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Antioxidant defense strategies of some pear cultivars onto different rootstocks under NaCl stress

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Abstract

Salt stress is one of the most significant abiotic factors limiting plant production worldwide. In this study, the tolerance of Ankara and Deveci pear varieties to NaCl stress was examined on commonly used rootstocks. According to our results, leaf surface area, leaf water potential, and hydrogen peroxide amount decreased under NaCl stress, while root/shoot ratio, proline content, and glutathione reductase activity in the leaves increased. The A×11 and A×29 combinations were identified as the most affected in terms of leaf surface area. The root/shoot ratio increased on OH×F rootstocks but decreased on Fox 11 and BA 29 rootstocks. GR activity was found to be higher on varieties grafted onto OH×F 97 rootstock, with the highest activity detected in the D×97 combination under severe stress. Total phenolic compounds and total flavonoid content were not affected by NaCl stress. Arbutin, chlorogenic acid, catechin, and rutin showed variable results under NaCl stress. In the more salt-tolerant Deveci variety, the amount of arbutin in leaves was higher compared to other phenolic compounds. Overall, the higher amount of arbutin, which is a key phenolic compound in pears, in the Deveci variety suggests that this compound may contribute to the tolerance mechanism.

Introduction

Salt stress, also known as salinity, has become a significant global issue, affecting 23% of cultivated areas, according to Shahid et al. (2018). The impact of salinity is exacerbated in arid and semi-arid climate regions with insufficient rainfall, coastal areas near oceans and seas, soils with poor drainage conditions, and situations where fertigation systems are used.

Salt stress, which arises from the accumulation of highly toxic ions such as sodium (Na^+) and chloride (Cl^-) in irrigation water and cultivated soil, adversely affects critical economic parameters such as growth, development, and yield in sensitive plants (Rouphael et al., 2018). The negative effects of salt stress on plants occur primarily through osmotic and ionic stress pathways. As a result of these stresses, oxidative stress mediated by reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) increases, leading to significant damage to membranes and other cellular structures (Santander et al., 2020).

To counteract oxidative damage and eliminate excessive ROS, plants activate endogenous defense systems, including enzymatic and non-enzymatic compounds such as phenolic compounds (phenolic

acids, flavonoids, etc.) (Dumanović et al., 2020; Hasanuzzaman et al., 2012). Indeed, it has been determined that salt stress triggers the biosynthesis of phenylpropanoids (Kumar et al., 2023) and increases the biosynthesis of various phenolic compounds (Petridis et al., 2012). Studies have shown that salt stress leads to changes in the levels of phenolic compounds that are important for the species (Calzone et al., 2023). For example, in salt-stressed olives, the amount of the important phenolic compound oleuropein increased, while the amount of hydroxytyrosol decreased (Petridis et al., 2012). This is because the content of phenolic compounds in salt-stressed plants varies depending on the species, variety, and level of stress (Calzone et al., 2023).

The grafting technique, used for centuries in fruit growing, allows cultivation on suitable rootstocks. Rootstocks can influence the yield and quality characteristics of the grafted varieties, as well as alter their tolerance to various stress factors, including salt stress (Asayesh et al., 2023; Koleska et al., 2018). Additionally, rootstocks can affect the biochemical mechanisms and alter the content and amount of phenolic compounds (Andreotti et al., 2006).

European pears (*Pyrus communis* L.) are sensitive to salt stress and exhibit growth and developmental regression when exposed to salinity for relatively long periods (Musacchi et al., 2006), as well as leaf damage (Okubo and Sakuratani, 2000). However, these effects vary depending on the rootstock used, as is the case with many fruit species (Aydınlı et al., 2024). Major phenolic compounds found in European pears include arbutin, chlorogenic acid, *p*-coumaric acid, catechin, epicatechin, rutin, and quercetin (Andreotti et al., 2006; Li et al., 2012; Tanrıöven and Ekşi, 2005). Under osmotic stress, arbutin and its derivatives are noted to play a protective role against environmental stress in pears (Larher et al., 2009). Additionally, higher levels of arbutin have been found in the tissues of pear varieties that are tolerant to *Erwinia amylovora*, a significant biotic stress factor (Günen et al., 2005).

To the best of our knowledge, studies on salt stress in European pears have so far been limited to rootstock or variety levels, with a focus on physiological mechanisms (Musacchi et al., 2006; Zafari et al., 2018). While it has been observed that variety × rootstock combinations activate antioxidant defense systems under drought stress (Asayesh et al., 2023), this situation remains unclear under salt stress conditions. Additionally, it is uncertain whether prominent phenolic compounds in European pears contribute to tolerance against salt stress. In recent years, European pear orchards in Türkiye frequently use OH×F and Fox series as well as BA 29 rootstocks. Local pear varieties such as Ankara and Deveci are predominant in the cultivation areas. Therefore, this study aims to determine the tolerance of major variety × rootstock combinations to salt stress and to assess the extent to which they utilize antioxidant defense strategies under NaCl stress.

Materials and Methods

Plant material and stress treatment

In the study, eight different scion × rootstock combinations were used (Table 1). Before stress treatments, grafting was performed using the T-budding technique in August of the preceding year. Subsequently, plants were uprooted from the nursery at the beginning of the winter dormancy period and planted in mid-March into 18-liter pots containing a mixture of garden soil + peat + sand (2:1:1). The research was conducted in temperature-controlled greenhouses belonging to the Fruit Research Institute (MAREM) located in Eğirdir, Türkiye. To induce salt stress in the plants, four different NaCl concentrations were added to the irrigation water. 0 mM NaCl represented the control, 20 mM NaCl indicated light stress, 40 mM NaCl denoted moderate stress, and 80 mM NaCl represented severe stress. Stress treatments began in mid-July and continued for approximately nine weeks. To mitigate osmotic stress in the plants, incremental doses of NaCl were systematically introduced, each increment being 20 mM. The experiment was terminated approximately nine weeks after NaCl treatments started when stress-related damage was observed in the leaves. At the end of the experiment, mature leaves were collected for biochemical analyses, frozen, and stored at -80°C.

Table 1. The scion-rootstock combinations and their abbreviations used in the experiment

Scion	Rootstock	Abbreviation of scion-rootstock combinations
Ankara	OH × F 97	A × 97
	OH × F 333	A × 333
	Fox 11	A × 11
	BA 29	A × 29
Deveci	OH × F 97	D × 97
	OH × F 333	D × 333
	Fox 11	D × 11
	BA 29	D × 29

Morphological traits

Leaf surface 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 (Placom, KP-90 N (Koizumi Co., Japan)). To obtain fresh shoot weight, plants were cut at the grafting point at the end of the experiment, and their weights were measured. For determining the fresh root weight, plants were removed from their pots, the growing medium in the root zone was carefully removed, and fresh root weights were measured. After these procedures, fresh root weights were measured using a precision scale.

Physiological traits

Determination of leaf water potential (Ψw)

LWP measurements were conducted using a pressure chamber (Instrument Model 1000 (PMS

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

Biochemical traits

Before the extraction procedures, the plant materials (except for those used for enzyme analyses) were lyophilized using a BW-10N Vacuum Freezing Dryer (Bluewave, Bluewave Industry Co., China).

Determination of proline content of leaves

The proline content in leaf samples was determined according to Bates et al. (1973). Readings were taken at 520 nm using a spectrophotometer (Shimadzu UV-1800 (Shimadzu Scientific Instruments, Columbia)), and the results were expressed as µmol proline g^{-1} DW.

Determination of oxidative stress markers: H_2O_2 concentration of leaves

The amount of H_2O_2 was determined according to Velikova et al. (2000). The absorbance values of the samples were measured at a wavelength of 390 nm using a spectrophotometer (Shimadzu UV-1800 (Shimadzu Scientific Instruments, Columbia)). The results were expressed as µmol H_2O_2 kg^{-1} DW.

Enzymatic antioxidants

Determination of glutathione reductase (GR) activities of leaves

The GR (glutathione reductase) activity of the leaves was measured according to Foyer and Halliwell (1976). In this method, 2 g of fresh leaf samples were extracted with 50 mM potassium phosphate buffer (pH 7.3). The samples were then centrifuged at 10,000 rpm for 15 min at 4°C, and 100 µl of the supernatant was taken and mixed with 900 µl of 0.025 M sodium phosphate buffer (pH 7.8). The absorbance of the samples was recorded at 340 nm and expressed as mol min^{-1} g^{-1}.

Non-enzymatic antioxidants

Total phenolic compounds (TPC)

The determination of total phenolic compounds was carried out using the method of Singleton and Rossi (1965). A 0.5 g sample of freeze-dried leaves was homogenized with 5 ml of 80% MeOH containing 1% HCl at room temperature for 15 min using a mechanical shaker at 200 rpm. The mixture was then centrifuged at 3,000 rpm at 22°C. An aliquot of 0.2 ml from the upper phase was taken and sequentially mixed with 1.5 ml of Folin reagent and 1.5 ml of sodium bicarbonate, and the absorbance was read at 765 nm. A standard solution of gallic acid with different concentrations was used, and the results were expressed as mg GAE g^{-1} DW (dry weight).

Total flavonoid content (TFC)

The TFC in the leaves was measured according to Zhishen et al. (1999). Briefly, 1 g of freeze-dried leaf sample was extracted with an 80% MeOH solution. From the extracted plant material, 1 ml was taken and diluted with 4 ml of distilled water. Immediately after, 5% $NaNO_2$ was added, and after 5 min, $AlCl_3$ was introduced. At the 6-min mark, 2 ml of 1 M NaOH was added, and the mixture was finally made up to 10 ml with distilled water. Rutin was used as a standard, and the results were expressed as mg RUTIN g^{-1} DW by measuring absorbance at 510 nm.

Total antioxidant capacity (TAC)

TAC in the leaves was determined using the phosphomolybdenum method (Prieto et al., 1999). To the 0.3 ml of the extraction solution, 3 ml of reagent solution (0.6 M sulfuric acid + 28 mM sodium phosphate + 4 mM ammonium molybdate) was added. The samples were vortexed to ensure a homogeneous mixture and then incubated at 95°C for 90 min. After incubation, the samples were cooled to room temperature, and their absorbance was measured at 695 nm. The results were expressed as mg AAE g^{-1} DW based on the absorbance readings and the calibration curve obtained from ascorbic acid standards.

Extraction of phenolic compounds and analytical procedures

The extraction of phenolic compounds from the samples was performed according to the method developed by Escarpa and González (1998). A 100 mg sample of freeze-dried leaves, which were powdered in liquid nitrogen, was extracted using a solution containing 3% formic acid and 1% 2,6-di-tert-butyl-4-methylphenol (BHT) in an ultrasonic water bath (cooled with ice). The samples were then centrifuged at 9,000 rpm for 7 min at 5°C, filtered through a 0.45 µm syringe filter, and injected into the system.

Phenolic compounds were analyzed using an Agilent 1200 series high-performance liquid chromatography (HPLC) system with multiple wavelengths. The system included an ODS-3 column (5.0 µm diameter, 4.6 mm x 250 mm length) used for the separation of phenolic compounds, along with a pump, an autosampler, and a multi-wavelength detector. The method for determining the quantities of phenolic compounds was based on Zhang et al. (2010). This method used two solutions: deionized water with 10% formic acid (Solvent A) and acetonitrile with 10% formic acid and 1.36% deionized water (Solvent B). The gradient profile was as follows: 95% A (0 min), 85% A (25 min), 78% A (42 min), 64% A (60 min), and 95% A (65 min). A post-run time of 10 min was applied. A 20 µl sample was injected into the system. The column temperature was set at 30°C, and the pump flow rate was 1 ml min^{-1}. Arbutin and catechin were detected at 280 nm; chlorogenic acid at 320 nm; and rutin at 365 nm. Phenolic standards were introduced into the system

at concentrations ranging from 0-100 µg ml^{-1}, and the amounts were calculated based on the areas determined from the calibration curve.

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. Differences between treatments were determined using the LSD Multiple Comparison Test. Significant differences were accepted at $p \leq 0.05$; $p \leq 0.01$; $p \leq 0.001$, and represented by different letters.

Results and Discussion

NaCl stress affects leaf surface area and root/shoot ratio in grafted pears

The initial morphological responses of plants exposed to NaCl stress are a reduction in leaf surface area and the restriction of root and shoot growth (Parida and Das, 2005; Wang and Nii, 2000). Both *Pyrus* spp. and other horticultural plants have been reported to exhibit reduced leaf area and affected root/shoot ratio under salt stress (García et al., 2024; Paganová et al., 2022). In this study, the leaf surface area was negatively affected by NaCl stress and significantly decreased in certain combinations (Table 2). The smallest leaf area was observed in plants grafted onto the Fox 11 rootstock, followed by those grafted onto the BA 29 rootstock (Figure 1A). The reduction in leaf surface area is thought to be due to the accumulation of toxic concentrations of ions like Na^+ and Cl^- and early leaf drop (Munns and Tester, 2008; Paganová et al., 2022). Ultimately, the first toxic symptoms and leaf drop were observed in the A×11 and A×29 combinations. In the D×11 combination, there was no change in leaf area despite NaCl stress. Coban and Ozturk (2020) reported smaller leaves in the Deveci variety grafted onto clonal rootstocks compared to plants grafted onto Fox 11. Additionally, the Fox 11 rootstock has smaller leaves compared to other rootstocks included in the study (Aydınlı et al., 2024). The lack of significant reduction in leaf surface area under NaCl stress in the D×11 combination can be explained by its small leaf structure even under optimal conditions.

In plants exposed to salt stress, the root/shoot ratio increases (Munns and Tester, 2008). Indeed, in our study, the root/shoot ratio increased following NaCl treatments compared to control plants (Table 2). The relative increase in the root/shoot ratio in OH×F combinations, while a decrease was observed in Fox 11 and BA 29 combinations (Figure 1B). Additionally, the NaCl stress had a significant effect on the root/shoot ratio (Table 2). Although the stress level did not affect it, NaCl stress increased the root/shoot ratio according to control. Roots are the first organs to cope with high soil salinity and play a crucial role in plant tolerance to salt stress (Zrig et al., 2023). One of the important tolerance mechanisms to salt stress is the exclusion of harmful ions by the roots. Musacchi et al. (2006) indicated that such a strategy might exist in the OH×F series rootstock Farold 40. According to our results, the relative increase in the root/shoot ratio in OH×F rootstock combinations can be explained by ongoing root growth as a result of such a tolerance mechanism.

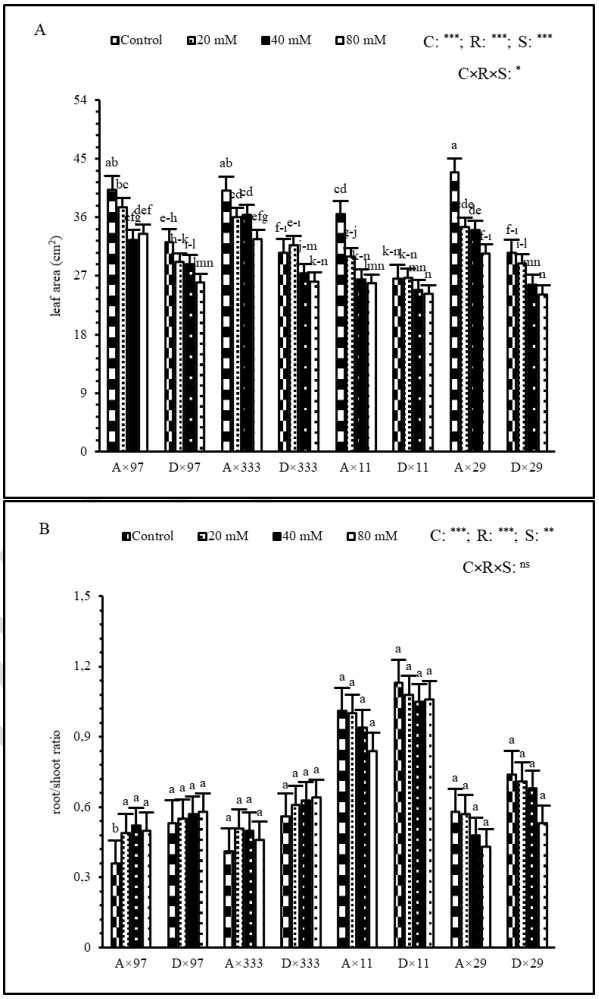

Figure 1. Effect of NaCl stress on leaf area (A) and root/shoot ratio (B) of eight pear cultivar × rootstock combinations. Letters show significant differences between each other. ***, **, * and ns denote the difference in significance level of $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$ and not significant, respectively.

Effect of NaCl stress on leaf water potential (Ψw) in grafted pears

Ψw is used as an indicator of physiological responses in plants under salt stress. It is known that salinity has a reducing effect on Ψw (Arif et al., 2020). NaCl stress leads to a decrease in Ψw, with increasing stress severity causing a significant reduction in Ψw (Table 2). In combination with the OH×F 97 rootstock, especially under severe NaCl stress, Ψw is significantly reduced (Figure 2A). In the A×11 combination, all levels of NaCl stress have a reducing effect on Ψw. In the D×11 combination, although Ψw decreases in plants exposed to light NaCl stress compared to controls, it does not change under moderate and severe stress levels. In fact, Ψw reduction is generally greater in sensitive plants under salt stress (Fozouni et al., 2012). Conversely, in

Table 2. The effect of NaCl stress on leaf area, root/shoot ratio, leaf water potential (LWP), proline (Pro), hydrogen peroxide (H_2O_2), and glutathione peroxidase (GR) activity in grafted pears

Parameters	Control	20 mM	40 mM	80 mM
Leaf area (cm^2)	34.95±0.56^a	31.83±0.44^b	29.51±0.43^c	27.88±0.40^d
Root/shoot	0.60±0.04^b	0.67±0.04^a	0.69±0.03^a	0.68±0.04^a
LWP (-MPa)	-3.69±0.08^a	-3.76±0.06^{ab}	-3.90±0.05^{bc}	-4.01±0.06^c
Pro (µmol g^{-1})	0.58±0.16^b	0.54±0.19^b	0.53±0.17^b	0.99±0.12^a
H_2O_2 (µmol kg^{-1})	7.75±0.54^a	7.83±0.51^a	7.28±0.42^{ab}	6.17±0.34^b
GR (mol min^{-1} g^{-1})	48.97±1.18^c	55.42±1.17^b	60.02±1.28^a	60.15±1.33^a

the Deveci combination grafted onto the Fox 11 rootstock, which we considered sensitive, Ψw increased after mild stress levels. Okubo et al. (2000) noted that Ψw values changed after the ninth week in their long-term salt stress study on *Pyrus* spp., which could be attributed to a difference in the mechanism. Additionally, Ψw in *P. pyraster* under long-term NaCl stress has been reported to be unaffected (Paganová et al., 2022). In our study, Ψw measurements were taken at the end of the experiment (approximately the sixtieth day). This suggests that our results could reflect changes in the mechanism under long-term salinity.

Could proline accumulation in grafted pears under NaCl stress be a sign of susceptibility?

One of the cellular responses of plants to saline conditions is the production of compounds known as compatible solutes. One of the most important of these compounds is proline (Mansour and Ali., 2017). It is known that proline accumulation increases under salt stress and that tolerant plants show a higher increase (Demiral and Türkan., 2006). The proline content in the leaves of pears exposed to NaCl stress increased significantly, especially under severe stress (Table 2). Additionally, the highest proline accumulation in the leaves was observed in plants grafted onto Fox 11 and BA 29 rootstocks. On the other hand, the proline content in the leaves of pears grafted onto Fox 11 and BA 29 rootstocks increased significantly under severe NaCl stress conditions (Figure 2B). According to Larher et al. (2009), there are large variations in the types of soluble accumulations in plant species and varieties and their contributions to low osmotic potential. Additionally, different researchers have indicated that while proline accumulation varies by species and varieties, it may not play a critical role in osmotic adjustment of cells (Bendaly et al., 2016). Tolerance to salinity is not a significant feature in some plant species (Mansour and Ali., 2017). Kim et al. (2016) reported that proline accumulation is much higher in sensitive genotypes under salt stress. Similarly, proline accumulation decreased in tolerant varieties under salt stress, while it significantly increased in sensitive varieties (Poury et al., 2023). In this regard, our results are consistent with the literature.

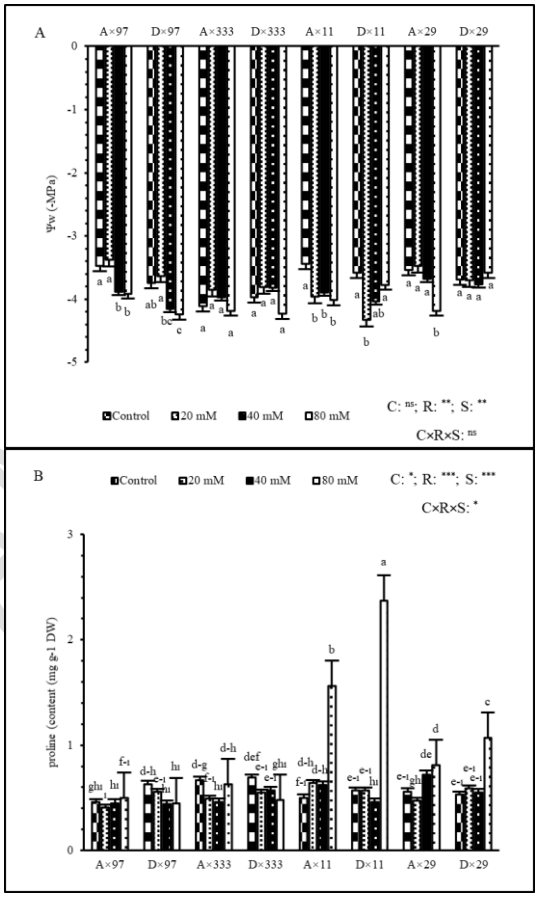

Figure 2. Effect of NaCl stress on Ψw (A) and leaves proline content (B) of eight pear cultivar × rootstock combinations. Letters show significant differences between each other. ** and ns denote the difference in significance level of $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$ and not significant, respectively.

H_2O_2 amount in leaves decreases with combinations of tolerance to NaCl stress

Plants exposed to salt stress also experience oxidative stress, a secondary stress caused by osmotic and ionic stress. Oxidative stress results in an increase in the production of ROS, such as H_2O_2, which are highly harmful to plant cells (Chatterjee et al., 2017). In this study, the amount of H_2O_2 in the leaves of pears exposed to severe NaCl stress (6.17 µmol kg^{-1}) decreased significantly (Table 2). When evaluating according to the variety × rootstock combinations, the amount of H_2O_2 in the leaves significantly or relatively decreased in plants grafted onto OH×F rootstocks, while it increased relatively in plants grafted onto Fox 11 and BA 29 rootstocks (Figure 3A).

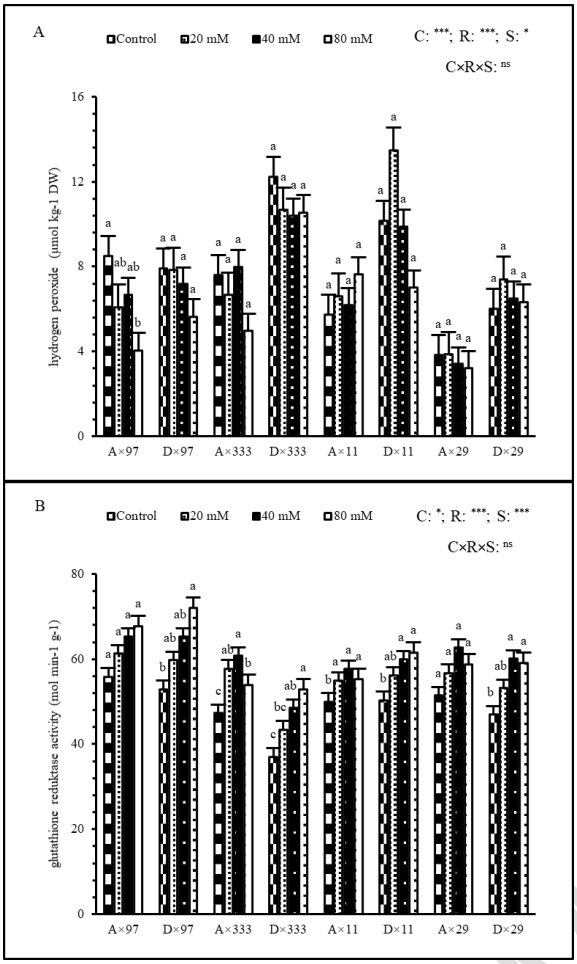

Figure 3. Effect of NaCl stress on leaf hydrogen peroxide (H_2O_2) content and glutathione peroxidase (GR) activity of eight pear cultivar × rootstock combinations. Letters show significant differences between each other. ***, * and ns denote the difference in significance level of $p \leq 0.001$, $p \leq 0.05$ and not significant, respectively.

Enzymatic antioxidant responses such as glutathione reductase activity may contribute to tolerance in grafted pears under NaCl stress conditions

Physiological research has shown that glutathione reductase (GR), an enzymatic antioxidant, is a central enzyme working to eliminate ROS continuously produced in various compartments under environmental conditions, including salinity (Sofy et al., 2020). Studies have shown that GR activity increases under salt stress conditions, and the increase is higher in tolerant plants (Ouertani et al., 2022). Under NaCl stress conditions, the GR activity in pear leaves generally increased with the severity of stress (Table 2). The highest GR activity was observed in plants grafted onto the OH × F 97 rootstock. In the D×97, D×333, and D×11 combinations, GR activity in the leaves increased significantly or relatively with increasing NaCl stress (Figure 3B). In the combinations with the Ankara variety (excluding A×97), the highest GR activity was observed under moderate NaCl stress conditions. In our study, H_2O_2 levels in the leaves decreased significantly or relatively in combinations grafted onto OH×F rootstocks, while they generally increased relatively in combinations grafted onto Fox 11 and BA 29 rootstocks. Additionally, GR activity increased significantly in tolerant combinations, particularly those with the Deveci variety, which is considered tolerant to NaCl stress. This suggests that the enzymatic antioxidant defense mechanism is effectively working to reduce oxidative damage in *P. communis* under NaCl stress.

Effect on non-enzymatic antioxidants in grafted European pears under NaCl stress

Phenolic compounds are non-enzymatic potential antioxidants that play a role in reducing ROS damage caused by salt stress. Among the class of phenolic compounds, the most commonly synthesized metabolites are phenolic acids and flavonoids (Wáskiewicz et al., 2013). Studies have reported varying results on the interaction between salt stress and phenolic compounds. For example, in strawberries, the TPC and TFC did not change under salt stress (Denaxa et al., 2022). In this study there was no effect of NaCl stress on the TPC, TFC, and TAC in terms of variety × rootstock × salinity interaction (Table 3). Additionally, it was found that NaCl stress had no impact on non-enzymatic antioxidants (Figure 4A). In contrast, the rootstock factor significantly affected these compounds, with the highest content observed in plants grafted onto BA 29 and Fox 11 rootstocks (Figure 4B). Very low amounts were detected in plants grown on OH×F rootstocks. Among the varieties, the Ankara variety was found to have higher TPC and TFC levels (Figure 4C). While phenolic compounds can sometimes increase salt stress tolerance, they generally play a key role in tolerance to other abiotic and biotic stresses (Castillo et al., 2022). Indeed, the TPC increased in *P. communis* under drought stress (Asayesh et al., 2023). This suggests that phenolic compounds may not be as effective in the tolerance mechanism of *P. communis* under NaCl stress compared to other tolerance components.

Table 3. Effect of variety, rootstock, salinity, and their interactions on total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), and some phenolic compounds in leaves

Parameters	Cultivar (C)	Rootstock (R)	Salinity (S)	C×R×S
Arbutin	***	***	ns	*
Chlorogenic acid	***	***	**	**
Catechin	***	**	***	**
Rutin	***	**	***	**
TPC	*	***	ns	ns
TFC	***	***	ns	ns
TAA	ns	***	ns	ns

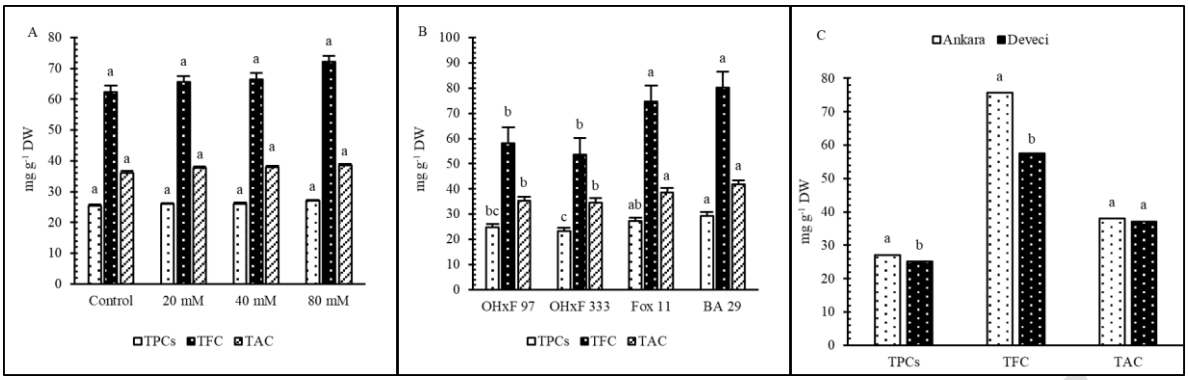

Figure 4. Effect of NaCl stress on total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) in the leaves of grafted plants at different stress (A), rootstock (B), and variety (C) levels. Letters show significant differences ($p \leq 0.05$) between each other.

The main phenolic compounds found in pears are arbutin and chlorogenic acid (Andreotti et al., 2006). Among the four phenolic compounds investigated in this study, the most abundant in the leaves were arbutin and chlorogenic acid. Arbutin and its derivatives have been reported to play a protective role against significant biotic stress, such as fire blight disease and osmotic stress in pears (Günen et al., 2005; Larher et al., 2009). Figure 5 shows the phenolic compound contents in the leaves of pears subjected to NaCl stress. All phenolic compounds were significantly affected by the interaction between variety × rootstock × salinity (Table 3). NaCl treatments caused significant changes in the amounts of chlorogenic acid, catechin, and rutin in pear leaves (Figure 6A). The amount of chlorogenic acid in the leaves significantly decreased under severe NaCl stress, while catechin and rutin levels increased with moderate and severe NaCl stress (Figure 6A). In combinations involving the BA 29 rootstock, higher amounts of arbutin (27.83 mg g^{-1}), chlorogenic acid (10.71 mg g^{-1}), catechin (0.62 mg g^{-1}), and rutin (4.42 mg g^{-1}) were found (Figure 6B). Conversely, combinations with the OH×F 333 rootstock had the lowest amounts of these compounds. When evaluated by variety, the Deveci variety stood out for its arbutin content (25.78 mg g^{-1}), while the Ankara variety excelled in chlorogenic acid (10.64 mg g^{-1}), catechin (0.64 mg g^{-1}), and rutin (4.91 mg g^{-1}) contents (Figure 6C).

The amount of arbutin in the leaves increased under moderate NaCl stress in the D×11 combination (31.44 mg g^{-1}) compared to the control (Figure 5A). Chlorogenic acid content significantly decreased under severe NaCl stress in combinations with the BA 29 rootstock, while it increased with light (12.08 mg g^{-1}) and moderate NaCl stress (9.36 mg g^{-1}) in the A×11 and D×11 combinations (Figure 5B). NaCl stress reduced chlorogenic acid content in the A×97 combination. The lowest chlorogenic acid amount was found in the A×333 combination under moderate NaCl stress (6.48 mg g^{-1}). Catechin content increased only with severe NaCl stress in the D×29 and A×333 combinations compared to the control, while it increased under all levels of NaCl stress

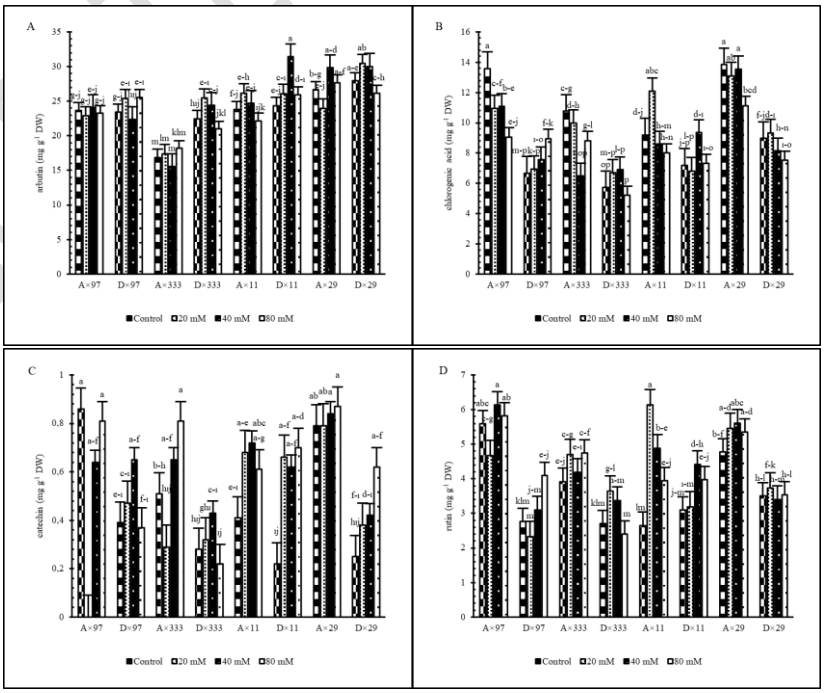

Figure 5. Effect of NaCl stress on leaves arbutin (A), chlorogenic acid (B), catechin (C), and rutin (D) contents of eight pear cultivar × rootstock combinations. The letters indicate differences ($p \leq 0.05$) between each other.

in the D×11 combination (Figure 5C). Rutin content in the leaves of the A×11 combination increased with NaCl stress (Figure 5D). The highest rutin amount was found under moderate NaCl stress (4.41 mg g^{-1}) in the D×11 combination. In combination with the OH×F 97 rootstock, higher rutin levels were obtained with moderate NaCl stress (6.13 mg g^{-1}) in the Ankara variety and with severe NaCl stress (4.10 mg g^{-1}) in the Deveci variety.

In our study, the amount of arbutin in pear leaves under NaCl stress was not significantly affected, and it increased relatively at light and moderate stress levels. Plants exposed to moderate environmental stress are known to increase the amount of certain phenolic compounds as an effective defense strategy (Zobayed et al., 2007). Our results align with this literature in this respect. Another notable result in our study is the higher accumulation of arbutin in the Deveci variety, which we identified as tolerant to NaCl stress. This suggests that arbutin, which contributes to tolerance against biotic stress in pears, may also contribute to the tolerance mechanism under NaCl stress. Additionally, these results suggest that phenolic components may contribute more to the tolerance mechanism in pears under NaCl stress than the total phenolic compounds.

Conclusion

Based on the morphological characteristics examined in the study, such as leaf area and root/shoot ratio, it can be said that plants grafted onto OH×F rootstocks are more tolerant to NaCl stress. Indeed, the combinations most affected by leaf area were found to be A×11 and A×29. Additionally, it was observed that the root/shoot ratio was negatively affected in varieties grafted onto sensitive rootstocks like Fox 11 and BA 29. The accumulation of proline, which is an important criterion for salt tolerance in developed plants, did not appear to be effective in the tolerance of *P. communis* to NaCl stress. Alongside the significant or relative decrease in oxidative stress markers under NaCl stress, the increase in antioxidant enzyme activity indicates that the enzymatic antioxidant tolerance mechanism of *P. communis* under salt stress is functioning effectively. The lack of change in the total phenolic compound and total flavonoid content under NaCl stress suggests that these compounds may not be effective in the tolerance mechanism of *P. communis*. However, the higher accumulation of arbutin in the leaves of the Deveci variety, which is more tolerant to NaCl stress, indicates that, rather than the total of secondary compounds, specific components play a role in the tolerance mechanism.

In conclusion, when evaluating the study as a whole, it is predicted that the Deveci variety grafted onto the OHxF 97 rootstock manages NaCl stress better compared to others. This combination could be used at soil salinity levels around the threshold of 4 dS m^{-1} and similar values.

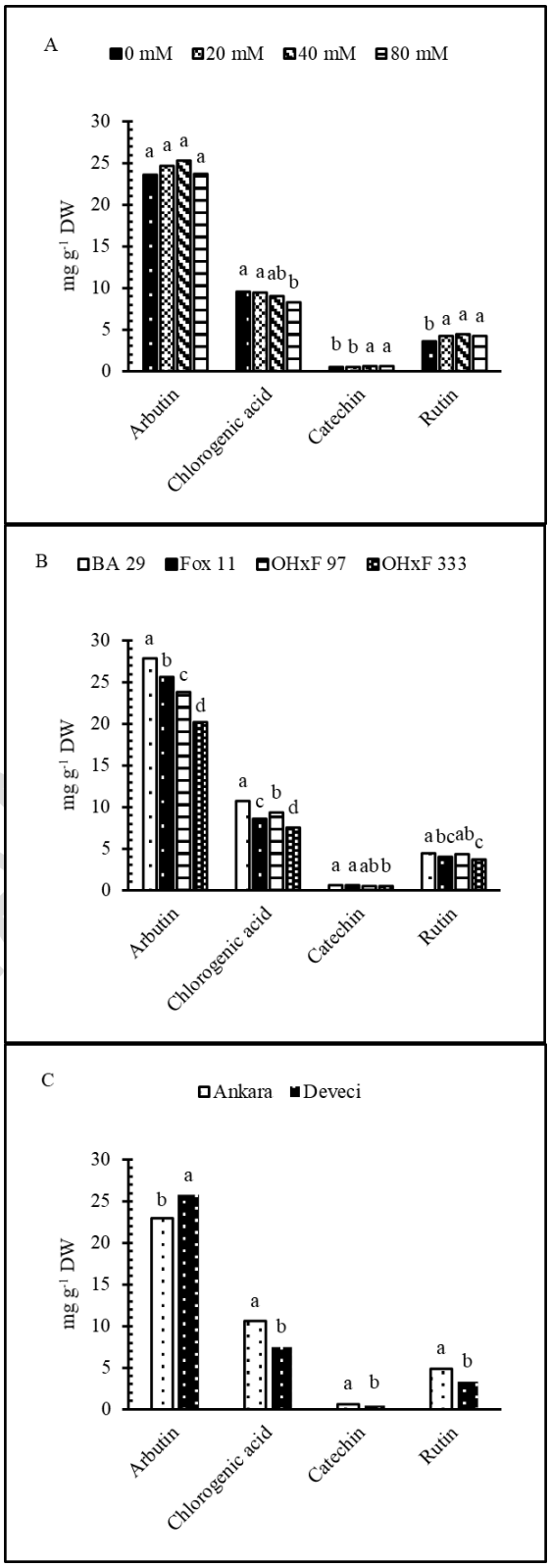

Figure 6. The effect of NaCl stress on certain phenolic compounds in the leaves of grafted plants at the levels of stress (A), rootstock (B), and variety (C). The letters indicate differences ($p \leq 0.05$) between each other.

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Author Contributions

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Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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