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In vitro Determination of Salt Stress Responses of OH×F 333 and OH×F 97 Pear Clonal Rootstocks

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ABSTRACT
In this study, the responses of OH×F 333 and OH×F 97 pear clonal rootstocks to salt stress were investigated. For this purpose, in vitro, plants of OH×F 333 and OH×F 97 pear clonal rootstocks were cultured in ½ MS medium containing different concentrations of salt (0, 50, 100, 150, and 200 mM NaCl). To adapt the plants to salt stress, the doses of NaCl added to the medium were gradually increased at weekly intervals. In the experiment, in parallel with the increasing salt stress, the regeneration rate and shoot number values decreased, while the degree of damage increased significantly. It was determined that most of the shoots died in the application of 200 mM NaCl. When the effects of different salt concentrations on biochemical parameters were examined, it was determined that total phenolics, total flavonoids, proline, and soluble protein contents decreased, while lipid peroxidation increased in parallel with salt concentrations. However, it was determined that there was an increase in the total phenolic and flavonoid contents in 150 mM NaCl application.

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ÖZET
Bu çalışmada OH×F 333 ve OH×F 97 armut klon anaçlarının tuz stresine gösterdiği tepkiler araştırılmıştır. Bu amaçla OH×F 333 ve OH×F 97 armut klon anaçlarının in vitro bitkileri kademeli olarak artırılan farklı konsantrasyonlarda tuz (0, 50, 100, 150 ve 200 mM NaCl) içeren ½ MS ortamında kültüre alınmıştır. Denemede in vitro koşullarda tuz konsantrasyonları arttıkça, rejenerasyon oranı ve sürgün sayısı değerleri azalmış, zararlanma derecesi ise önemli derecede artmıştır. 200 mM NaCl uygulamasında çoğu sürgünün canlılıklarını yitirdikleri tespit edilmiştir. Farklı tuz konsantrasyonlarının biyokimyasal parametreler üzerindeki etkisi incelendiğinde toplam fenolik madde, toplam flavonoid madde, prolin ve çözünebilir protein içeriklerinin azaldığı, lipid peroksidasyon içeriğinin ise tuz konsantrasyonlarına paralel şekilde arttığı belirlenmiştir. Ancak toplam fenolik ve flavonoid madde içeriklerinde 150 mM NaCl uygulamasında tekrar bir yükselişin olduğu tespit edilmiştir.

Bahçe Bitkileri

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Anahtar Kelimeler
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Prolin
Tuz stresi
Toplam fenolik madde
Toplam flavonoid madde

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INTRODUCTION
The decrease in natural resources in the world day by day reveals new searches in agriculture as in every field. The decrease in agricultural areas and product productivity due to the increasing world population and the effect of various stress factors shows that human beings will face a serious nutritional problem in the coming years. The efforts to obtain the highest efficiency from the existing production areas have accelerated depending on the increase in the world population. In addition, scientists have stated that deterioration in the climate system will cause negative consequences. Türkiye is among the countries that will

be most affected by climate change due to global warming. If the necessary precautions are not taken, the factors that contribute to the disruption of the natural balance will progressively intensify, ultimately resulting in climate changes caused by global warming. Because, due to human reasons, the increase in greenhouse gas accumulations and particles in the atmosphere, the destruction of the natural environment, and the depletion of the ozone layer, will cause a global temperature increase (Öztürk, 2002; Hayaloğlu, 2018). Temperature increases, on the other hand, will activate many stress factors such as decreased precipitation, drought, and salinization of the soil, and this will have negative consequences. In particular, these abiotic stressors have limiting effects on global food security, quality, and plant productivity (Hayaloğlu, 2018; Yıldırım et al., 2021). Soil quality and health are one of the most important factors for fruit cultivation (Koç and Yakupoğlu, 2022). Approximately 20% of the irrigable agricultural lands in the world are adversely affected by soil salinity. This problem has increased with the excessive use of fertilizers, inappropriate use of irrigation water, natural environmental conditions, and global climate change (Zhao et al., 2021). Salinity is one of the important environmental stress factors that negatively affect the development, quality, and yield of plants. In crop production, approximately 50% of crop losses occur due to abiotic stress factors. An area of 6% in total, 30% of the irrigable areas, is faced with soil salinity today. On the other hand, improper cultural practices (such as fertilization, and irrigation) cause an increase in salinity (Hasaruzzaman et al., 2013).

Salt stress is an abiotic stress factor that adversely affects all stages of the plant such as germination, development, flowering, and fruit set. High Na concentrations in saline soil limit plant water uptake and nutrient absorption. Lack of water and nutritional imbalance cause osmotic stress and ionic stress problems. Various physiological and molecular changes occur with salt stress and it causes problems in plant growth and development by limiting photosynthesis (Van Zelm et al., 2020; Gong, 2021). This shows that salt stress is an important abiotic stress factor. Studies have reported that damages occur in pear rootstocks under salt stress due to the transport of Na and Cl ions to the leaves (Matsumoto et al.,2006a).

To meet the increasing agricultural production need, it is necessary to carry out plant production in unsuitable areas. Although there are some differences in salt tolerance between plant species and cultivars, it is known that the majority of cultivated plants are generally sensitive to salinity (Kuşvuran et al., 2008). Studies on the development of salt-tolerant rootstocks or cultivars have gained importance in growing under salt-stress conditions in fruit growing. In addition, the tolerance levels of existing rootstocks and cultivars to salt stress should also be determined. In this regard, Asian (*Pyrus betulaefolia* Bunge., *P. pyrifolia* Nakai, and *P. xerophila* Yu) and Mediterranean pear species (*P. amygdaliformis* Vill. and *P. elaeagrifolia* Pall) were irrigated with 75 mM and 150 mM NaCl solutions for 30 days and salt tolerance levels were determined. According to the research, it was determined that Mediterranean pear species are more tolerant to salt than Asian species (Matsumoto et al., 2006b). Some studies have been conducted to determine the salt stress tolerance of some pear rootstocks (Aydınlı, 2021; Javadisaber et al., 2024). However, detailed studies are needed to determine tolerance mechanisms against stress conditions. There are significant differences according to genotypes, especially in the synthesis of biochemical substances such as phenolics, flavonoids, and proline.

In this study, it was aimed to determine the responses of OH×F 333 and OH×F 97 pear clonal rootstocks to salt stress at different concentrations in vitro. For this purpose, some morphological (shoot number, shoot length, degree of damage) and biochemical (total phenolic, total flavonoid, proline, soluble protein, lipid peroxidation) analyses were performed on rootstocks.

MATERIAL and METHOD

Material

In this study, shoot tips taken from OH×F 333 and OH×F 97 pear clonal rootstocks were used as material. The experiment was carried out at Isparta University of Applied Sciences, Faculty of Agriculture, Department of Horticulture, Tissue Culture Laboratory in 2020-2021. Autoclavable glass magentas were used for cultures in the study. Forceps, scalpels, and sterile filter papers were used as materials at all stages of in vitro culture. MS medium and agar used in the study were purchased from Duchefa Biochemie B.V. (Haarlem, The Netherlands). NaCl, BAP, IBA, sucrose, gallic acid, catechin, and proline were obtained from Merck (Merck KGaA, Germany).

Method

Sterilization

The prepared media were distributed in glass magentas with an internal volume of 100 ml, with approximately 25 ml of medium. Then, the media were sterilized by keeping them in an autoclave set at 121 ℃ for 15 min (Şan et al., 2015).

The 2-3 cm shoot tips used in the study were taken in May 2020 and first washed under tap water for 15 min. After being kept in 70% ethanol for a min., it was washed with sterile distilled water to remove alcohol. In the last stage, shoot tips were kept in a 20% sodium hypochlorite solution (15% Cl content) containing a few drops of Tween 20 for 18 min by shaking. At the end of

the period, the shoot tips taken into the laminar air flow cabinet were washed with sterile distilled water 3 times for 5 min each to remove sodium hypochlorite (Tuncel & Şan, 2023). The sterile shoot tips were left on sterile filter paper and their moisture was removed. Then, the shoot tips prepared in 0.5-1 cm length were planted in nutrient media.

Salt stress experiment

Sterilized shoot tips were prepared about 0.5-1 cm in length and planted in nutrient media in magenta containers. MS medium (Murashige & Skoog, 1962) was used in all stages of the experiment.

Microshoots were propagated by subcultures at 4-week intervals until sufficient shoot numbers were reached for the salt stress experiment. In the propagation step, 1.5 mg/L BAP, 0.1 mg/L IBA, and 30 g/L sucrose were added to the MS medium. After the pH of the medium was adjusted to 5.7, 7 g/L agar was added and sterilized. In vitro, cultures at the propagation stage were incubated in a climate chamber set at 22 ℃ and 16 hours of light and 8 hours of darkness. The experiment was initiated once the required number of shoots was reached in the research.

In the experiment, approximately 1-1.5 cm of in vitro shoots were planted in the medium. In the study, different concentrations of NaCl (0, 50, 100, 150, and 200 mM) were added to the ½ MS medium (Table 1). The pH of the nutrient media was adjusted to 5.7. The shoots were incubated in a climate chamber set at 22 ℃ and 16 hours of light/8 hours of darkness (Çalhan, 2020). The incubation process took a total of 8 weeks under salt stress conditions. The conclusion of the experiment involved the investigation of certain morphological traits, such as regeneration rate, shoot length, the number of shoots, and the degree of damage. At the same time, the experiment included the determination of biochemical parameters such as total phenolics, total flavonoids, lipid peroxidation, proline, and soluble protein contents.

Table 1. Applications of different NaCl concentrations on OH×F 333 and OH×F 97 pear clonal rootstock
Çizelge 1. OH×F 333 ve OH×F 97 armut klon anaçlarına farklı NaCl konsantrasyonları uygulamaları

Treatments*	Incubation conditions
Control (0 mM NaCl)	Incubation in ½ MS basal medium for 8 weeks
50 mM NaCl	Incubation in ½ MS medium without NaCl for a week, then in medium containing 50 mM NaCl for 7 weeks
100 mM NaCl	Incubation in ½ MS medium without NaCl for a week, in the medium containing 50 mM NaCl for a week, and then in the medium containing 100 mM NaCl for 6 weeks
150 mM NaCl	Incubation in ½ MS medium without NaCl for a week, in a medium containing 50 mM NaCl for a week, in a medium containing 100 mM NaCl for a week, and then in a medium containing 150 mM NaCl for 5 weeks
200 mM NaCl	Incubation in ½ MS medium without NaCl for a week, in a medium containing 50 mM NaCl for a week, in a medium containing 100 mM NaCl for a week, in a medium containing 150 mM NaCl for a week and then in a medium containing 200 mM NaCl for 4 weeks

* 30 g L^{-1} sucrose, 7 g L^{-1} agar, 1.5 mg L^{-1} BAP and 0.1 mg L^{-1} IBA were added to all nutrient media.

Measurement and Analysis

Regeneration rate: It was determined by the ratio of new shoot-forming explants to the total number of explants cultured and expressed as %.

Shoot length: Shoot lengths were measured with the help of a digital caliper and determined by calculating the average.

Number of shoots: The shoots that emerged on the main explant were counted averaged and determined as number/explant.

The degree of damage: The degree of damage seen in the explants in the experiment was scored according to the following scale (Sivritepe et al., 2008).

1: Very severe damage (complete drying of explants)

2: Severe damage (chlorosis and local drying of explants),

3: Moderate damage (severe chlorosis on explants and developmental arrest),

4: Less damage (chlorosis and poor growth of explants),

5: No damage (explants are healthy and growth is good.).

Total phenolic analysis: Total phenolic contents were made by modifying the Folin-Ciocalteau method specified by Singleton and Rossi (1965). The results were calculated according to the gallic acid standard and are expressed as mg GAE g^{-1} fresh weight (FW).

Total flavonoid analysis: Total flavonoid analysis was carried out by modifying the method specified by Zhishen et al. (1999). The results were calculated according to the catechin standard and expressed as mg CE g^{-1} FW.

Soluble protein analysis: Soluble protein analysis was performed by modifying the method specified by Hartree (1972). Results were calculated according to BSA (Bovine Serum Albumin) standardization and are expressed in mg g^{-1} FW.

Lipid peroxidation analysis: Lipid peroxidation was carried out by modifying the method determined by Jiang et al (2010). Results are expressed as nmol g^{-1} FW.

Proline analysis: Proline analysis was performed according to the method described by Li et al. (2012). The results were calculated according to the D-Proline standard and expressed as µmol g^{-1} FW.

Experimental Design and Data Analysis

The experiment was planned according to the factorial randomized plots experimental design with 3 replications and 10 explants in each replication. A total of 150 microshoots were used for each rootstock in the study. The obtained data were subjected to variance analysis in the Minitab package program (MINITAB 17 inc). The difference between the significant means was determined according to the Tukey test and shown with different letters (Mathews, 2004). The results were given as standard errors in the tables.

RESULTS

Morphological Features

In the study, the effects of salt stress on regeneration rate, shoot number, shoot length, and degree of damage were investigated in OH×F 333 and OH×F 97 pear clonal rootstocks. Analysis of variance was applied to the obtained data and the results are presented in Table 2.

Table 2. The effects of different NaCl applications on morphological values in OH×F 333 and OH×F 97 pear clonal rootstocks

Çizelge 2. OH×F 333 ve OH×F 97 armut klonal anaçlarında farklı NaCl uygulamalarının morfolojik değerler üzerine etkileri

Rootstocks	Salt stress applications	Regeneration rate (%)	Shoot length (mm)	Shoot number	Degree of damage (0-5 range)
OH×F 333	Control	90±6.1 ab*	15.3±1.48bc	3.94±0.44 ab	4.6±0.25 a
	50 mM NaCl	70±12.2 abcd	17.5±1.69 abc	2.70±0.29 cd	3.6±0.25 ab
	100 mM NaCl	80±6.1 abc	17.9±0.73 abc	2.20±0.20 cde	4.6±0.25 a
	150 mM NaCl	40±10.0 de	20.3±1.67 abc	1.50±0.16 e	4.2±0.37 a
	200 mM NaCl	20±12.2 e	17.6±1.62 abc	1.10±0.10 e	1.2±0.20 c
OH×F 97	Control	100±0.0 a	17.4±1.14 abc	4.15±0.29 a	4.6±0.25 a
	50 mM NaCl	80±9.3 abc	21.5±0.89 ab	2.90±0.23 bc	4.2±0.20 a
	100 mM NaCl	60±6.1 bcd	21.9±1.43 a	2.00±0.18 cde	3.4±0.51 ab
	150 mM NaCl	50±7.9 cde	20.9±1.96 abc	1.60±0.15 de	2.6±0.51 bc
	200 mM NaCl	15±6.1 e	14.5±0.63 c	1.15±0.06 e	1.2±0.20 c
Applications Mean	Control	95±3.3 a	16.4±0.93 b	4.04±0.25 a	4.6±0.16 a
	50 mM NaCl	75±7.4 a	19.5±1.12 ab	2.80±0.18 b	3.9±0.18 ab
	100 mM NaCl	73±5.8 a	19.9±1.00 ab	2.10±0.13 c	4.0±0.33 ab
	150 mM NaCl	45±6.2 b	20.6±1.22 a	1.55±0.10 cd	3.4±0.40 b
	200 mM NaCl	18±6.5 c	16.1±0.97 b	1.13±0.06 d	1.2±0.13 c
Rootstocks Mean	OH×F 333	61±6.8	17.8±0.69	2.29±0.23	3.64±0.28 a
	OH×F F 97	61±6.5	19.2±0.79	2.36±0.23	3.20±0.29 b

* The difference between the means shown with different letters for each rootstock is statistically significant. ($p \leq 0.05$)

In the evaluation of the regeneration rate, it was observed that the interaction between the rootstock and application had notable significance (Table 2). It was observed that the regeneration rates decreased as the salt concentration increased in both rootstocks. In the study, the highest regeneration rates were observed in both OH×F 333 and OH×F 97 rootstocks in the control application (90% and 100%, respectively). It was determined that the lowest regeneration rate was 20% in OH×F 333 rootstock and 15% in OH×F 97 rootstock in 200 mM NaCl application. When the application means were examined, the difference between the applications was found to be significant. The average regeneration rate was determined as 95% in the control application and 18% in the 200 mM NaCl application. There was no statistically significant difference between rootstocks in terms of regeneration rate.

Rootstock x application interaction was found to be statistically significant in terms of shoot length. While there was no difference between applications in OH×F 333 rootstock, 200 mM NaCl application in OH×F 97 rootstock significantly reduced shoot length compared to control. Considering the application averages, the difference was found to be statistically significant. The highest shoot length was determined as 20.6 mm in 150 mM NaCl concentration, and the lowest shoot length was 16.1 mm in 200 mM NaCl application. There was no statistical difference between rootstocks in terms of shoot length.

In the study, rootstock x application interaction was significant in terms of shoot number. The highest shoot number was observed in the control application in both OH×F 97 and OH×F 333 rootstocks (4.15 and 3.94, respectively). It was determined that there was a decrease in the number of shoots of the plants in parallel with the increase in the applied salt concentrations. It was determined that the lowest shoot number values were 1.10 in OH×F 333 rootstock and 1.15 in OH×F 97 rootstock in 200 mM NaCl application. Considering the application averages, the difference was found to be statistically significant. It was determined that the highest shoot number was 4.04 in the control application, and the lowest shoot number was 1.13 in the 200 mM NaCl application. There was no statistically significant difference between rootstocks in terms of the number of shoots.

In terms of the degree of damage, rootstock x application interaction was found to be statistically significant. The signs of damage to the plants were manifested as weak shoot development, chlorosis, and drying of the leaves. When the values in Table 2 were examined, it was determined that the highest damage was observed in 200 mM NaCl application with a value of 1.2 for both rootstocks (Figures 1 and 2). In this application, it was observed that although some plants were alive, most plants died. Considering the application averages, the difference was found to be statistically significant. In parallel with the increase in salt concentration, the damage status of the plants also increased. When the rootstocks were compared in terms of damage, it was seen that OH×F 333 rootstock was relatively more tolerant to salt stress conditions than OH×F 97 rootstock (3.64 and 3.20, respectively).

Fig. 1. Growth of OH×F 97 rootstock in ½ MS medium containing different concentrations of NaCl
Şekil 1. Farklı konsantrasyonlarda NaCl içeren ½ MS ortamında OH×F 97 anacının gelişmesi

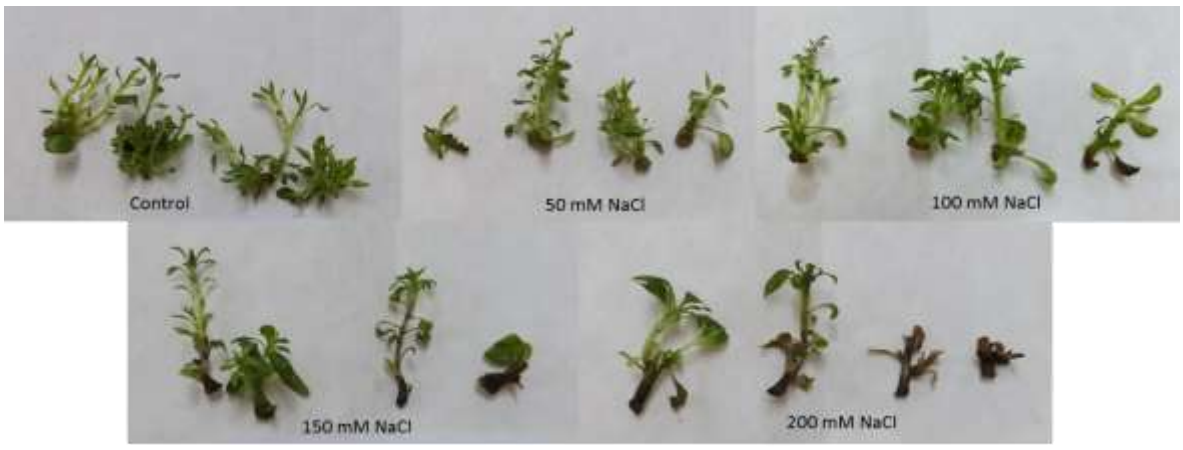

Fig. 2. Growth of OH×F 333 rootstock in ½ MS medium containing different concentrations of NaCl
Şekil 2. Farklı konsantrasyonlarda NaCl içeren ½ MS ortamında OH×F 333 anacının gelişmesi

Biochemical Properties

In the study, the effects of salt stress treatments on total phenolic, total flavonoid, lipid peroxidation, and proline contents of OH×F 333 and OH×F 97 pear clonal rootstocks were investigated. The results are given in Table 3.

In salt stress studies, because of high damage to plants in media containing 200 mM NaCl, sufficient plant samples could not be taken for biochemical analysis. Therefore, biochemical analyses were not performed in 200 mM NaCl application. In the study, rootstock x application interaction was found to be statistically significant in terms of total phenolic content. The

highest total phenolic was determined in OH×F 333 rootstock in the control application (4.66 mg g^{-1}). It was detected in 150 mM NaCl application (1.98 mg g^{-1}) in OH×F 97 rootstock. The lowest total phenolic contents were found in OH×F 333 and OH×F 97 rootstocks in 100 mM NaCl application (1.99 mg g^{-1} and 0.83 mg g^{-1}, respectively). In the study, as the salt concentrations increased, a decrease was observed in the total phenolic content. However, in the application of 150 mM NaCl, an increase was detected again in both rootstocks. The difference between the salt stress application averages was found to be statistically significant. It was determined that the highest total phenolic content was in the control application, while the lowest content was determined at 100 mM NaCl concentration. The difference between rootstocks was also significant. Accordingly, while the total phenolic content of OH×F 333 rootstock was 3.02 mg g^{-1}, this value was found to be 1.60 mg g^{-1} in OH×F 97 rootstock.

Table 3. The effects of NaCl applications on biochemical properties of OH×F 333 and OH×F 97 pear clonal rootstocks.

Çizelge 3. NaCl uygulamalarının OH×F 333 ve OH×F 97 klon armut anaçlarının biyokimyasal özelliklerine etkileri

Rootstocks	Salt stress applications	Total phenolics (mg g^{-1} FW)	Total flavonoids (mg g^{-1} FW)	Soluble protein (mg g^{-1} FW)	MDA (nmol g^{-1} FW)	Proline (µmol g^{-1} FW)
OH×F 333	Control	4.66±0.13 a*	0.53±0.01 ab	0.76±0.06 cd	23.0±1.41 ab	111.0±2.41 a
	50 mM NaCl	2.12±0.02 c	0.44±0.01 b	0.82±0.04 cd	26.9±1.19 a	48.4±1.48 b
	100 mM NaCl	1.99±0.10 cd	0.43±0.02 b	0.64±0.08 de	23.2±1.61 ab	13.9±0.10 b
	150 mM NaCl	3.22±0.09 b	0.55±0.02 ab	0.45±0.03 e	22.9±0.47 ab	40.5±0.90 b
OH×F 97	Control	1.87±0.06 cd	0.49±0.03 ab	1.38±0.02 a	16.1±0.70 c	23.1±1.16 b
	50 mM NaCl	1.66±0.10 d	0.67±0.14 ab	0.97±0.05 bc	20.7±1.19 bc	18.8±1.60 b
	100 mM NaCl	0.83±0.03 e	0.44±0.05 b	1.11±0.02 b	22.9±0.37 ab	21.7±1.81 b
	150 mM NaCl	1.98±0.05 cd	0.90±0.21 a	0.81±0.03 cd	21.3±0.90 b	24.2±2.89 b
Applications mean	Control	3.30±0.43 a	0.51±0.02 ab	1.07±0.10 a	19.5±1.14 b	67.3±19.80 a
	50 mM NaCl	1.20±0.10 c	0.55±0.07 ab	0.90±0.04 b	23.8±1.08 a	33.6±2.15 b
	100 mM NaCl	1.40±0.18 d	0.44±0.03 b	0.90±0.08 b	23.0±0.70 a	17.8±1.92 b
	150 mM NaCl	2.60±0.20 b	0.72±0.11 a	0.70±0.05 c	22.1±0.40 ab	32.4±3.89 b
Rootstocks mean	OH×F 333	3.02±0.23 a	0.50±0.01 b	0.70±0.04 b	24.0±0.49 a	53.6±11.40 a
	OH×F 97	1.60±0.10 b	0.62±0.07 a	1.10±0.05 a	20.2±0.75 b	22.0±1.04 b

* The difference between the means shown with different letters for each rootstock is statistically significant. ($p \leq 0.05$)

Rootstock x application interaction was found to be significant in terms of total flavonoid content. The highest total flavonoid content was observed in 150 mM NaCl application (0.55 mg g^{-1} in OH×F 333, 0.90 mg g^{-1} in OH×F 97) on both rootstocks. However, it was determined that salt stress applications did not significantly affect the total flavonoid content in OH×F 333 rootstock. The difference between the salt stress application averages was found to be statistically significant. The highest value was determined in the application of 150 mM NaCl, while the lowest value was observed in the application of 100 mM NaCl. The difference between rootstocks in terms of total flavonoid content was statistically significant. In this respect, the total flavonoid content of OH×F 97 rootstock was higher than that of OH×F 333 rootstock (0.62 mg g^{-1} and 0.50 mg g^{-1}, respectively).

Rootstock x application interaction was found to be statistically significant in terms of soluble protein content. In the study, the highest soluble protein content was found in 50 mM NaCl application for OH×F 333 (0.82 mg g^{-1}) and control (1.38 mg g^{-1}) for OH×F 97. The lowest soluble protein content was detected in the application of 150 mM NaCl on both OH×F 333 and OH×F 97 rootstocks (0.45 mg g^{-1} and 0.81 mg g^{-1}, respectively). The difference between the salt stress application averages in terms of soluble protein was found to be statistically significant. It was determined that the highest soluble protein content was in the control application. The difference between rootstocks in terms of soluble protein content was found to be statistically significant. Accordingly, while the soluble protein content was 1.10 mg g^{-1} in OH×F 97 rootstock, this value was determined as 0.70 mg g^{-1} in OH×F 333 rootstock.

Rootstock x application interaction was found to be statistically significant in terms of lipid peroxidation. The highest lipid peroxidation content was observed in OH×F 333 rootstock with a value of 26.9 nmol g^{-1} in 50 mM NaCl application. In OH×F 97 rootstock, the highest lipid peroxidation was determined in 100 mM NaCl application (22.9 nmol g^{-1}). The difference between the salt stress application averages in terms of lipid peroxidation was found to be statistically significant. The highest lipid peroxidation was detected in the application of 50 and 100 mM NaCl (23.8 and 23.0 nmol g^{-1}, respectively). The lowest value was determined to be 19.5 nmol g^{-1} in the control

application. When the rootstocks were compared, higher values were obtained in OH×F 333 rootstock (24.0 nmol g^{-1}) compared to OH×F 97 rootstock (20.2 nmol g^{-1}) in terms of lipid peroxidation.

When the proline contents of the rootstocks were examined, the rootstock x application interaction was found to be significant. In the study, the highest proline content was observed in the control application (111.0 µmol g^{-1}) in OH×F 333 rootstock. In OH×F 97 rootstock, there was no statistical difference between applications. When the salt stress application averages were evaluated, it was determined that the highest proline content was (67.3 µmol g^{-1}) in the control application. The difference between rootstocks in terms of proline content was found to be statistically significant, and the proline content of OH×F 333 rootstock (53.6 µmol g^{-1}) was found to be higher than that of OH×F 97 rootstock (22.0 µmol g^{-1}).

DISCUSSION

In this study, we determined the tolerance or sensitivity levels of OH×F 333 and OH×F 97 pear clonal rootstocks, which are widely used in pear cultivation, to salt stress at different concentrations in vitro.

Morphological Features

Plants are generally sensitive to saline conditions. Therefore, salt stress negatively affects the vegetative development of plants in many ways. In the study, when the effects of different salt concentrations on the regeneration rate under in vitro conditions were evaluated, it was observed that there was a statistically significant decrease in the regeneration rates in parallel with the increase in salt concentrations. In some studies, similar findings to the results were obtained. Krasensky and Jonak (2012) found that there was a decrease in the fresh and dry weight of onion plants under increased salt stress conditions. Similarly, Zambia (2019) stated that the development slowed down in parallel with the increase in salt concentration in peas.

In the study, it was determined that the number of shoots decreased significantly in parallel with the increase in the salt stress level. Supporting the results, Rahman et al. (2007) reported that the application of salt stress in vitro conditions reduced the number of shoots in *P. communis* rootstocks compared to the control. Similarly, Shiyab et al. (2003) stated that salt stress applications (control, 50, 100, 150, 200, and 300 mM NaCl) decreased shoot growth in sour orange. The researchers stated that at 200 and 300 mM NaCl concentrations, the growth parameters of the plants were greatly affected and the shoots lost their vitality.

In the study, it was observed that shoot lengths increased as NaCl concentrations increased in both rootstocks. However, it was determined that shoot length was shorter in the 200 mM NaCl application than in the control application because growth and development completely stopped. It is thought that the increase in shoot length values in parallel with the salt concentration may be a result of the decrease in the number of shoots. In parallel with the study, Javadisaber et al. (2024) and Sotiropoulos et al. (2006) reported that they obtained the longest shoots from high salt concentrations. However, it is reported that there is a decrease in shoot length in general under salt stress. Studies conducted on pear (Sotiropoulos et al. 2006) and quince (Sotiropoulos et al. 2007) reported that the length of micro shoots decreased with increasing salt concentrations. Dajic (2006) reported that photosynthesis slowed down with the decrease in shoot growth of plants exposed to salt stress. It was also stated in the study that turgor decreased and mineral transport was difficult. In addition, it has been reported that the increased Na concentration in plants under salt stress causes damage to the shoots (Uyar, 2016).

In the study, it was determined that the damage increased in direct proportion to the increase in salt concentrations. It was observed that chlorosis, browning, drying, and shortening of shoot length occurred in the shoots of plants grown in in vitro conditions where different salt concentrations were applied. Javadisaber et al. (2024), in their study on pear genotypes, stated that the level of damage in micro shoots increased in parallel with the increase in salt concentration. In another study, it was determined that Asian wild pear species (*P. betulaefolia* Bunge, *P. pyrifolia* Nakai, and *P. xerophila* Yu) were more affected by salt stress than Mediterranean wild pear species (*P. amygdaliformis* Vill. and *P. elaeagrifolia* Pall.) (Matsumoto et al., 2006b).

Biochemical Properties

Plants make changes in their metabolism to adapt to different environmental conditions. These changes are symptoms such as chlorosis of leaves, early flowering, shedding of leaves, or curling of leaves. Phenolic compounds, which play an active role in this process, are effective in signaling stress factors in the plant and synthesizing chemical substances. It also plays an active role in the opening and closing of stomata in leaves, early maturation, and changes in respiratory activity (Hacıkamiloğlu, 2023). In this study, the total phenolic content decreased in parallel with the salt stress level in both rootstocks. However, it was determined that there was an increase again in the application of 150 mM NaCl. Supporting the results, Çalhan (2020) reported that the total phenolic content decreased in parallel with the increasing salt concentration in myrtle genotypes, while the total phenolic content increased again at high salt

concentrations. In other studies, it has been reported that there is an increase of up to three times in the total phenolic content of sugar cane and olive with the increase in salt stress (Wahid & Ghazanfar, 2006; Petridis et al., 2012). However, it has been stated in the studies that the total phenolic content decreases or is not affected under salt stress conditions and that these compounds may differ according to the salt concentration. Bourgou et al (2010), in their study on the *Nigella sativa* plant, reported that the total phenolic content decreased as a result of salt stress applications. Similarly, Shaheen et al. (2012) reported that the total phenolic content of the eggplant decreased under salt stress. The results we obtained in this study were found to be compatible with the literature.

Flavonoids, on the other hand, are a subgroup of phenolic compounds and participate in the plant defense system against different stress conditions (Harborne & Williams, 2000). In the findings obtained in this study, it was determined that the total flavonoid content did not show a statistically significant difference according to salt concentrations. However, an increase was detected in 150 mM NaCl application only in OH×F 97 rootstock compared to the control. In the study conducted by Gengmao et al. (2015), it was reported that the total flavonoid content of sunflowers increased under salt stress conditions compared to the control application. However, there are studies reporting reductions in the total flavonoid content of plants under salt stress. Petropoulos et al (2017) reported that the total flavonoid content of high salt concentrations increased little or had no stress effect. In a study conducted on marigolds, it was determined that the total flavonoid content decreased in salt stress application compared to the control (Khalid et al., 2010). In this study, a partial decrease was observed in the total flavonoid content of OH×F 333 and OH×F 97 pear clonal rootstocks under salt stress conditions, and an increase was observed in 150 mM NaCl application.

Since plants activate oxidative stress under salt stress, membrane lipid peroxidation causes damage (Ye et al., 2000). With this damage, lipid peroxidation occurs. The damage to the membrane with lipid peroxidation is irreversible and becomes malondialdehyde, the most important product. In this study, it was determined that lipid peroxidation was not statistically affected in OH×F 333 rootstocks in salt stress applications, while it increased in OH×F 97 rootstocks in 100 and 150 mM NaCl applications compared to the control. Ertürk et al., (2007) applied salt stress to cherry rootstocks in vitro and stated that both lipid peroxidation and antioxidative enzyme activities increased under salt stress. Again, supporting the results, it has been reported that lipid peroxidation increases under salt stress conditions in strawberry and *Pyrus betulafolia* species (Tanou et al., 2009; Wu & Zou, 2009). Researchers have reported that oxidative stress occurs, necrotic lesions occur on leaves, H_2O_2/O_2 accumulation occurs in tissues, and lipid peroxidation increases, especially in 200 mM NaCl application.

When plants are under stress, they synthesize and accumulate various osmotic regulators to protect themselves from stress.

Proline, which is one of these osmotic regulators, is an amino acid that has different functions such as being an energy source as well as having antioxidant properties as well as its osmotic effect (Ben Ahmed et al., 2008). As a result of this research, it was seen that salt stress applications did not affect the proline content of OH×F 97 rootstocks statistically. On the other hand, it was determined that the proline content of OH×F 333 rootstock decreased significantly with salt stress applications. In the studies, it was stated that the accumulation of proline increased with the increase in the stress level in plants under salt stress. As a result of examining the tolerance effect of proline under stress conditions, it has been argued that this situation may not be valid in some plant species under salt stress (Mansour & Ali, 2017). As a matter of fact, in the salt stress study conducted on 46 genotypes of *Panicum virgatum*, it was determined that the proline content increased 5000 times in some salt-sensitive genotypes, while this increase was at a small level in tolerant lines (Kim et al., 2016).

It is known that plants protect themselves from reactive oxygen species (ROS) by stabilizing their protein structure with the help of osmolytes under salt-stress conditions (Zhu, 2001). Considering the soluble protein content in this study, it was observed that there were significant decreases in parallel with the increase in salt concentration. Studies have reported that stress factors cause increases or decreases in soluble protein content. It has been reported that soluble protein contents increase in onions (Bekheet et al., 2006) and decrease in sorghum (Parlak & Özaslan Parlak, 2006) under increasing salt stress conditions. The response of plants to stress conditions may vary according to the tolerance levels of genotypes.

As a result, both OH×F 97 and OH×F 333 clonal rootstocks were found to be sensitive to high doses of salt stress. However, it was determined that they showed little improvement under salt stress, which can be considered as high as 100 mM NaCl. When the rootstocks are compared with each other, although there is no statistical difference according to the morphological and biochemical analysis results, it has been determined that the OH×F 333 rootstock is slightly more prominent than the OH×F 97 rootstock. Especially in terms of damage degree, OH×F 333 clonal rootstock gave better values than OH×F 97 clonal rootstock. However, more detailed studies should be done in vitro and in vivo conditions to obtain more precise results.

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The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The author declares no conflicts of interest.

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