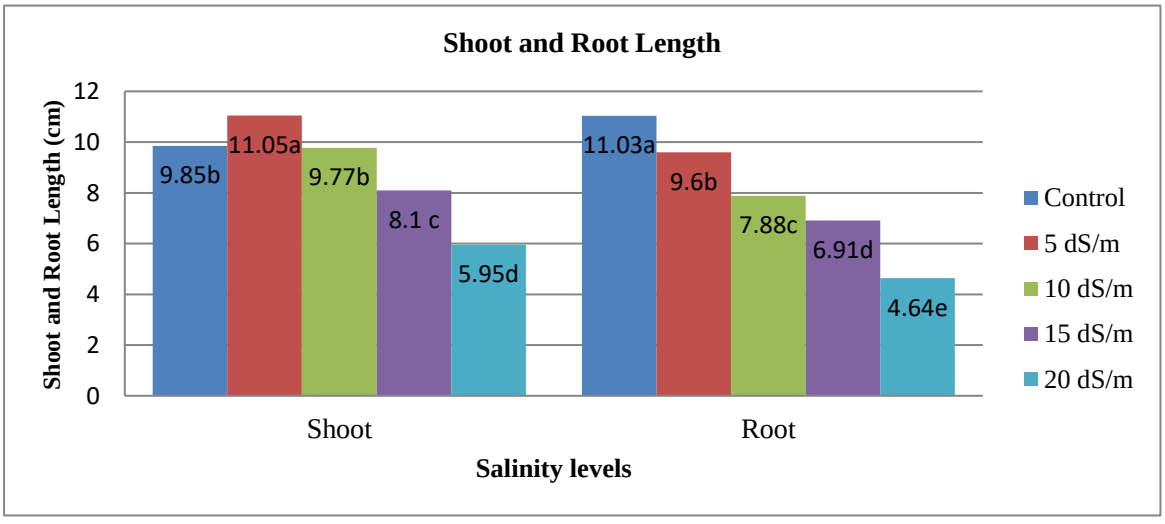

Figure 3.5. The main effect of salinity on shoot and root length (bars with different letters are significantly different at $p<0.05$)

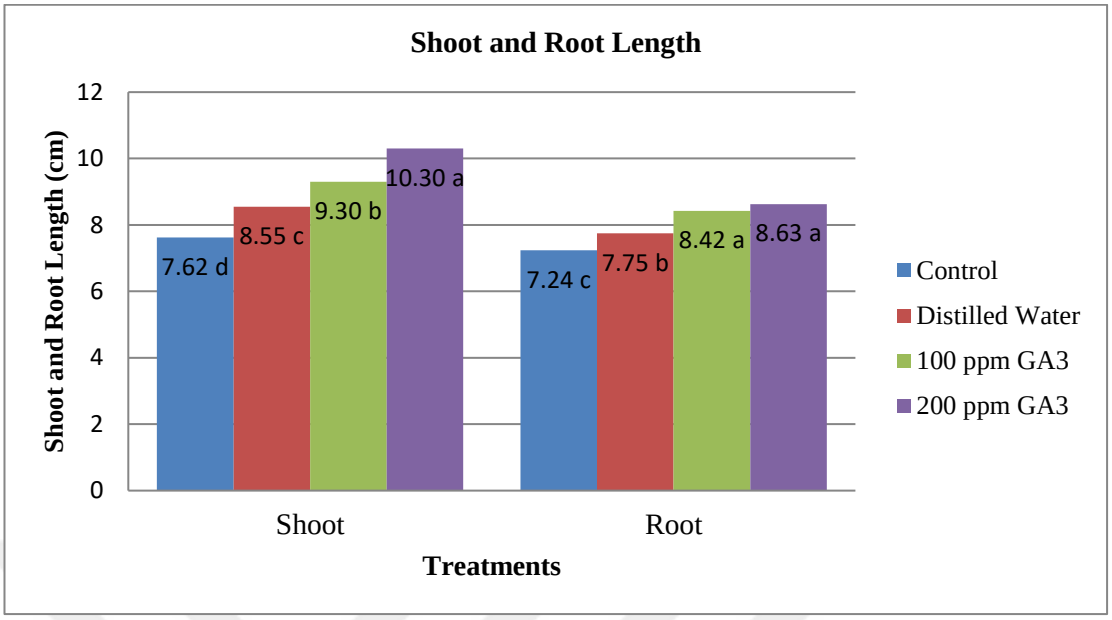

Figure 3.6. The main effect of seed treatments on shoot and root length (bars with different letters are significantly different at $p<0.05$)

The interaction between cultivars and salinity had a significant effect on shoot and root length (Figure 3.7). While the highest shoot length was achieved from the Hostyn and Lofa cultivars at 5 dS/m (11.51 and 10.6 cm, respectively), the longest root was achieved from the Hostyn and Lofa cultivars under control salinity level (11.07 and 10.98 cm, respectively). The lowest shoot and root length of both cultivars were found in 20 dS/m salinity level. Control and 10 dS/m salinity levels made no difference on shoot length of Hostyn and Lofa cultivars. While significant decreases were obtained in shoot length especially at 15 and 20 dS/m, the decreases in root length were determined with increasing salinity levels.

Figure 3.8 showed the interaction between cultivars and seed treatments. The rising concentration of GA_3 applied on seeds increased the shoot and root length of Hostyn cultivar (Figure 3.8). The best result for shoot and root length of Hostyn cultivar were obtained from 200 ppm GA_3 treatments, while the shortest shoot and root length were obtained from the control salinity level (Figure 3.8). However, the highest shoot and root length of Lofa cultivar were obtained from 100 and 200 ppm GA_3 treatments.

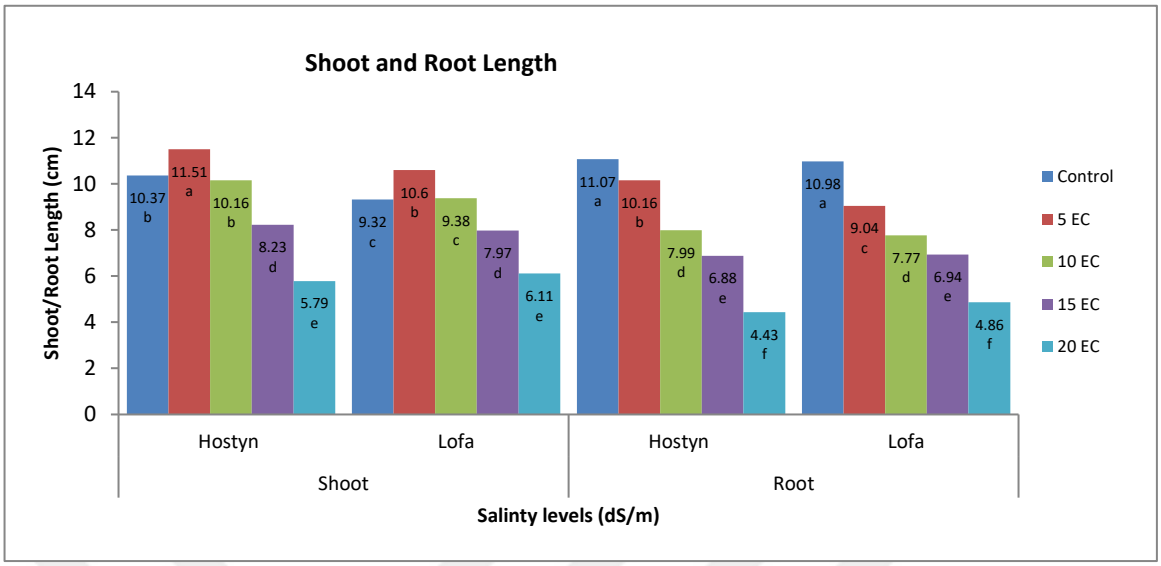

Figure 3.7. Effect of cultivars and salinity interaction on shoot and root length (bars with different letters are significantly different at p<0.05)

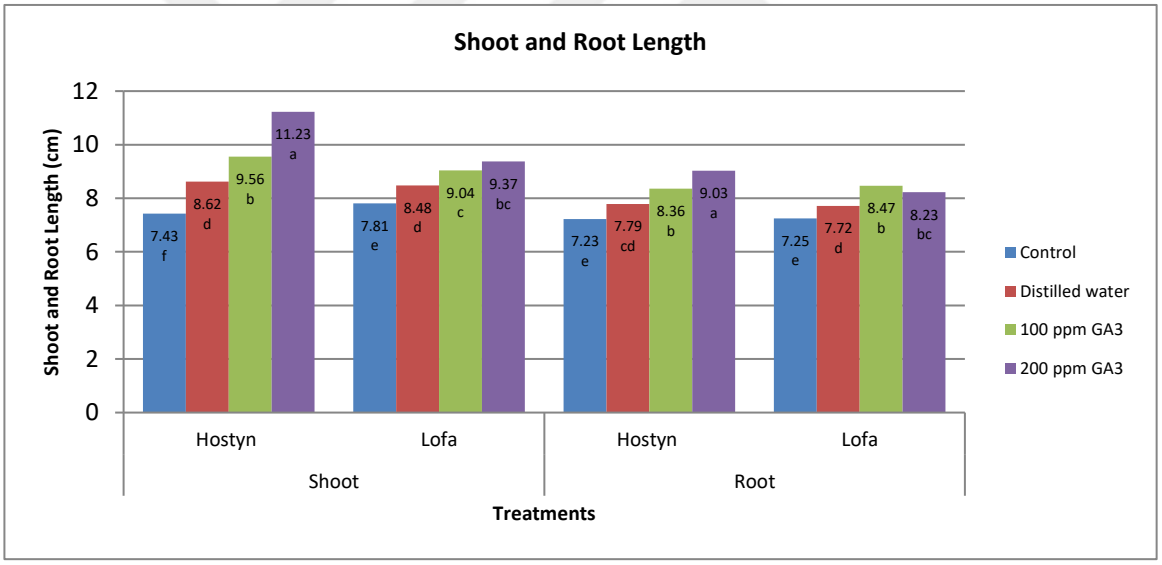

Figure 3.8. Effect of cultivars and seed treatments interaction on shoot and root length (bars with different letters are significantly different at p<0.05)

Considering the salinity and seed treatments interaction, both shoot and root length increased at 200 ppm GA_3 treatment for all salinity levels as compared to untreated seeds Table 3.4). The 200 ppm GA_3 treatment also increased shoot length as compared to the distilled water treatments.

Table 3.4 showed interaction among cultivars, salinity and seed treatment for shoot and root length. The highest shoot length of in Hostyn cultivar was found in 200 ppm GA_3 treatment at 5 dS/m salinity level (14.67 cm), while the highest shoot length of Lofa

cultivar was found in 100 and 200 ppm GA_3 treatments at 5 dS/m salinity level (11.26 and 11.34cm, respectively). The lowest shoot length of Hostyn and Lofa cultivars (3.81 and 5.20 cm, respectively) were found at 20 dS/m salinity level of untreated seeds. The 200 ppm GA_3 treatment increased shoot length of Hostyn cultivar at all salinity levels as compared to the control and distilled water treatments, while 100 and 200 ppm GA_3 treatments enhanced shoot length of Lofa cultivar at 5, 10, 15 and 20 dS/m salinity levels as compared to the control treatment. Seed treatments (Distilled water, 100 and 200 ppm GA_3) increased shoot length of both cultivars at 10, 15 and 20 dS/m salinity levels. The highest root length of Hostyn cultivar was also recorded from the seeds primed with 200 ppm GA_3 at 5 dS/m salinity level. In Lofa cultivar, the highest root length was recorded from distilled water, 100 ppm GA_3 and 200 ppm GA_3 treated seeds at control salinity level. Root lengths increased with seed treatments at 20 dS/m salinity level as compared to the control.

Table 3.4. The effect of interaction among cultivars, salinity and seed treatments on shoot and root length

NaCl levels (dS/m)	**Treatment**	**Shoot length (cm)**			**Root length (cm)**		
		Hostyn	**Lofa**	**Mean**	**Hostyn**	**Lofa**	**Mean**
Control	Control	8.76 klm	9.02 h-l	8.89 efg	10.42 de	9.77 ef	10.10 c
	Distilled water	9.52 g-k	8.94 ı-l	9.23 de	10.57 cde	11.13 bcd	10.85 b
	100 ppm GA_3	10.41 efg	9.43 g-k	9.92 c	11.75 b	11.66 bc	11.71 a
	200 ppm GA_3	12.80 b	9.89 f-ı	11.34 b	11.53 bcd	11.37 bcd	11.45 ab
5	Control	9.41 g-k	10.00 fgh	9.71 cd	9.00 f-ı	9.14 fgh	9.08 de
	Distilled water	10.24 fg	9.79 f-j	10.02 c	9.00 f-ı	8.05 h-m	8.53 ef
	100 ppm GA_3	11.71 c	11.26 cde	11.48 b	9.65 ef	9.46 efg	9.56 cd
	200 ppm GA_3	14.67 a	11.34 cd	13.00 a	12.96 a	9.48 efg	11.22 ab
10	Control	8.38 lm	8.31 lm	8.35 gh	7.52 j-m	7.59 j-m	7.55 gh
	Distilled water	9.65 f-k	9.50 g-k	9.57 cd	7.88 ı-m	7.00 l-o	7.44 gh
	100 ppm GA_3	10.52 def	9.55 f-k	10.03 c	8.18 h-l	8.61 f-j	8.39 ef
	200 ppm GA_3	12.08 bc	10.14 fg	11.11 b	8.39 g-k	7.87 ı-m	8.13 fg
15	Control	6.80 op	6.50 opq	6.65 ı	6.13 nop	5.99 opq	6.06 ı
	Distilled water	7.95 mn	8.08 lm	8.01 h	7.07 l-o	7.19 k-n	7.13 h
	100 ppm GA_3	8.73 klm	8.43 lm	8.58 fgh	7.36 klm	7.30 klm	7.33 h
	200 ppm GA_3	9.43 g-k	8.88 j-m	9.15 def	6.95 mno	7.30 klm	7.12 h
20	Control	3.81 s	5.20 r	4.50 k	3.07 t	3.75 st	3.41 k
	Distilled water	5.75 qr	6.10 pq	5.93 j	4.45 rs	5.20 pqr	4.83 k
	100 ppm GA_3	6.40 opq	6.54 opq	6.47 ıj	4.86 qr	5.34 pqr	5.10 j
	200 ppm GA_3	7.19 no	6.58 opq	6.89 ı	5.34 pqr	5.14 pqr	5.24 j
Mean		**9.21**	**8.67**		**8.10**	**7.92**	

*Within the same column and row, means followed by different letters are significantly different at $p<0.05$

3.3. Seedling Fresh and Dry Weight

Variance analysis results revealed that all parameters had a significant effect on seedling fresh and dry weight (Table 3.5; $p<0.05$).

Table 3.5. Analysis of variance for the effects of cultivars, salinity and seed treatments and their interactions on seedling fresh and dry weight

Source	DF	Seedling Fresh Weight			Seedling Dry Weight		
		Mean Square	F	Sig.	Mean Square	F	Sig.
Cultivar (A)	1	36.864	10.291	**0.002**	1.106	45.010	**0.000**
Salinity (B)	4	803.065	224.182	**0.000**	1.427	58.102	**0.000**
Treatment (C)	3	11.698	3.266	**0.024**	0.083	3.388	**0.020**
A × B	4	40.104	11.195	**0.000**	0.101	4.132	**0.004**
A × C	3	14.927	4.167	**0.008**	0.098	4.006	**0.009**
B × C	12	7.081	1.977	**0.032**	0.090	3.681	**0.000**
A × B × C	12	7.402	2.066	**0.024**	0.067	2.746	**0.003**
Error	120	3.582			0.025		
Total	159						

Dry and fresh weight of seedling decreased drastically at 15 and 20 dS/m salinity levels (Figure 3.9). On the other hand, the highest seedling fresh weight (24.61 mg) was found at the salinity level of 5 dS/m, while the highest seedling dry weight was obtained from the control, 5 and 10 dS/m salinity levels (1.89-1.90 mg). The seedlings at 5 dS/m salinity showed the highest fresh and dry weights. The treatment of GA_3 did not immediately increase the fresh and dry weight of the seedlings (Figure 3.10). While the highest seedling fresh weight was recorded in distilled water and control treatments, the highest seedling dry weight was recorded in the control and 200 ppm GA_3 treatments. The seeds primed with 100 ppm GA_3 treatments produced the lowest fresh and dry weight.

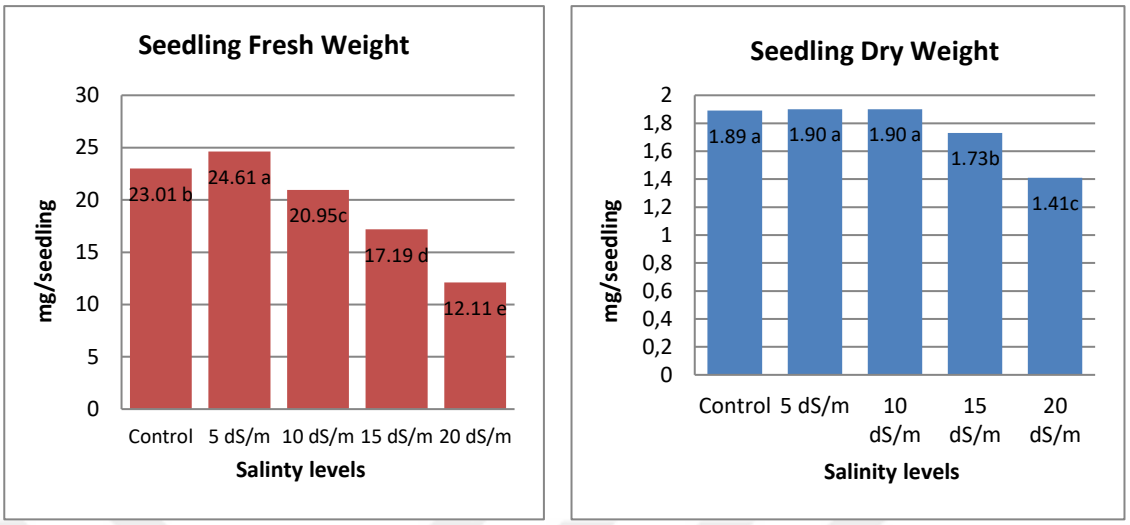

Figure 3.9. The main effect of salinity on seedling fresh and dry weight (bars with different letters are significantly different at p<0.05)

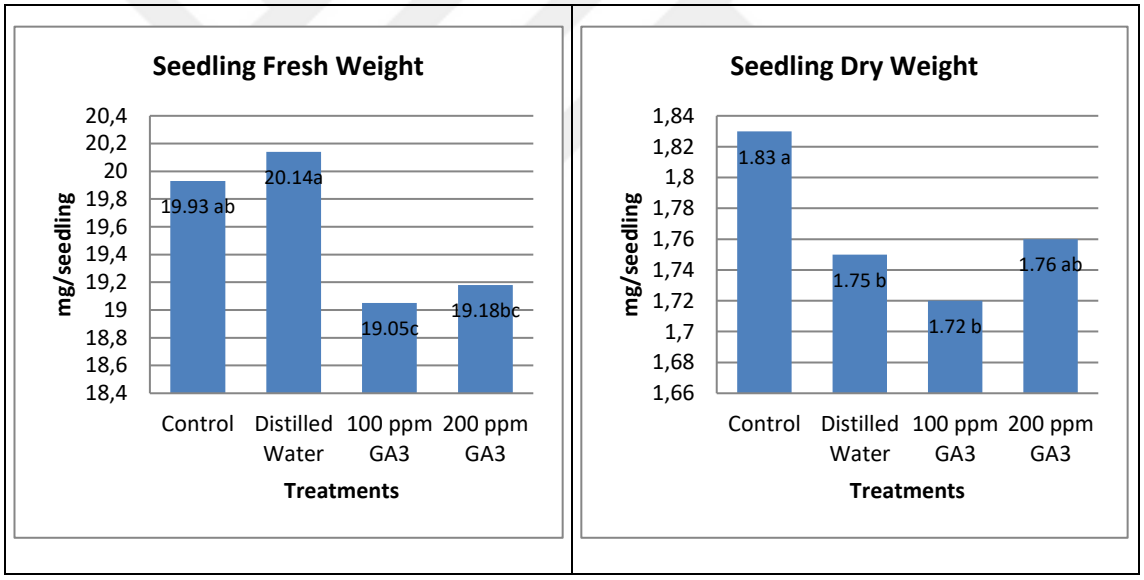

Figure 3.10. The main effect of seed treatments on seedling fresh and dry weight (bars with different letters are significantly different at p<0.05)

Interaction between cultivars and salinity showed that the highest fresh and dry weights of Hostyn cultivar were obtained from the control, 5 and 10 dS/m salinity levels (Figure 3.11). The highest fresh and dry weights of Lofa cultivar were obtained from 5 dS/m and 10 dS/m salinity levels. The lowest fresh and dry weights were achieved from 20 dS/m salinity level of both Hostyn and Lofa cultivars. Interaction between cultivars and seed treatments showed that the highest seedling fresh weight (21.48 mg) was obtained from the distilled water treatment of Hostyn cultivar (Figure 3.12). On the other hand, the

highest dry weight of Hostyn cultivar was determined in control and 200 ppm GA_3 treatments. In Lofa cultivar, the highest fresh weight was obtained from control and 200 ppm GA_3 treatments. Seedling dry weight of Lofa cultivar ranged between 1.64 -1.70 and differences in dry weights among the treatments were not significant. Interaction between salinity and seed treatment showed that the highest seedling fresh weight of 25.19 mg was obtained from the control treatment at 5 dS/m salinity level (Table 3.6). Differences among fresh weights at control, 5, 15 and 20 dS/m salinity levels were not found to be significant. However, control and distilled water treatments at 10 dS/salinity level displayed higher fresh weight than 100 and 200 ppm GA_3 treatments. The highest dry weights were obtained from control and 200 ppm GA_3 treatments at control and 5 dS/m salinity levels, from control and distilled water treatments at 10 dS/m salinity level.

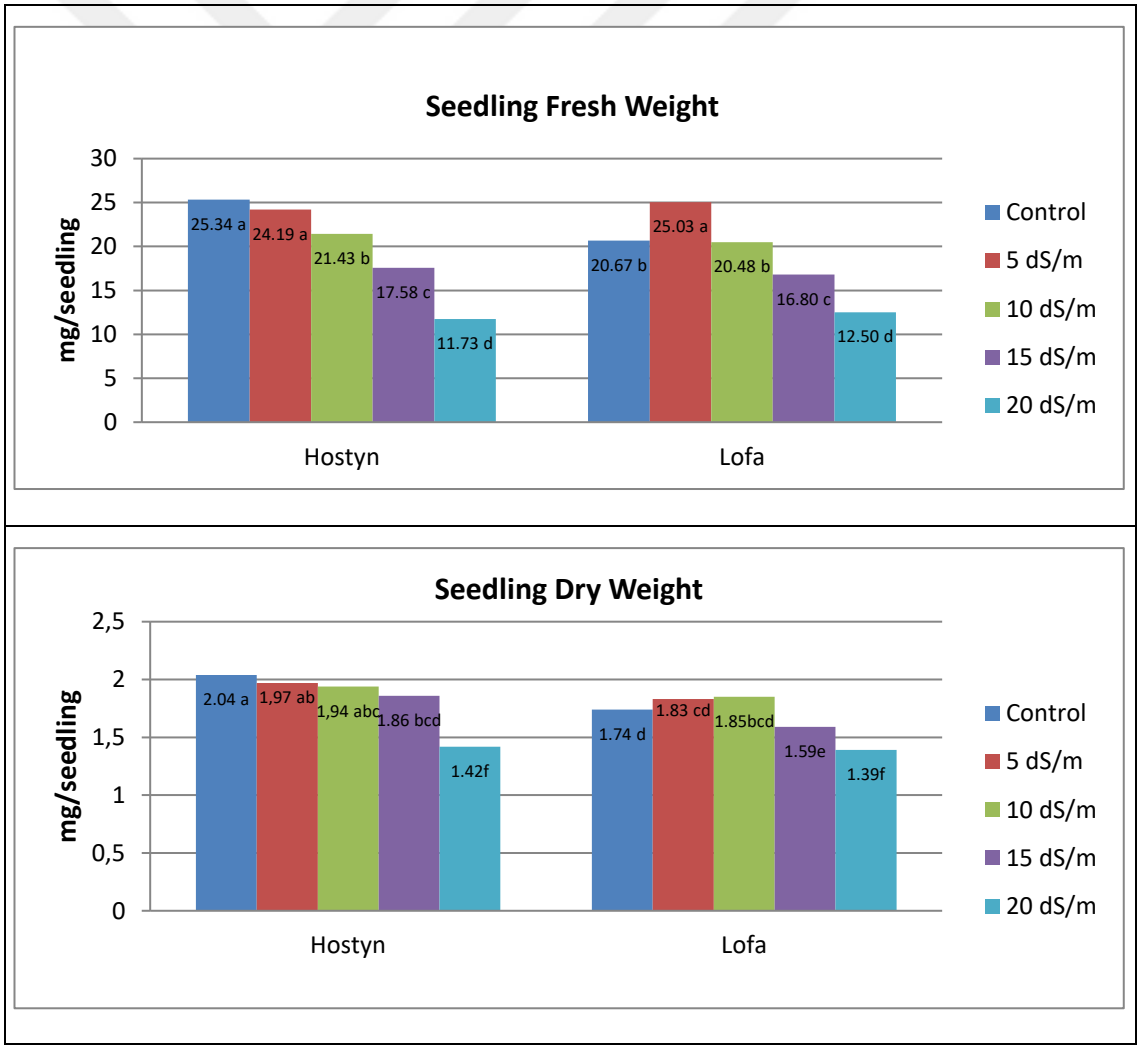

Figure 3.11. Effect of interaction between salinity and cultivars on seedling fresh and dry weight (bars with different letters are significantly different at $p<0.05$)

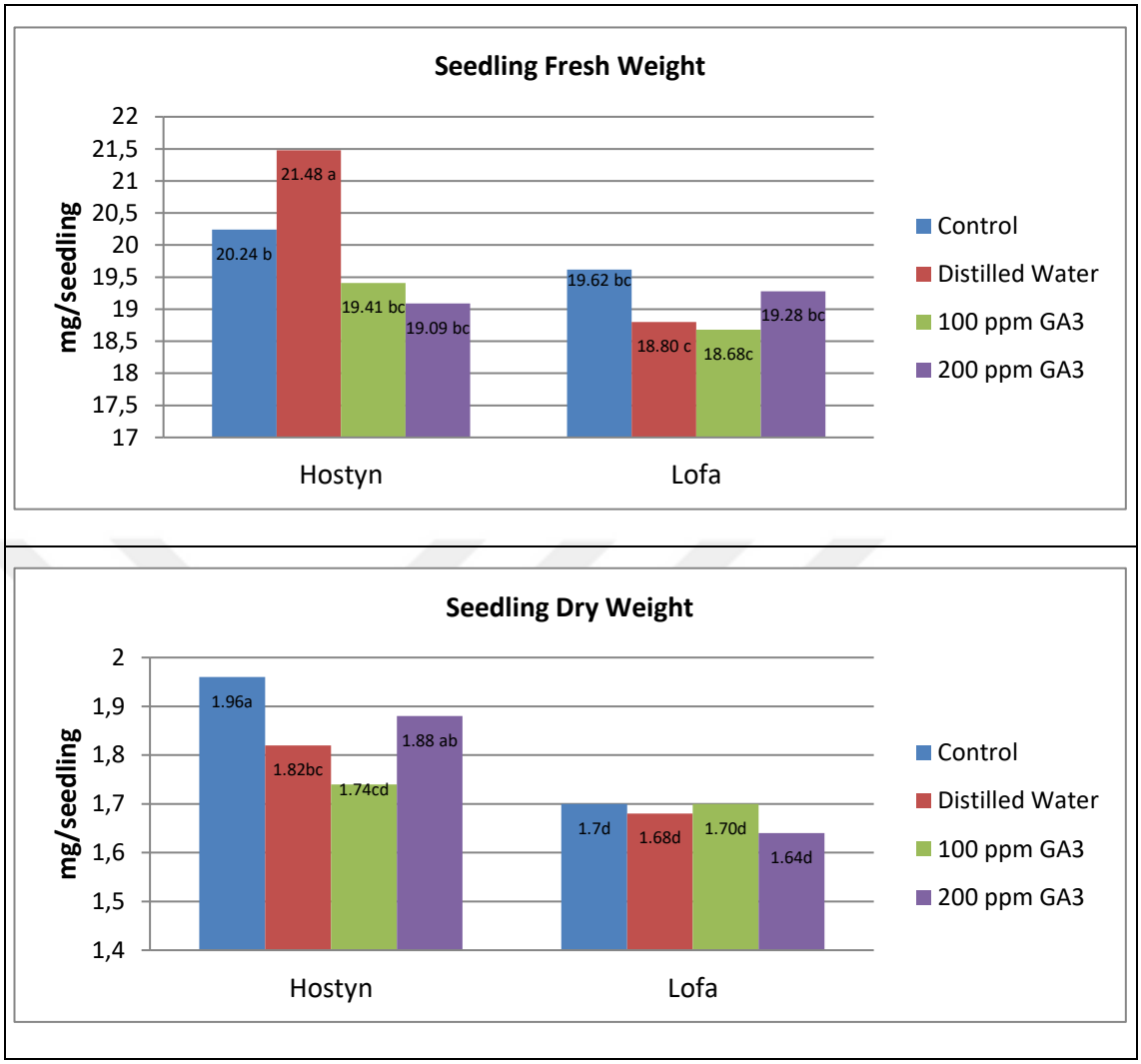

Figure 3.12. Effect of interaction between seed treatments and cultivars on seedling fresh and dry weight (bars with different letters are significantly different at $p<0.05$)

Table 3.6 showed interaction among cultivars, salinity and seed treatments. The highest fresh weight of Hostyn was found in control (26.37 mg), followed by 100 ppm GA_3 treatment (26.02 mg) at control salinity level, distilled water (25.27 mg) and 200 ppm (25.20 mg) GA_3 treatments at 5 dS/m salinity level and distilled water treatment (24.95) at 10 dS/m salinity level. Furthermore, the highest fresh weight of Lofa was found in control treatment at 5 dS/m salinity level (27.97 mg) and in 200 ppm GA_3 treatment at 5 dS/m salinity level (25.07 mg). Meanwhile, the highest dry weight of Hostyn was found in control and 200 ppm GA_3 treatments at control salinity level and control treatment at 5 dS/m salinity level.

Table 3.6. Effect of interaction among cultivars, salinity and seed treatments on fresh and dry weight of *Festulolium* cultivars

NaCl level (dS/m)	Treatments	Fresh Seedling Weight (mg/seedling)			Dry Seedling Weight (mg/seedling)		
		Hostyn	Lofa	Mean	Hostyn	Lofa	Mean
Control	Control	26.37 ab	21.55 e-ı	23.96 ab	2.30 a	1.92 b-h	2.11 a
	Distilled water	24.67 bcd	20.42 f-j	22.55 bc	1.73 f-j	1.68 g-k	1.70 f
	100 ppm GA_3	26.02 abc	19.12 ıjk	22.57 bc	1.95 b-g	1.65 h-k	1.80 def
	200 ppm GA_3	24.30 b-e	21.58 e-ı	22.94 bc	2.17 ab	1.73 f-j	1.95 a-d
5	Control	22.90 c-g	27.97 a	25.19 a	2.10 abc	1.98 b-f	2.04 ab
	Distilled water	25.27 a-d	23.25 b-f	24.26 ab	1.90 c-h	1.70g-j	1.80 def
	100 ppm GA_3	23.40 b-f	24.30 b-e	23.85 abc	1.88 c-h	1.85 c-h	1.86c-f
	200 ppm GA_3	25.20 a-d	25.07 a-d	25.14 a	2.00 b-f	1.78 d-ı	1.89 b-e
10	Control	22.45 d-h	21.13 f-ı	21.79 c	2.00 b-f	1.90 c-h	1.95 a-d
	Distilled water	24.95 a-d	21.32 e-ı	23.14 abc	1.95 b-g	2.03 bcd	1.99 abc
	100 ppm GA_3	19.12 ıjk	19.45 h-k	19.29 de	1.88 c-h	1.77 d-ı	1.82 c-f
	200 ppm GA_3	19.17 ıjk	20.00 g-j	19.59 d	1.95 b-g	1.70 g-j	1.83 c-f
15	Control	18.85 ıjk	15.80 lm	17.32 ef	2.02 b-e	1.43 kl	1.73 ef
	Distilled water	19.30 ıjk	16.32 kl	17.81 def	2.02 b-e	1.53 ı-l	1.78 def
	100 ppm GA_3	16.58 kl	17.67 jkl	17.12 f	1.65 h-k	1.75 e-ı	1.70 f
	200 ppm GA_3	15.60 lmn	17.40 jkl	16.50 f	1.75 e-ı	1.67 h-k	1.71 ef
20	Control	10.62 o	12.15 o	11.39 g	1.35 l	1.28 l	1.31 g
	Distilled water	13.20 mno	12.65 o	12.93 g	1.48 jkl	1.48 jkl	1.48 g
	100 ppm GA_3	11.92 o	12.85 no	12.39 g	1.35 l	1.48 jkl	1.41 g
	200 ppm GA_3	11.15 o	12.35 o	11.75 g	1.52 ı-l	1.35 l	1.44 g
Mean		**20.05**	**19.10**		**1.848**	**1.681**	

*Within the same column and row, means followed by different letters are significantly different at $p<0.05$

3.4. Seedling Vigor Index (SVI)

The analysis of variance revealed that cultivars, salinity and seed treatments as well as the interaction between cultivars and salinity, cultivars and seed treatments, salinity and seed treatments significantly affected seedling vigor index (Table 3.7; $p<0.01$).

Table 3.7. Analysis of variance for effects of cultivars, salinity and seed treatments and their interactions on seedling vigor index

		Seedling Vigor Index		
Source	**DF**	**Mean Square**	**F**	**Sig.**
Cultivar (A)	1	8.742	6.560	**0.012**
Salinity (B)	4	519.711	389.955	**0.000**
Treatment (C)	3	101.446	76.118	**0.000**
A × B	4	4.519	3.391	**0.011**
A × C	3	11.500	8.628	**0.000**
B × C	12	3.793	2.846	**0.002**
A × B × C	12	1.904	1.428	0.162
Error	120	1.333		
Total	159			

The salinity stress was inversely related to the SVI (Figure 3.13). The highest SVI was obtained from the control and 5 dS/m salinity level. Increasing salinity levels decreased SVI values. At 20 dS/m salinity level, the seedlings showed the lowest SVI with 8.8. The application of GA_3 to seeds promoted the SVI. The highest SVI (16.48) was obtained from 200 ppm GA_3 treatment, followed by 100 ppm GA_3, distilled water and control (the lowest, 12.7) treatments (Figure 3.13).

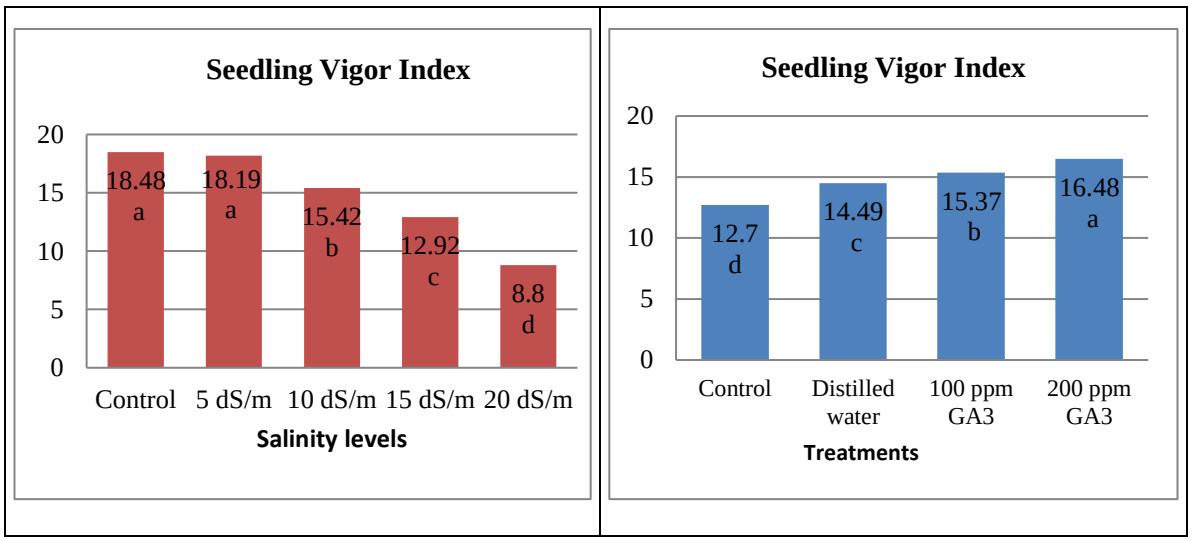

Figure 3.13. The main effect of salinity and seed treatments on seedling vigor index (bars with different letters are significantly different at $p<0.05$)

Interaction between cultivars and salinity tended to have inverse correlation with SVI (Figure 3.14). When the salinity level increased, SVI tended to decrease in both cultivars. The lowest SVI was obtained from 20 dS/m salinity level in Hostyn cultivar with 7.96 (Figure 3.10). SVI of Lofa cultivar was higher than SVI of Hostyn cultivar at 20 dS/m

salinity level. Interaction between seed treatment and cultivar showed that increasing GA_3 doses also increased SVI in Hostyn cultivar but not in Lofa cultivar. The results of the interaction between salinity and seed treatment showed that the highest SVI was obtained from 5 dS/m salinity level of 200 ppm GA_3 treatment (21.29) (Table 3.8). At the salinity levels of 0, 5 and 10 dS/m, 200 ppm GA_3 treatments had higher SVI than the control and distilled water treatments.

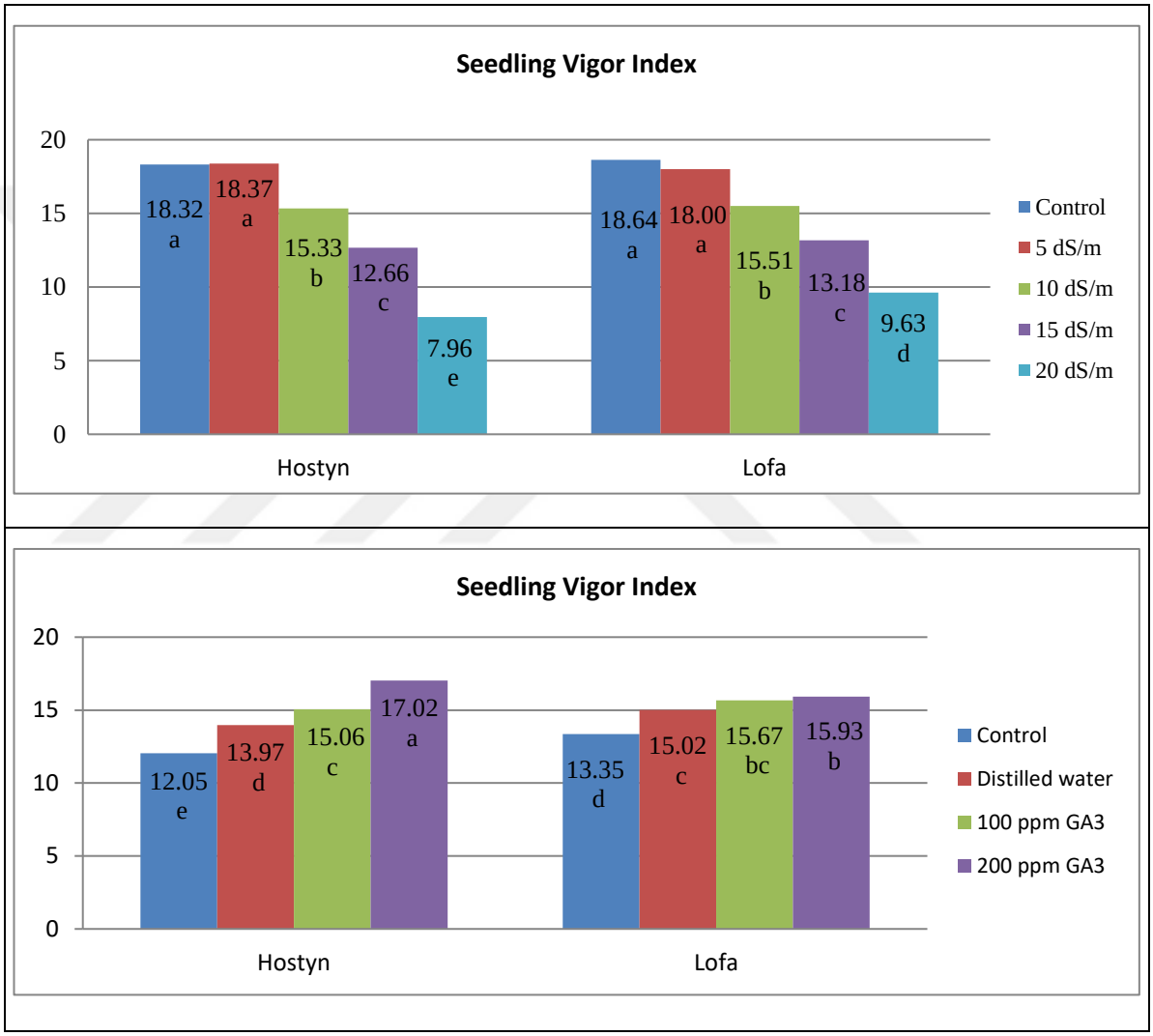

Figure 3.14. Effect of cultivars × salinity and cultivars × seed treatments interactions on seedling vigor index (bars with different letters are significantly different at $p<0.05$)

Table 3.8. The effect of interaction among cultivars, salinity and seed treatments on seedling vigor index

NaCl level (dS/m)	Treatments	Seedling Vigor Index		
		Hostyn	Lofa	Mean
Control	Control	16.70	17.67	17.18 de
	Distilled water	16.88	18.85	17.86 cd
	100 ppm GA_3	19.07	18.99	19.03 bc
	200 ppm GA_3	20.62	19.04	19.83 b
5	Control	15.10	16.84	15.97 ef
	Distilled water	17.00	16.69	16.85 de
	100 ppm GA_3	18.25	19.04	18.64 bc
	200 ppm GA_3	23.12	19.45	21.29 a
10	Control	13.03	13.92	13.47 h
	Distilled water	14.72	15.68	15.20 fg
	100 ppm GA_3	15.92	16.05	15.99 ef
	200 ppm GA_3	17.63	16.38	17.01 de
15	Control	10.42	10.88	10.65 ı
	Distilled water	12.67	13.75	13.21 h
	100 ppm GA_3	13.53	13.79	13.66 h
	200 ppm GA_3	14.00	14.31	14.15 gh
20	Control	5.02	7.45	6.24 k
	Distilled water	8.57	10.12	9.35 j
	100 ppm GA_3	8.54	10.47	9.50 ıj
	200 ppm GA_3	9.70	10.49	10.10 ıj
Mean		**14.53**	**14.99**	

*Within the same column and row, means followed by the different letters are significantly different at $p<0.05$

3.5. Proline Content

Analysis of variance for proline content showed that cultivars, salinity and seed treatments as well as the interaction between cultivars and salinity, salinity and seed treatments significantly affected proline content of the seedlings (Table 3.9; $p<0.01$).

Table 3.9. Analysis of variance for the effects of cultivars, salinity and seed treatments and their interactions on proline content

		Proline		
Source	**DF**	**Mean Square**	**F**	**Sig.**
Cultivar (A)	1	11.979	72.503	**0.000**
Salinity (B)	3	180.849	1094.563	**0.000**
Treatment (C)	3	5.726	34.658	**0.000**
A × B	3	4.358	26.375	**0.000**
A × C	3	0.074	0.451	0.717
B × C	9	4.198	25.411	**0.000**
A × B × C	9	0.143	0.862	0.561
Error	96	0.165		
Total	127			

The salinity stress positively affected proline content. The increasing salinity levels increased the seedling proline contents (Figure 3.15). At 20 dS/m salinity level, the seedlings showed the highest proline content (5.93 mg/g DW). Seed treatments caused an increase in proline content. The 200 ppm GA_3 treatment had the highest proline content (3.11 mg/g DW), followed by 100 ppm GA_3, distilled water and control (the lowest, 2.11 mg/g) treatments.

Interaction between cultivar and salinity indicated that the amount of proline increased due to the increasing salinity levels in Hostyn cultivar, nevertheless, a significant increase of proline was found at 10 and 15 dS/m salinity levels in Lofa cultivar (Figure 3.16). The highest proline content was found at 15 dS/m salinity level of Hostyn cultivar. The lowest proline content was obtained from the control and 5 dS/m salinity levels of Lofa cultivar and control salinity level of Hostyn cultivar.

Analysis of variance showed the interaction between salinity and seed treatments. The highest proline content was achieved from 200 ppm GA_3 treatment at 15 dS/m salinity level (Table 3.10). At 10 and 15 dS/m salinity levels, GA_3 treatments increased the proline content of seedlings as compared to the control treatments.

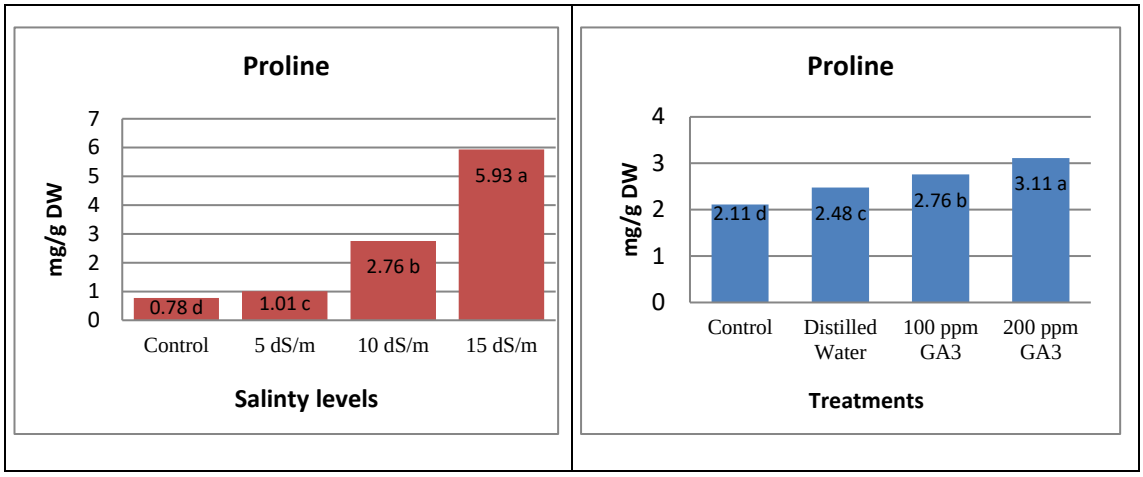

Figure 3.15. The main effect of salinity and seed treatments on proline content (bars with different letters are significantly different at p<0.05)

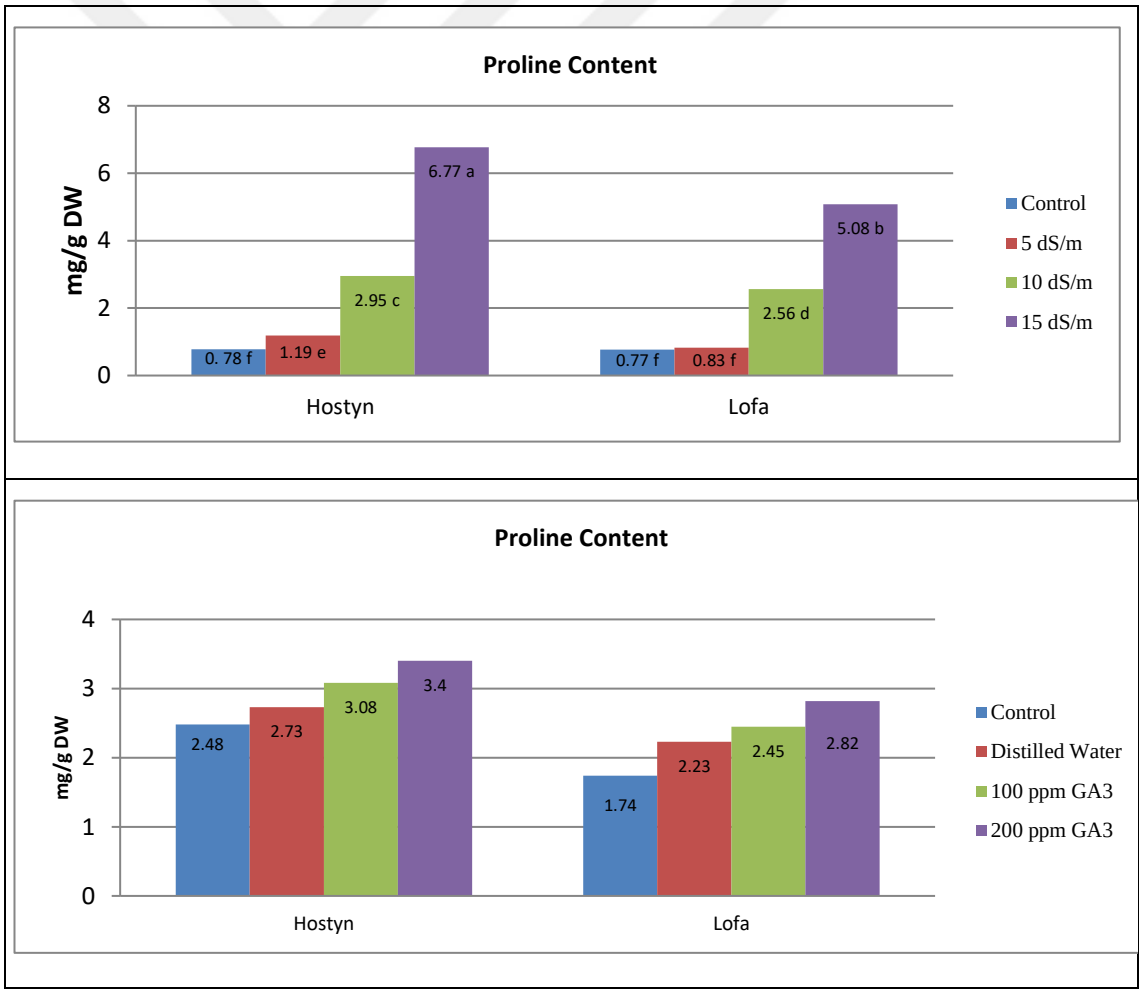

Figure 3.16. Effect of cultivars × salinity and cultivars × seed treatments interactions on proline content (bars with different letters are significantly different at p<0.05)

Table 3.10. Effect of interaction among cultivars, salinity and seed treatments on proline content

NaCl level (dS/m)	Treatments	Proline Content (mg/g DW)		
		Hostyn	Lofa	Mean
Control	Control	0.73	0.86	0.79 ıj
	Distilled water	0.96	1.05	1.00 hıj
	100 ppm GA_3	0.85	0.59	0.72 ıj
	200 ppm GA_3	0.59	0.59	0.59 j
5	Control	1.37	0.81	1.09 hı
	Distilled water	0.88	0.75	0.81 ıj
	100 ppm GA_3	1.51	1.02	1.26 h
	200 ppm GA_3	0.99	0.73	0.86 hıj
10	Control	2.48	2.12	2.30 g
	Distilled water	2.96	2.26	2.61 fg
	100 ppm GA_3	2.89	2.77	2.83 f
	200 ppm GA_3	3.48	3.10	3.29 e
15	Control	5.33	3.20	4.27 d
	Distilled water	6.14	4.88	5.51 c
	100 ppm GA_3	7.09	5.39	6.24 b
	200 ppm GA_3	8.52	6.86	7.69 a
Mean		2.92	2.31	

*Within the same column and row, means followed by the different letters are significantly different at $p<0.05$

CHAPTER IV

DISCUSSION-CONCLUSION AND RECOMMENDATIONS

4.1. Discussion

Germination and seedling growth are critical stages of crop establishment (Tsegay and Andargie, 2018). Without a high germination percentage, there would not enough crop establishment (Nimir et al., 2014). GP indicated the percentage of seeds that germinated until the end of the observation time (at least 2 mm radicles length). In both cultivars, seeds mostly germinated well until the salinity level of 15 dS/m. The highest GP was found at salinity levels of 5, control and 10 dS/m (88.7%, 88.6%, 87.4%, respectively). The lowest germination percentage was obtained from 20 dS/m salinity level. The inhibitory effect of salt stress on germination percentage were also reported by Tsegay and Andargie (2018) and Ibrahim et al. (2019) in *Zea mays, Pisum sativum, Lathyrus sativus* and wheat. Ali et al. (2021) reported that the reduction in GP might be due to decrease in water uptake and enzyme activity caused by salinity.

Both seed treatment with 200 ppm GA_3 and distilled water increased the percentage of *Festulolium* germination as compared to the control. This result is in line with the finding of Ma et al. (2018) who determined significant effects of seed priming with GA_3 on germination parameters of perennial grass *Leymus chinensis*. It was determined that 50 mM GA_3 treatments increased the percentage of germination up to around 79%. On the other hand, seed priming with 200 mM GA_3 showed less performance (with a germination percentage of around 70%). It was indicated that seed priming with GA_3 hormone at appropriate concentrations could lead to high germination rates (Ma et al., 2018).

Salinity also significantly affected mean germination time. MGT of *Festulolium* cultivars significantly increased with increasing salinity levels. This result complies with the findings of Kandil et al. (2014) and Sheikh-Mohamadi et al. (2017). In general, 200 ppm GA_3 treatment at all salinity levels caused a decrease in MGT as compared to control

treatment (non-treated seed) in both cultivars. Younesi and Moradi (2015) obtained the lowest MGT at priming with GA_3 under salinity stress. Tsegay and Andargie (2018) reported that priming with 0.2 g/L GA_3 reduced MGT in *Zea mays*, *Pisum sativu*m and *Lathyrus sativus.*

Salt stress is proven to have a negative effect on shoot and root growth. Shoot length of both cultivars decreased at 15 and 20 dS/m salinity levels, while root length decreased with increasing salinity levels. The shortest root and shoot length were found in the seedlings that was subjected to 20 dS/m salinity level. The root growth inhibition was more prominent than shoot. Present findings agreed with a previous study conducted in *Isatis indigotica* (Jiang et al., 2020). It was reported in studies carried out in *Sorghum bicolor* and *Festuca arundinacea* that shoot and root length decreased due to increasing salinity levels (Nimir et al., 2014; Shiade and Bolet, 2020). The decrease in shoot and root length may be resulted from the toxic effects of NaCl or an osmotic and oxidative stress (Sheikh-Mohamadi et al. 2017; Ibrahim et al., 2019). Seedling fresh and dry weight also decreased at salinity levels of 15 and 20 dS/m.

The longest shoot and root length were achieved in seeds that treated with GA_3. The 200 ppm GA_3 treatment increased shoot length in Hostyn cultivar at all salinity levels as compared to control and distilled water treatments, while 100 and 200 ppm GA_3 treatments enhanced shoot length of Lofa cultivar at 5, 10, 15 and 20 dS/m salinity levels as compared to control treatment. Iqbal and Ashraf (2013) indicated that seed pre-treatment with GA_3 alleviated the inhibitory effect of salinity and 150 mg/L GA_3 pre-treatment was the most effective treatment to increase shoot growth in wheat. Shahzad et al. (2021) indicated that better seedling length of GA_3 treated maize might be due to the role of GA_3 in stimulating the cell elongation.

An increasing tendency in fresh and dry weight with seed treatments was not encountered in this study. Similar to present findings, Dhillon et al. (2021) also reported no significant effect of seed treatments on seedling dry weight. It was suggested that the reason for no effect of hormone priming on seedling dry weight despite positive impacts on root and shoot length could be that the experimental duration was too short (2 weeks) to reflect the impact on seedling dry weight. However, Muniandi et al. (2018) reported that the size of basal diameter decreased drastically in all GA_3 treated plants as compared to the control plants which resulted lighter and weaker but taller plants in kenaf. In this experiment,

GA_3 treatments did not significantly increase seedling fresh and dry weights, although GA_3 treatments increased shoot lengths.

Seedling vigor index (SVI) is a growth parameter in seed germination studies, which measure the potential for rapid and uniform emergence (Arnott et al., 2021). GA_3 pre-treatments promoted the SVI. The highest SVI of both cultivars was obtained from 200 ppm GA_3 treatments, followed by 100 ppm GA_3, distilled water and control treatments. Salinity levels significantly affected SVI. With a less salt stress given to the seed, the *Festulolium* seeds germinated simultaneously at the first beat. Conversely, when the dose of salt applied to seed was higher, the value of SVI decreased due to the inhibition of plant germination and growth. GA_3 treatments increased SVI significantly at all salinity levels. Similarly, Ibrahim et al. (2019) reported that seed treatment by GA_3 positively affected SVI under salt stress. During seed germination, GA_3 may result in early and vigorous germination through induced synthesis of hydrolytic enzymes (α-amylase) which converts starch into glucose providing energy for seed germination (Seethalakshmi et al., 2022)

Elevated salinity increased proline content of *Festulolium* cultivars. Several reports have indicated that proline contents increased with increasing salinity levels (Sheikh-Mohamadi et al., 2017; Wang et al., 2019; Shihab and Hamza, 2020). Seed priming using GA_3 enhanced proline accumulation at salinity levels of 10 and 15 dS/m. Similar results were also reported by Siddiqui et. al. (2008), Jiao et al. (2019) and Wang et al. (2019). In contrast to present findings, Ahmad et al. (2021) stated that GA_3 pre-treatments decreased proline content in shoots and roots of *Pisum sativum*. Proline plays a protective role acting as an important osmoprotectant, and also It stabilizes proteins, membrans and sub-cellular structures and protects cells from oxidative damage by reactive oxygen species (Hnilickova et al., 2021). The significance of proline accumulation is controversial. While some researchers indicated that an increase in proline content correspondent to improvement in its tolerance to salinity, others indicated that proline was a sign of stress but did not increase tolerance to salinity (Siddiqui et al., 2008; Wang et al., 2019; Al-Harthi et al., 2021; Hnilickova et al., 2021).

4.2. Conclusion and Recommendations

In summary, salinity stress reduced the germination and growth of both *Festulolium* cultivars. Seed treatments with distilled water and 200 ppm GA_3 increased GP as compared to the control treatment. Salinity stress increased MGT. In both cultivars, 200 ppm GA_3 treatment decreased MGT of both cultivars as compared to control treatment. On the other hand, at the salinity levels of control, 5, 10 and 15 dS/m, all seed treatments (distilled water, 100 and 200 ppm GA_3) shortened MGT as compared to the control treatment in Lofa cultivar. Salinity stress caused a decrease on shoot and root length. Seed treatments (distilled water, 100 and 200 ppm GA_3) increased shoot length at 10, 15 and 20 dS/m salinity levels in both cultivars. The highest root length in both cultivars was recorded from the seeds primed with 200 ppm GA_3 but differences between seed treatments at all salinity levels varied according to cultivars. An increasing tendency in fresh and dry weight with seed treatments cannot be noted in both cultivars. While salinity negatively affected SVI, seed treatments promoted the SVI. The highest SVI in *Festulolium* cultivars was obtained from 200 ppm GA_3 treatment, followed by 100 ppm GA_3, distilled water and control treatments. GA_3 treatments increased SVI significantly at all salinity levels. Elevated salinity increased proline content of *Festulolium* cultivars. Seed priming using GA_3 enhanced proline accumulation at salinity levels of 10 and 15 dS/m.

According to this germination experiment results, 200 ppm GA_3 treatment increased GP, shoot length, SVI and proline content and decreased MGT in both *Festulolium* cultivars. However, further experiments such as enzyme synthesis, breakdown of stored food reserves and protein synthesis of *Festulolium* cultivars are recommended to fully understand the effect of GA_3 treatments on salinity stress

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