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Research Article

Morphological and Physiological Changes under NaCl Stress in Some *Pyrus* and *Quince* Rootstocks

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Tolerance

Abstract: In this study, the aim was to determine some morphological, physiological, and biochemical changes in non-grafted plants of OHxF 97, OHxF 333, Fox 11, and BA 29 rootstocks under NaCl stress. NaCl (0 mM, 20 mM, 40 mM, and 80 mM) was applied to the rootstocks planted in 18-liter pots with irrigation water repeated over two years. Under NaCl stress, plant height, plant diameter, and leaf area decreased in all rootstocks. Additionally, Fox 11 and BA 29 rootstocks were more adversely affected by NaCl stress to leaf necrosis. The amounts of chl a, chl b, and total chl decreased in Fox 11 rootstock with moderate and severe stress treatments. Carotenoid content in the leaves, especially under severe stress conditions, showed a decrease in *Pyrus* rootstocks. Under NaCl stress, the leaves of Fox 11 were rich in proline. MDA content generally increased with NaCl stress compared to the control in Fox 11 and BA 29. Although significant changes in plant nutrients were generally not observed with NaCl, a significant decrease in the amount of K^+ in the leaves of Fox 11 was identified. Consequently, Fox 11 and BA 29 rootstocks exhibit sensitivity to NaCl stress, whereas OHxF rootstocks demonstrate greater tolerance.

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1. Introduction

Salinization is a significant abiotic stress factor that adversely affects available agricultural lands. 7% of the world's terrestrial areas, 20% of arable lands, and half of irrigable agricultural lands are facing salinity (Gupta and Huang, 2014; Kumar et al., 2015). Furthermore, the growing apprehension among scientists regarding the escalating incidence of salinity stress, particularly in arid and semi-arid regions, is propelling extensive research into salt stress in the foreseeable future.

Salinity exerts direct effects on plants by inducing osmotic and ion stress, while its indirect effects (secondary effects) manifest through oxidative stress (Petridis et al., 2012; Wei et al., 2017). Osmotic stress is the initial stress resulting from an increase in salt concentration in the root rhizosphere (Tuteja, 2007). As a result of osmotic stress, factors such as a reduction in leaf water potential (LWP)

and stomatal conductance (Shabala and Munns, 2012; Navada et al., 2020), photosynthesis inhibition (Meloni et al., 2003), and elevated production of reactive oxygen species (ROS) (Jia et al., 2019) contribute to growth interruption (Munns and Tester, 2008). Ion stress induced by toxic ions such as sodium (Na^+) and chloride (Cl^-) (Sofy et al., 2020), particularly the excessive accumulation of Na^+ ions (Niu et al., 2017), contributes to the indirect effects. Oxidative stress arises from the excessive generation of harmful ROS (Arif et al., 2020). To withstand these stress factors, plants employ tolerance mechanisms and develop various physiological and biochemical adaptations. Important tolerance mechanisms include a) ion homeostasis and partitioning of ions, b) ion transport and uptake, c) synthesis of osmoprotectants and compatible solutes, d) activation of antioxidant enzymes and synthesis of antioxidant compounds, e) synthesis of polyamines, f) production of nitric oxide, g) hormone modulation (Gupta and Huang, 2014).

Plants are classified into halophytes and glycophytes based on their adaptation abilities to salt, with most agriculturally important species falling into the glycophyte class (Gupta and Huang, 2014). Pear (*Pyrus* spp.), one of the important temperate climate species, shows sensitivity when exposed to prolonged relatively low salinity (Okubo et al., 2000). Additionally, growth retardation and leaf damage occur under osmotic and ionic stress (Hasegawa et al., 2000)

Although pear cultivation in Türkiye is currently conducted in non-saline soils, according to Musacchi et al. (2006) the use of drip irrigation methods, the inclusion of fertigation systems in practices, and cultivation in areas close to the coast may lead to secondary salt stress and, consequently, salt damage. Minimizing this damage is seen as a priority and developing salt-tolerant rootstocks or determining the tolerance/sensitivity levels of existing plant materials used in production are important considerations. Against this backdrop, the current study aims to determine the tolerance mechanisms of OH x F 97, OH x F 333, Fox 11, and BA 29 rootstocks, against salt stress induced by irrigation water containing different concentrations of NaCl. The study generates data on the responses of rootstocks and their tolerance/sensitivity status based on various morphological, physiological, and biochemical parameters.

2. Material and Methods

2.1. Plant material

The study included one-year-old non-grafted plants of rootstocks OHxF 97 (Old Home x Farmingdale 97), OHxF 333 (Old Home x Farmingdale 333), Fox 11, and BA 29, which are used as rootstocks in pear growing. Clonal rootstocks of pear (*Pyrus communis* L.) were propagated through tissue culture, while the quince (*Cydonia oblonga* L.) clonal rootstock was propagated using the stool-bed technique. At the onset of the vegetation period, the plants were transplanted into a cultivation medium consisting of garden soil (sieved) + sand + peat (2:1:1) in 18-liter containers. In June, the plants were moved into a climate-controlled greenhouse where the treatments would take place.

2.2. NaCl treatments

The NaCl treatments were initiated in mid-July. The study included four different NaCl concentrations, including a control (irrigation water ~ 3 mM NaCl), light stress (20 mM), moderate stress (40 mM), and severe stress (80 mM). The amount of water given to the plants was determined based on the previously established field capacity. The irrigation interval was set at 4-5 days in the research. To prevent osmotic shock in the plants, NaCl doses of 20 mM were gradually applied. After approximately sixty days of NaCl treatments, damage related to NaCl stress was observed in the leaves, and the trial was concluded. For biochemical analyses, fully developed leaves from the middle part of each plant were collected at the end of the study, immersed in liquid nitrogen, and stored at -80°C until the analyses were conducted. Following this process, each plant was cut at the root collar.

The study was designed as a randomized complete factorial experiment with three replicates, with each replicate consisting of five plants, following a factorial design in the experimental plots. This research was repeated for two years.

2.3. Measurement of morphological parameters

Plant heights were measured in meters from the graft point to the top of the leading shoot. Shoot diameter was measured with calipers from the midpoint of the shoots. Leaf area measurements were conducted on 10 randomly selected leaves from each replicate at the end of the experiment. The surface areas of the samples were recorded in "cm^2" using a digital planimeter (Koizumi KP-90 N). The scale developed by Sivritepe et al. (2008) was employed to assess the damage occurring in the leaves and shoots of plants exposed to NaCl stress.

2.4. Measurement of physiological parameters

LWP measurements were conducted using a pressure chamber (PMS Instrument Company, Model 1000) between 12:00 and 14:00 on at least two fully mature leaves randomly selected from a plant in each treatment. To ensure the samples reached a stable state, leaves were wrapped in aluminum foil before measurements (Küçükyumuk et al., 2015).

2.5. Biochemical analyses

The total chlorophyll (total chl), chlorophyll a (chl a), chlorophyll b (chl b), and carotenoid (car) concentrations in the leaves were determined according to the method described by Arnon (1949). Fresh leaf samples were subjected to extraction with 80% (v/v) acetone. A portion of the extracted sample was taken, and the absorbances of chl a at 663 nm, chl b at 645 nm, and car at 470 nm were measured using a spectrophotometer (Shimadzu, UV-1800). The following equations were used in the calculations (Lichtenthaler and Wellburn, 1983):

$$\text{chl a} = (11.75 \times A663 - 2.23 \times A645) \times 20 / \text{mg sample weight} \quad (1)$$

$$\text{chl b} = (18.61 \times A645 - 3.96 \times A663) \times 20 / \text{mg sample weight} \quad (2)$$

$$\text{car} = ((1000 \times A470 - 2.27 \times \text{chl a} - 81.4 \times \text{chl b}) / 227) \times 20 / \text{mg sample weight} \quad (3)$$

The proline content in freeze-dried leaf samples was determined according to Bates et al. (1973). Readings were taken at 520 nm using a spectrophotometer, and the results were expressed as µmol proline per g dry weight.

Lipid peroxidation, expressed as malondialdehyde (MDA) content, was assessed according to Hernandez and Almansa (2002). Readings at 532 nm and 600 nm were recorded on the spectrophotometer, and the results were presented as nmol per g.

For macro-nutrient analysis of leaves, the Kjeldahl method (Gerhardt Vapodest 40) with a live burning technique was used for N^{2+}. Microwave live burning methods were employed for P^{4+}, K^{+}, Ca^{2+}, and Mg^{2+} analyses in dried leaf samples. Measurements of prepared samples were conducted using an ICP-AES (Spectro Arcos Blue2) device (Kaçar and İnal, 2008).

2.6. Statistical analyses

The study was conducted with a factorial experimental design in randomized complete blocks, with three replications, and five plants were used in each replication. Statistical analyses were performed using JMP 11 software. The Shapiro-wilk test was used to determine whether the data showed normal distribution. Non-parametric tests, including the Kruskal-Wallis test, were applied to data that deviated from a normal distribution to evaluate the results. Differences between treatments were determined using the LSD Multiple Comparison Test ($P<0.05$; $P<0.01$; $P<0.001$).

3. Results

3.1. Effect of NaCl stress on morphological variables

In all *Pyrus* rootstocks exposed to NaCl stress, plant height generally decreased over both years, depending on genotype and stress level (Figure 1A and Figure 1B). Particularly, the plant height of the Fox 11 was significantly restricted in severe stress treatments in both years of the study. Specifically, when compared to control, the largest percentage decreases were determined to be 71.91% and 69.31% in Fox 11 in successive years, as shown in Figure 1A and Figure 1B. For the BA 29, representing the *Quince* species in the study, significant changes in plant height were not observed in both years under NaCl. Typically, the impact of NaCl treatments on plant height revealed noticeable decreases, with variations observed across years and a consistent decline noted with increasing stress levels (Figure 1A and Figure 1B).

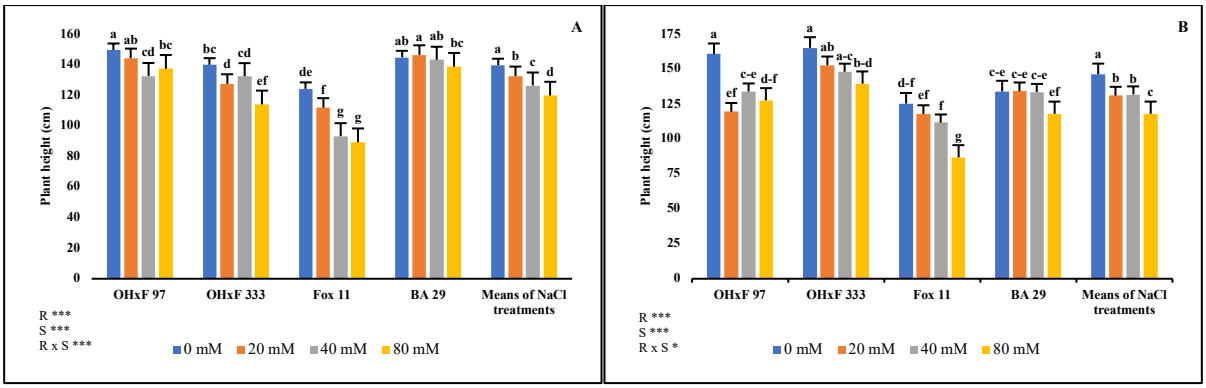

Figure 1. Plant height of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=15). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. *** and * represent the significance level of differences at p<0.001, p<0.05, and non-significant, respectively.

In the first year, the plant diameter of the OHxF 97 rootstock significantly decreased in the moderate (6.47 mm) and severe stress (6.52 mm) compared to the control (7.52 mm) and light stress (7.52 mm) treatments (Figure 2A). The plant diameter of the OHxF 333 rootstock decreased significantly at all stress levels compared to the control. However, the plant diameter in Fox 11 and BA 29 rootstocks was not affected by NaCl. In the second year, it was determined that NaCl stress led to a proportional decrease in plant diameter in all *Pyrus* rootstocks (Figure 2B).

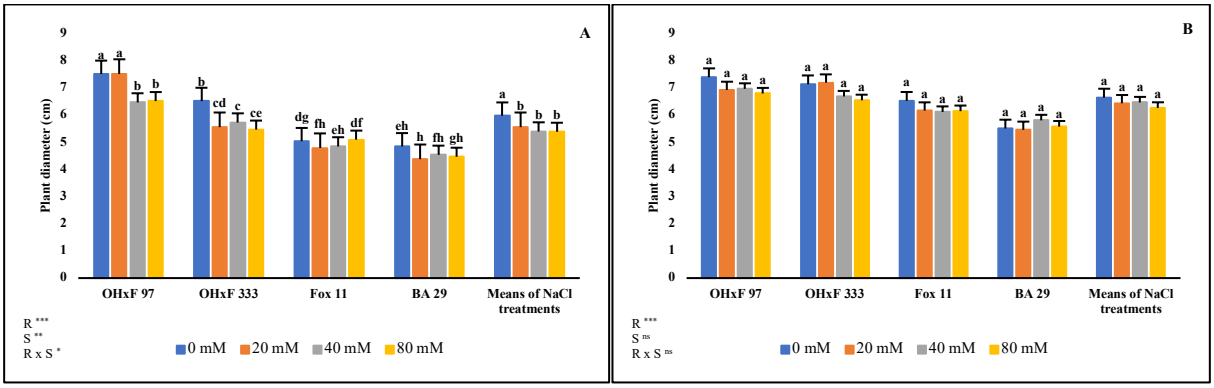

Figure 2. Plant diameter of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=15). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. ***, **, *, and ns represent the significance level of differences at p<0.001, p<0.01, p<0.05, and non-significant, respectively.

In the first year, the leaf area was affected by NaCl stress in all rootstocks except Fox 11 (Figure 3A). Especially in the BA 29 rootstock, leaf area significantly decreased at all stress levels (light stress - 27.13 cm^2; moderate stress - 26.93 cm^2; severe stress - 23.47 cm^2) compared to the control (30.4 cm^2). In the second year of the study, moderate and severe NaCl stress reduced leaf area in all rootstocks (Figure 3B), with the highest percentage decreases observed in the severe stress treatments of OHxF 333 (31.48%) and OHxF 97 (23.32%), respectively. The study revealed a significant reduction in leaf area due to NaCl stress, and the smallest leaves were observed because of severe stress.

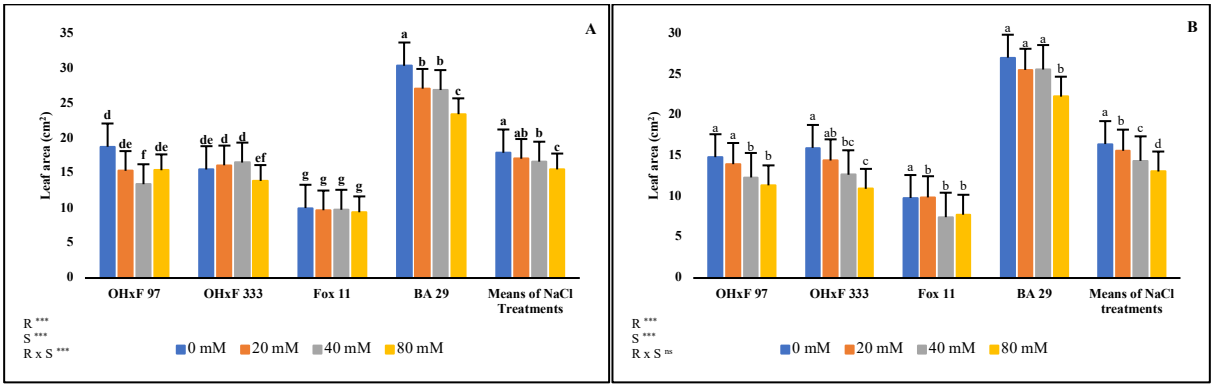

Figure 3. Leaf area of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=30). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. *** and ns represent the significance level of differences at $p<0.001$ and $p<0.05$, respectively.

The leaf necrosis observed in plants exposed to NaCl stress is presented in Figure 4A and Figure 4B. According to this, no signs were observed with light stress treatments in plants treated with NaCl for approximately sixty days. Additionally, there was no indication of NaCl damage at moderate stress levels in OHxF rootstocks. However, Fox 11 and BA 29 suffered significantly under moderate and severe NaCl stress. For instance, in the case of the Fox 11 rootstock, the leaf necrosis, which was 1.87 and 1.33 in moderate stress treatments in successive years, was determined as 3.0 and 2.67 in severe stress treatments. In BA 29, the leaf necrosis, which was 0.67 and 0.80 in successive years, was measured as 2.0 and 1.60 under severe stress.

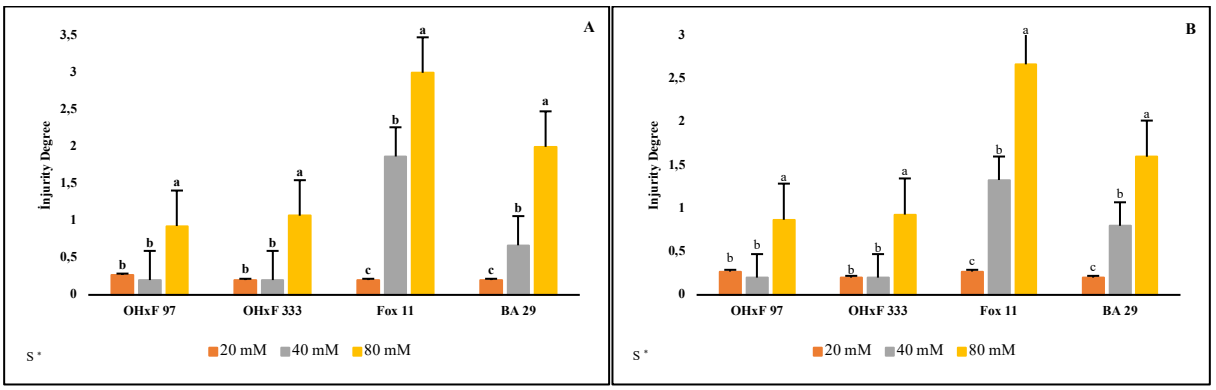

Figure 4. Injurity degree of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=3). Letters represent the effect of NaCl treatment on rootstocks. *, represents the significance level of differences at $p<0.05$.

3.2. Effect of NaCl stress on physiological variables

The effect of NaCl treatments on LWP is presented in Figure 5A and Figure 5B. LWP values at the end of the first year trial reveal that is not affected by NaCl treatments in all rootstocks. However, in the treatment averages, LWP increased under moderate and severe stress conditions. In measurements taken at the end of the second year trial, the LWP significantly decreased in severe stress treatments for

OHxF 333 (-4.61 MPa) and Fox 11 (-4.13 MPa) rootstocks. In BA 29, the treatments did not affect the LWP.

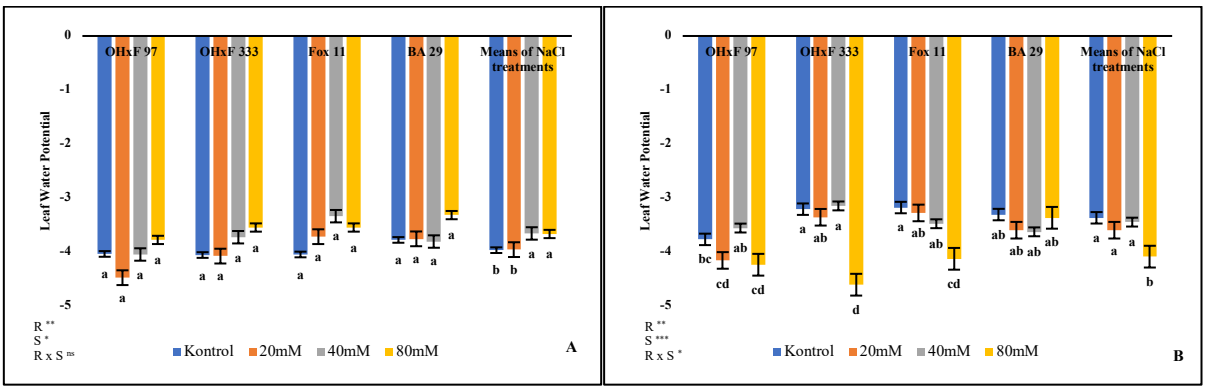

Figure 5. Leaf water potential of rootstocks under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=6). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. ***, **, *, and ns represent the significance levels of $p<0.001$, $p<0.01$, $p<0.05$, and non-significant, respectively.

3.3. Effect of NaCl stress on biochemical variables

In the first year, the amounts of chl a, chl b, total chl, and carotenoid in the leaves were not affected by the treatments (Table 1). In the second year, however, the mentioned parameters were influenced by the rootstock x treatment interaction. While the amount of chl a generally remained unchanged in OHxF and BA 29 under stress treatments, it significantly decreased in plants exposed to moderate (2.87 mg g^{-1}) and severe (2.93 mg g^{-1}) stress in the Fox 11. A similar situation was observed in the amounts of chl b and total chl, where a decrease was found in plants subjected to moderate (chl b; 1.01 mg g^{-1} – total chl; 3.88 mg g^{-1}) and severe (chl b; 1.07 mg g^{-1} – total chl; 3.99 mg g^{-1}) stress in the Fox 11. The amount of carotenoid in the leaves significantly increased in the OHxF rootstocks (OHxF 97; 4.17 mg g^{-1}; OHxF 333; 4.09 mg g^{-1}) at the moderate stress level compared to other stress levels. The lowest carotenoid amount in *Pyrus* rootstocks occurred in plants exposed to severe stress (OH x F 97; 1.64 mg g^{-1} - OH x F 333; 1.51 mg g^{-1} - Fox 11; 1.94 mg g^{-1}) and was significantly different from other treatments.

Table 1. The influence of NaCl treatments on the concentration of photosynthetic pigments in leaves

	Photosynthetic Pigment Concentration							
NaCl (mM)	**chl a (mg g^{-1})**		**chl b (mg g^{-1})**		**total chl (mg g^{-1})**		**car (mg g^{-1})**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
OH x F 97								
0	2.97	2.80bc	0.91	1.03b	3.89	3.83b	3.37	3.49abc
20	4.05	2.02de	1.31	0.81c	5.36	2.83cd	4.67	3.06bcd
40	3.73	2.63bc	1.18	1.00bc	4.91	3.63bc	4.25	4.17a
80	3.42	2.31cde	1.15	0.89bc	4.57	3.20bc	4.05	1.64f
OH x F 333								
0	3.01	2.54bcd	0.90	0.90bc	3.91	3.45bc	3.34	2.82cd
20	3.37	2.52bcd	1.04	0.89bc	4.41	3.41bc	3.83	3.07bcd
40	2.82	2.65bc	0.86	0.91bc	3.68	3.55bc	3.10	4.09a
80	3.04	2.72bc	0.97	0.93bc	4.01	3.65b	3.53	1.51f
Fox 11								
0	2.92	4.17a	0.98	1.38a	3.90	5.55a	3.49	3.58ab
20	3.44	3.93a	1.15	1.33a	4.59	5.26a	4.04	3.08bcd
40	2.92	2.87bc	0.98	1.01bc	3.90	3.88b	3.46	3.10bcd
80	3.18	2.93b	1.14	1.07b	4.32	3.99b	3.95	1.94ef
BA 29								
0	3.28	1.18fg	0.98	0.38d	4.26	1.56ef	3.58	2.97bcd
20	3.74	1.07g	1.12	0.32d	4.86	1.39f	4.17	3.34bc
40	3.65	1.35fg	1.08	0.42d	4.73	1.77ef	4.06	3.26bcd
80	2.91	1.75ef	0.85	0.53d	3.76	2.28de	3.30	2.56de

Table 1. The influence of NaCl treatments on the concentration of photosynthetic pigments in leaves (continued)

	Photosynthetic Pigment Concentration							
NaCl (mM)	**chl a (mg g^{-1})**		**chl b (mg g^{-1})**		**total chl (mg g^{-1})**		**car (mg g^{-1})**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
Means of treatments								
0	3.04b	2.67	0.94b	0.92	3.99b	3.60	3.45b	3.22b
20	3.65a	2.39	1.16a	0.84	4.80a	3.22	4.18a	3.14b
40	3.28ab	2.37	1.02ab	0.84	4.30ab	3.21	3.72b	3.66a
80	3.14b	2.43	1.03ab	0.85	4.17b	3.28	3.71b	1.91c
Means of rootstocks								
OH x F 97	3.54	2.44b	1.14a	0.93b	4.68	3.37b	4.08	3.09
OH x F 333	3.06	2.61b	0.94b	0.91b	4.00	3.52b	3.45	2.87
Fox 11	3.12	3.47a	1.06ab	1.20a	4.18	4.67a	3.73	2.92
BA 29	3.39	1.34c	1.01ab	0.41c	4.40	1.75c	3.78	3.03

Letters represent the interaction between rootstock and NaCl stress and the variation among treatment averages.

The proline levels in the leaves increased significantly or relatively in all rootstocks under NaCl stress (Figure 6A and Figure 6B). In both years, the highest proline levels were observed in the severe stress treatment of the Fox 11 (182.3-232.15 µmol g^{-1}), showing a significant difference compared to other treatments. OHxF 97 followed Fox 11 in terms of proline amount in both years, and different results were observed among treatments for this rootstock over the years.

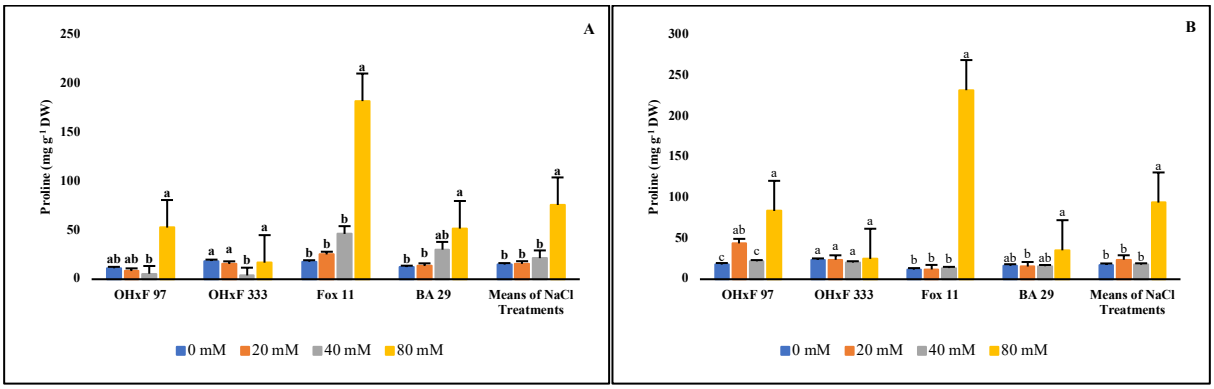

Figure 6. Proline content of rootstocks leaves under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=3). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. The significance level of $p<0.05$.

In the first year of the study, the highest MDA levels were observed in OHxF 333 under light stress (2.95 nmol g^{-1}) and in BA 29 under both moderate and severe stress (3.3-2.92 nmol g^{-1}, respectively) (Figure 7A). In the second year, BA 29 exhibited the highest MDA levels across all stress treatments (2.33-2.36-3.12 nmol g^{-1}) (Figure 7B). Particularly, the MDA content significantly increased in Fox 11 and BA 29 rootstocks under severe stress compared to the control plants in the second year. A similar trend was observed in the overall averages of NaCl treatments in the second year. While no significant difference was found between the control and light to moderate stress treatments, the MDA content substantially increased in plants subjected to severe stress.

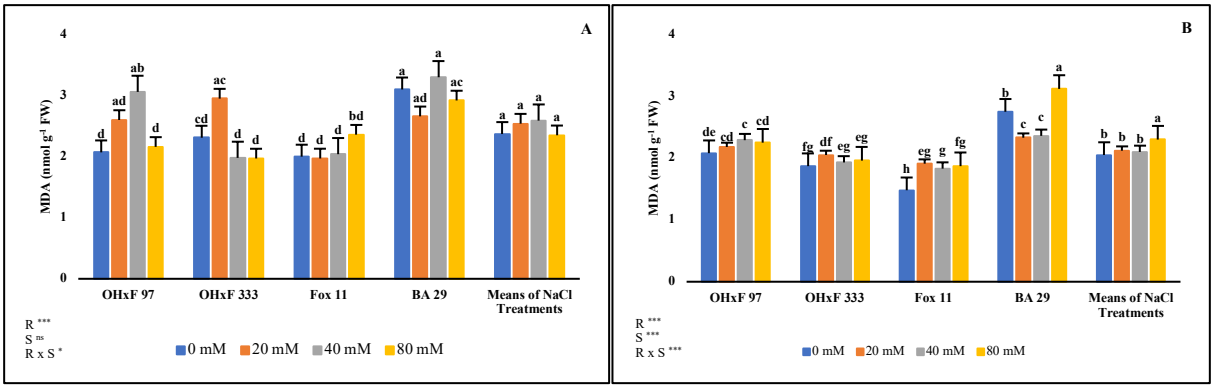

Figure 7. MDA content of rootstocks leaves under four different NaCl stress (A: First year and, B: Second year). Each value indicates the ± SE of three replicates (n=3). Letters represent the rootstock×NaCl interaction and the effect of NaCl treatment on rootstocks. ***, * and ns denote the significance level of $p<0.001$ and $p<0.05$, respectively.

The N^+ content in the leaves ranged from 2.09% to 2.38% in the first year, respectively for Fox 11 under moderate stress and OHxF 97 under severe stress. In the second year, it varied between 2.24% and 2.72%, with OHxF 97 under moderate stress and OHxF 333 in the control having the highest and lowest values, respectively (Table 2). While N^+ content was unaffected by NaCl treatments in the first year, there was a significant decrease in the second year due to NaCl treatments. Additionally, Fox 11 exhibited lower N^+ content (2.16%) compared to other rootstocks. For P^{4+} content, it ranged from 0.12% to 0.16% in the first year, with Fox 11 under moderate stress, OHxF 97, and BA 29 in the control showing variations. Moreover, P^{4+} content significantly decreased in the leaves of OHxF 333 under NaCl stress. In the second year, P^{4+} content fluctuated between 0.23% and 0.33%, with OHxF 333 under severe stress, OHxF 97, and Fox 11 under light stress having the highest values (Table 2). Particularly, under severe stress, a significant decrease in P^{4+} content in the leaves was observed.

NaCl treatments on the K^+ content in the leaves revealed a significant reduction in Fox 11 rootstock under both light and severe stress compared to the control. Furthermore, while the leaf K^+ content was unaffected by NaCl stress, Fox 11 (1.50%) had a higher K^+ content than other rootstocks (Table 2). Ca^{2+} content in the leaves ranged from 1.93% to 2.45% in the first year, with Fox 11 in the control group and OHxF 97 under severe stress having the highest values, respectively. In the second year, Ca^{2+} content varied between 1.61% and 2.34%, with Fox 11 and BA 29 under moderate stress showing the highest values. The Ca^{2+} content in the leaves increased significantly or relatively due to NaCl stress. However, throughout both years of the study, Fox 11 consistently exhibited the lowest Ca^{2+} content (2.14% to 1.76%) among the rootstocks (Table 2). Mg^{2+} content in the leaves ranged from 0.27% to 0.53% in the first year, with Fox 11 in the control and BA 29 under severe stress showing variations. In the second year, Mg2+ content fluctuated between 0.12% and 0.35%, with OHxF 333 under severe stress and BA 29 in the control having the highest values. Particularly, Mg^{2+} content was significantly affected by severe stress treatments (0.43%). Additionally, BA 29 consistently had the highest Mg^{2+} content in both years of the study (0.47% to 0.29%) among the rootstocks (Table 2).

Table 2. The influence of NaCl treatments on the concentration of specific macro nutrients in leaves

	Macro nutrients									
NaCl (mM)	**(%) N^{2+}**		**(%) P^{4+}**		**(%) K^+**		**(%) Ca^{2+}**		**(%) Mg^{2+}**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
OH x F 97										
0	2.22^{ns}	2.67^{a}	0.16^{ns}	0.32^{ns}	1.31^{df}	1.42^{ns}	2.21^{ns}	1.80^{ns}	0.33^{ns}	0.16^{ns}
20	2.31	2.42^{b}	0.15	0.33	1.31^{de}	1.44	2.35	1.75	0.39	0.23
40	2.24	2.24^{b}	0.15	0.26	1.33^{de}	1.23	2.30	2.28	0.32	0.24
80	2.38	2.28^{b}	0.15	0.27	1.43^{bd}	1.35	2.45	1.98	0.47	0.17

Table 2. The influence of NaCl treatments on the concentration of specific macro nutrients in leaves (continued)

	Macro nutrients									
NaCl (mM)	**(%) N^{2+}**		**(%) P^{4+}**		**(%) K^{+}**		**(%) Ca^{2+}**		**(%) Mg^{2+}**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
OH x F 333										
0	2.35	2.72^{ns}	0.15^{a}	0.31	1.30^{df}	1.46	2.01	1.62	0.38	0.13
20	2.32	2.60	0.14^{b}	0.29	1.14^{fg}	1.54	2.29	1.88	0.34	0.15
40	2.23	2.38	0.13^{b}	0.31	1.10^{g}	1.47	2.38	1.80	0.31	0.19
80	2.18	2.35	0.13^{b}	0.23	1.25^{eg}	1.36	2.29	1.94	0.31	0.12
Fox 11										
0	2.11	2.54^{ns}	0.14^{ns}	0.26	1.62^{a}	1.40	1.93	1.98	0.27	0.25
20	2.19	2.39	0.14	0.33	1.43^{bd}	1.43	2.20	1.79	0.36	0.24
40	2.09	2.33	0.12	0.26	1.53^{ac}	1.45	2.09	1.61	0.32	0.22
80	2.23	2.42	0.13	0.24	1.43^{bd}	1.42	2.35	1.64	0.41	0.21
BA 29										
0	2.15	2.49^{ns}	0.16^{ns}	0.30	1.22^{eg}	1.34	2.33	2.08	0.49	0.35
20	2.36	2.27	0.15	0.29	1.21^{eg}	1.31	2.36	1.98	0.40	0.24
40	2.31	2.30	0.15	0.29	1.55^{ab}	1.36	2.27	2.34	0.46	0.31
80	2.34	2.33	0.13	0.28	1.37^{ce}	1.28	2.42	1.96	0.53	0.26
Means of treatments										
0	2.21^{ns}	2.60^{a}	0.15^{a}	0.30^{a}	1.36^{ns}	1.40^{ns}	2.12^{b}	1.87^{ns}	0.37^{b}	0.22^{ns}
20	2.30	2.42^{b}	0.14^{b}	0.31^{a}	1.27	1.43	2.30^{a}	1.85	0.37^{b}	0.21
40	2.22	2.31^{b}	0.14^{b}	0.28^{ab}	1.38	1.38	2.26^{a}	2.01	0.35^{b}	0.24
80	2.29	2.35^{b}	0.14^{b}	0.26^{b}	1.37	1.35	2.38^{a}	1.88	0.43^{a}	0.19
Means of rootstocks										
OH x F 97	2.29^{a}	2.41^{ns}	0.15^{a}	0.29^{ns}	1.34^{b}	1.36^{ns}	2.33^{a}	1.95^{ns}	0.38^{b}	0.20^{b}
OH x F 333	2.27^{a}	2.51	0.14^{b}	0.29	1.20^{c}	1.46	2.24^{ab}	1.81	0.33^{b}	0.15^{c}
Fox 11	2.16^{b}	2.42	0.13^{b}	0.27	1.50^{a}	1.42	2.14^{b}	1.76	0.34^{b}	0.23^{b}
BA 29	2.29^{a}	2.35	0.15^{a}	0.29	1.34^{b}	1.32	2.34^{a}	2.09	0.47^{a}	0.29^{a}

Letters represent the interaction between R × S and the variation between treatment averages.

4. Discussion

Salt stress, one of the most significant abiotic stress factors, adversely affects almost all physiological and biochemical processes of plants (Misra and Gupta, 2005), thus imposing limitations on plant growth and productivity (Kotagiri and Kolluru, 2017). As known, rootstocks are used by plants to gain tolerance against soil-related stress factors (Tavallali and Karimi, 2019). The role of rootstocks is crucial in determining the performance of trees under saline conditions (Grattan and Grieve, 1999), and it has been suggested that the accumulation of salt in leaves is primarily controlled by the rootstock genotype (Santa-Cruz et al., 2002). Proper rootstock selection may reduce the adverse effects of salinity or enhance salt tolerance (Colla et al., 2010; Huang et al., 2013; Karimi and Nasrolahpour-Moghadam, 2016).

In woody plants, shoot development is hindered by high concentrations of NaCl in the growing medium (Gong et al., 2013). In our study, we observed a decrease in plant height in the *Pyrus* rootstocks under NaCl stress, with the highest percentage decrease found in the Fox 11 exposed to 80 mM NaCl. The findings are consistent with previous salt stress studies in pears (Okubo and Sakuratani, 2000; Okubo et al., 2000; Musacchi et al., 2006). A noteworthy aspect was observed in the BA 29 rootstock. Despite intense necrosis in its leaves due to NaCl stress, significant changes in plant height were not on this rootstock. According to Greenway and Munns (1980), one of the reasons for the decrease in growth under salt stress is the restriction of cytokinin transport from roots to shoots. The main regions where

cytokinin's are synthesized in plants are root apical meristems (Taiz and Zeiger, 2008). The BA 29, compared to other *Pyrus* rootstocks in the study, has a more extensive lateral root system and, consequently, more meristematic regions. Based on current insights, it is hypothesized that the root anatomical structure of the BA 29 rootstock plays a role in promoting longitudinal growth under NaCl stress.

Vegetative growth parameters in plants are adversely affected under saline conditions, and the decrease in stem/shoot diameter represents one of the most crucial criteria. Indeed, studies conducted on different species to date have shown that shoot diameter is influenced by salt stress (Nassar et al., 2016; Mehdi-Tounsi et al., 2017). In our study, shoot diameter is generally affected by NaCl stress. On the other hand, the shoot diameter of the Fox 11, which we thought could be sensitive to NaCl stress, was not affected by the stress. Under stress, the apical bud stops growing longitudinally but continues to grow laterally. According to our observations, NaCl stress first appeared in the Fox 11 rootstock, and the apical bud formed earlier. The lack of changes in stem diameter in the Fox 11 is associated with this situation.

The initial rapid response by plants to salt stress is a reduction in leaf surface area (Wang and Nii, 2000), and this is considered a mechanism to minimize water loss (Ribeiro et al., 2006). Previous studies have consistently shown that leaf area is adversely affected by salinity (Munns and Tester, 2008; Singh et al., 2016). In our study, we also found that the leaf area was negatively affected by NaCl stress, and this interaction was particularly significant in the BA 29 in both years.

When the accumulation of harmful ions such as Na^+ and Cl^- reaches toxic levels in tissues, plants experience ionic stress (Munns and Passioura, 1984), leading to toxic symptoms such as chlorosis and necrosis in mature leaves (Tester and Davenport, 2003; Munns et al., 2006). The tolerance level of a plant to salt stress can be determined by the damage index. This assessment reflects the overall response of plants to salinity (Wang et al., 2015). In our study, the highest damage occurred in the severe stress treatments of the Fox 11 and BA 29 rootstocks. Additionally, in each stress treatment, the highest damage was observed in the Fox 11, while the lowest damage was observed in the OHxF rootstocks. One of the significant tolerance mechanisms developed by plants against salt stress is the prevention of the transport of Na^+ and Cl^- ions to leaves (to toxic levels) by retaining them in woody tissues. Based on this, it is suggested that such a mechanism may exist, especially in the OHxF rootstocks, particularly OHxF 97.

At the initial stage of salt stress, the reduced water uptake capacity of root systems and the resulting osmotic stress due to high salt accumulation in the soil and plant occur (Munns, 2005; Munns and Tester, 2008). The decrease in LWP is considered a useful criterion for determining the water stress caused by salinity (Katerji et al., 2003). In their study exposing different *Pyrus* species to NaCl stress, Okuba et al. (2000) observed a decrease in LWP values until the ninth week. After the ninth week, they determined that the control LWP values were lower, and they stated that this condition did not arise from mechanism differences induced by salt stress.

Leaf chlorophyll content is an indicator of the overall health of plants (Zhang and Kirkham, 1994). A decrease in chlorophyll pigments in stressed plants is a significant indicator of oxidative stress, oxidation, and pigment breakdown (Sarker and Oba, 2018). Additionally, a decrease in photosynthetic pigments under salt stress has been directly associated with salt tolerance (Ashraf and Sarwar, 2002). In our study, the content of chl a, chl b, and total chl generally remained unchanged in OHxF and BA 29, while it significantly decreased in Fox 11, especially under moderate and severe stress conditions.

Carotenoids play an important protective role against lipid peroxidation in plants under abiotic stress (dos Santos et al., 2019). In our study, a significant decrease in carotenoid levels was observed in *Pyrus* rootstocks exposed to severe NaCl stress. However, an interesting result emerged in OHxF rootstocks, where the carotenoid content increased under moderate NaCl stress. This situation in OHxF rootstocks is thought to be related to the protective role of carotenoids against photooxidation under salt stress (Barthod et al., 2007; Zrig et al., 2011).

The presence of salt in the growing medium generally results in the accumulation of compounds with low molecular weight (Hasegawa et al., 2000). Simultaneously, one of the most important of these compounds, also known as compatible solutes, is proline (Ghoulam et al., 2002; Girija et al., 2002). There are significant differences in the types of accumulated soluble compounds and their relative contributions to low osmotic potential across plant species and varieties (Larher et al., 2009). Moreover, it has been noted that, depending on plant species and varieties, the accumulation of proline does not

play a highly significant role in cellular osmotic adjustment (Ghars et al., 2008; Bendaly et al., 2016). Salt-sensitive genotypes accumulate more proline in various species (Kim et al., 2016). In our study, the highest amount of proline was observed in Fox 11. Tuteja (2007) reported that salt-sensitive plants attempt to maintain osmotic balance by synthesizing compatible solutes.

MDA is a product of lipid peroxidation and tends to accumulate in large quantities in plants exposed to salt stress (de Azevedo Neto et al., 2006). Additionally, it is used as a marker in determining plant sensitivity to oxidative damage (Xu et al., 2016). On the other hand, it has been suggested that salt-sensitive plants contain higher amounts of MDA compared to tolerant or resistant plants (Ahmed et al., 2013). In our study, it can be said that the amount of MDA is affected by NaCl stress, and the impact is more pronounced in rootstocks we characterized as sensitive to stress. For example, in Fox 11, an increase in MDA levels in response to NaCl stress was observed in both years. A similar situation was observed in BA 29 under severe stress conditions. In this regard, the results obtained in the study are consistent with the findings of Wu and Zou (2009) in *Pyrus betulafolia*.

Potassium (K^+), an essential element for plants, plays a significant role in plant development (Kaçar, 1984). Due to the similar physicochemical structure of Na^+ and K^+, under salt stress, the transportation of Na^+ in high concentrations causes K^+ deficiency, leading to a significant decrease in K^+ levels in most glycophyte species (Greenway and Munns, 1980; Maathuis and Amtmann, 1999). K^+, Ca^{2+} and Mg^{2+} alleviate the adverse effects of salt stress on plant growth (Ahmad and Prasad, 2012; Sarwat et al., 2013). In the study, NaCl stress did not have a negative effect on Ca^{2+} and Mg^{2+}, but N^+ levels in OHxF 97, P^{4+} levels in OHxF 333, and K^+ levels in Fox 11 rootstock significantly decreased under NaCl stress. Additionally, it was generally determined that Fox 11 had the least nutrient content.

5. Conclusions

When the data obtained in the study is evaluated as a whole, it can be stated that the Fox 11 rootstock is sensitive to NaCl stress. Even at around the threshold salinity value in the soil of 4 dS m^{-1}, damage occurred in the Fox 11 rootstock due to NaCl stress, suggesting that the use of this rootstock in areas under salt threat may be problematic. Although not as much as Fox 11, it was determined that the BA 29 rootstock also showed sensitivity to NaCl stress. According to the results obtained in our study, it would not be wrong to say that the rootstocks that are more tolerant to NaCl stress are the OHxF rootstocks. It can be said that the tolerance parameters to NaCl stress work more effectively, especially in the OHxF 97 rootstock. The salinity level in the soil, especially below the threshold salinity value, indicates that OHxF 97 rootstock can be used in pear cultivation. However, considering characteristics such as growth vigour and early fruit setting, OHxF 333 rootstock is also considered an alternative to the mentioned rootstock in pear production.

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