

Role of enzymatic antioxidants on salinity tolerance of some rootstocks used in European pear (*Pyrus communis* L.) cultivation

Melih Aydın^{1*}, Fatma Yıldırım², Emel Kaçal¹, Sercan Önder³, Mesut Altındal¹ and Yaşar Karakurt³

¹ Fruit Research Institute, Eğirdir, Isparta, Türkiye, 32500.

² Dep. of Hort., Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye, 32000.

³ Dep. of Agri. Biotechnology, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye, 32000.

*Correspondence should be addressed to Melih Aydın: melih.aydinli@tarimorman.gov.tr

Abstract

Aim of study: Plants, whether halophytes or glycophytes, develop defensive strategies to mitigate the damage caused by salt stress. This study aimed to investigate the biochemical responses of selected pear rootstocks under sodium chloride (NaCl) stress.

Area of study: Fruit Research Institute, Eğirdir, Isparta, Türkiye.

Material and methods: One-year-old non-grafted rootstocks, pear OH×F 97, OH×F 333 (Old Home × Farmingdale) and Fox 11, and quince BA 29 were used. Four NaCl concentrations were applied: 0 mM (control), 20 mM, 40 mM, and 80 mM.

Main results: Relative water content (RWC%), stomatal conductance (g_s), and hydrogen peroxide (H_2O_2) levels in leaves significantly decreased with increasing NaCl stress across all rootstocks. Antioxidant enzyme activities and sodium (Na^+) content generally increased in response to higher stress levels. Based on RWC%, g_s , and Na^+ values, Fox 11 and BA 29 were the most negatively affected. In OH×F 97, activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), and glutathione reductase (GR) significantly increased under NaCl stress.

Research highlights: Enzymatic antioxidant defense mechanisms play a key role in salinity tolerance in European pear. OH×F rootstocks, particularly OH×F 97, are recommended for soils with salinity levels close to the threshold of 4 dS m⁻¹.

Keywords: *Pyrus* spp.; salinity; rootstock; antioxidant enzymes; tolerance.

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El papel de los antioxidantes enzimáticos en la tolerancia a la salinidad de algunos patrones utilizados en el cultivo de peras europeas (*Pyrus communis* L.)

Resumen

Objetivo del estudio: Las plantas, ya sean halófitas o glicófitas, emplean estrategias defensivas para contrarrestar los daños causados por el estrés salino. El objetivo del estudio fue especificar las respuestas bioquímicas de algunos patrones de peral bajo condiciones de estrés por cloruro de sodio (NaCl).

Área del estudio: Instituto de Investigación Frutícola, Eğirdir, Isparta, Turquía.

Material y métodos: Se utilizaron plantas no injertadas de un año de edad de los patrones de peral OH×F 97, OH×F 333 (Old Home × Farmingdale) y Fox 11 y membrillero BA 29. Se aplicaron cuatro concentraciones diferentes de NaCl (0 mM (control), 20 mM, 40 mM y 80 mM).

Resultados principales: El contenido relativo de agua (RWC%), la conductancia estomática (g_s) y los niveles de peróxido de hidrógeno (H_2O_2) en las hojas disminuyeron significativamente con el aumento del estrés por NaCl en todos los patrones. Las actividades de las enzimas antioxidantes y el contenido de Na^+ (sodio) generalmente aumentaron en relación con el nivel de estrés. Los patrones más afectados fueron Fox 11 y BA 29, respectivamente, según los valores de RWC%, g_s y Na^+ . Las actividades de las enzimas superóxido dismutasa (SOD), catalasa (CAT), glutatión peroxidasa (GPX), dehidroascorbato reductasa (DHAR), (ascorbato peroxidasa) APX y glutatión reductasa (GR) aumentaron significativamente en OH×F 97 bajo estrés por NaCl.

Conclusiones: Los componentes de defensa antioxidante enzimática contribuyen al mecanismo de tolerancia en perales europeos bajo estrés por NaCl. Los patrones OH×F (especialmente OH×F 97) pueden ser preferidos cuando el valor de salinidad umbral del suelo está alrededor de 4 dS m^{-1} .

Palabras clave: *Pyrus* spp.; salinidad; patron; enzimas antioxidantes; tolerancia.

Introduction

Salinity, one of the most significant environmental factors limiting horticultural crop productivity (Musacchi et al., 2006), is rapidly increasing in arable areas due to climate change and drought (Ahanger & Agarwal, 2017).

Salt stress restricts plant development by causing osmotic stress, ionic stress, and a secondary stress known as oxidative stress (Munns & Tester, 2008; Petridis et al., 2012; Wei et al., 2017). Tuteja (2007) defined osmotic stress as the initial response to elevated salt levels in the root rhizosphere. As a result of osmotic stress, leaf water potential (LWP), stomatal conductance (g_s), and photosynthesis are reduced (Meloni et al., 2003; Navada et al., 2020), while the production of reactive oxygen species (ROS) increases (Jia et al., 2019), ultimately inhibiting plant growth (Munns & Tester, 2008). Following osmotic stress, ionic stress develops due to the excessive accumulation of toxic ions, particularly sodium (Na^+) and chloride (Cl^-), in plant tissues (Sofy et al., 2020). Among these, the excessive accumulation of Na^+ causes specific ion toxicity (Niu et al., 2017). Oxidative stress, in turn, results from the disruption of ion homeostasis, leading to the overproduction of ROS such as superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radicals (Gill & Tuteja, 2010; Arif et al., 2020).

Plants have evolved various defense strategies to cope with the adverse effects of salt stress by modifying their metabolism. These include controlling ion uptake and transport to aerial parts (Parida & Das, 2005), synthesizing compatible solutes such as glycine betaine (GB) (Ashraf & Harris, 2004), and activating enzymatic and non-enzymatic antioxidant mechanisms (Gill & Tuteja, 2010). The level of tolerance often depends on the genotype (Fu et al., 2019).

The accumulation of non-toxic compatible solutes at high concentrations is a crucial biochemical response associated with salt stress tolerance (Sofy et al., 2020). One such solute is glycine betaine, a naturally occurring quaternary ammonium compound that protects plants against various environmental stresses, including salinity (Malekzadeh, 2015). Under salt stress, ROS production, particularly of H_2O_2 , increases in plants (Chatterjee et al., 2017), leading to alterations in the synthesis of essential amino acids, proteins, lipids, photosynthetic pigments, and enzymes (Singh et al., 2016). The resulting oxidative damage can be mitigated through the activation of enzymatic and non-enzymatic antioxidant defense systems (Demiral & Türkan, 2004). The key enzymatic antioxidants involved include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), and glutathione reductase (GR) (Gill & Tuteja, 2010; Sorkheh et al., 2012). Previous studies have demonstrated a close relationship between the activity of these enzymes and salinity tolerance in plants (Sekmen et al., 2007).

Pear (*Pyrus communis* L.), the second most widely cultivated pome fruit after apple, is considered a salt-sensitive species (Maas, 1993). Research on *Pyrus* species has shown growth inhibition and leaf necrosis under salt stress, with prolonged exposure even at moderate salinity levels leading to plant damage or death (Myers et al., 1995; Okubo et al., 2000).

Rootstocks play a critical role in mediating plant responses to salinity (Mehdi-Tounsi et al., 2017). Musacchi et al. (2006) reported that salt damage in leaves varied depending on whether *P. communis* scions were grafted onto *P. communis* or *Cydonia oblonga* Mill. rootstocks. Similarly, Storey (1995) and Moya et al. (2003) noted a correlation between rootstock vigour and salinity tolerance.

Currently, in fruit production, the widespread use of chemical fertilizers, along with drip irrigation and fertigation systems (Musacchi et al., 2006), combined with the effects of climate change, has led to secondary salinization in cultivated areas. As a glycophyte, pear is particularly sensitive to such salinization. However, most existing studies have utilized rootstocks with similar vigour, and comprehensive biochemical evaluations of salinity tolerance mechanisms remain limited.

In modern European pear cultivation, both pear and quince clonal rootstocks are widely used. In traditional or semi-intensive orchards, vigorous clonal pear rootstocks such as Old Home × Farmingdale (OH×F) and Fox series are commonly employed. For high-density or intensive systems, dwarfing quince rootstocks like BA 29 are preferred.

The aim of this study was to evaluate the performance of selected *P. communis* and *C. oblonga* rootstocks under NaCl-induced salinity and to assess the extent to which their inherent tolerance mechanisms are activated under stress conditions.

Material and methods

Plant material and stress treatments

This study was conducted at the Fruit Research Institute (MAREM), located in Eğirdir, Isparta, Türkiye (latitude 37°49'N, longitude 30°52'E, altitude 940 m). One-year-old non-grafted plants of the pear OH×F 97, OH×F 333, Fox 11, and quince BA 29 rootstocks were used. The pear rootstocks were propagated via tissue culture, while the quince rootstock (BA 29) was obtained from layer beds. In early spring, the plants were transplanted into 18-liter containers filled with a 2:1:1 mixture of sifted garden soil, sand, and peat. In June, the pots were transferred to a controlled greenhouse where the stress treatments were conducted. NaCl applications began in mid-July. To induce varying levels of salinity stress, three NaCl concentrations were applied: mild (20 mM), moderate (40 mM), and severe (80 mM). First-grade irrigation water (~3 mM NaCl) served as the control. NaCl was applied every 3 to 5 days along with irrigation water, based on the pre-determined field capacity of the pots. To avoid osmotic shock, the 20 mM NaCl dose was introduced gradually. After nine weeks of exposure, visible leaf damage due to salt stress was observed.

Physiological measurements: RWC% and g_s

Following the treatments, fully developed mature leaves were collected, and their fresh weight (FW) was recorded. The leaves were then immersed in distilled water for 24 h to reach full turgidity, after which the turgid weight (TW) was measured. Subsequently, the leaves were dried at 72 °C for 48 h, and their dry weight (DW) was recorded. RWC% was calculated using the formula described by Barrs and Weatherley (1962).

$$\text{RWC (\%)} = 100 \times (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}).$$

Stomatal conductance was measured using a porometer (Delta-T, Porometer-AP4). Measurements were taken from three fully expanded leaves located on different parts of the plants, with two replicates per treatment.

Biochemical analyses: GB, H_2O_2 , Antioxidant enzyme activities, and Na^+

At the end of the experiment, fully developed leaves from the middle of each plant were collected, immersed in liquid nitrogen, and stored at -80°C until analysis. Prior to extraction, the plant materials-except those used for enzyme and Na^+ analyses were lyophilized using a Blue Wave Multi Pipeline XO-10 freeze dryer.

Leaf samples were homogenized with 20 ml of deionized water, filtered, and stored at -20 °C until analysis. The amount of GB was determined according to Sairam et al. (2002). The absorbance values of the samples were measured at a wavelength of 365 nm using a spectrophotometer. The results were expressed as $\mu\text{g GB g}^{-1} \text{ DW}$.

H_2O_2 content was measured following the method of Velikova et al. (2000). Absorbance was recorded at 390 nm, and results were expressed as $\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{ DW}$.

The SOD and CAT activities (Wang et al., 2005), GPX activity (Zaharieva et al., 1999) and DHAR activity (Nakano & Asada, 1981) were expressed as $\text{U mg}^{-1} \text{ protein}$. APX activity (Nakano & Asada, 1981) and GR activity (Foyer & Halliwell, 1976) were expressed as $\text{mole min}^{-1} \text{ g}^{-1}$.

Dried leaf sample was extracted with 1M HNO_3 for Na^+ analysis (Zrig et al., 2011). After the sample was filtered, the Na^+ content was determined using ICP-AES with a Spectro Arcos Blue2 instrument.

Statistical analysis

The research was repeated for two years and arranged in a randomized complete block design with factorial experiment design in the random plots, with three replications and five plants per replication. Differences between treatments were analyzed using the LSD Multiple Comparison Test ($p \leq 0.05$; $p \leq 0.01$; $p \leq 0.001$), and the results were confirmed using the F test with the variance analysis method in the JMP 11 software. Additionally, Pearson linear correlation analysis (heatmap correlation) and multivariate principal component analysis (PCA) were conducted using OriginPro software (version 2021, OriginLab, Northampton, MA).

Results

Physiological changes in the rootstocks under NaCl stress

In OH×F 97, RWC% was not significantly affected by NaCl treatments in either year of the study (Fig. 1). In contrast, RWC% decreased under NaCl stress in the other rootstocks. Positive correlations were found between RWC% and g_s in both years, while negative correlations were observed between RWC% and CAT, APX, and Na⁺ (Fig. 2).

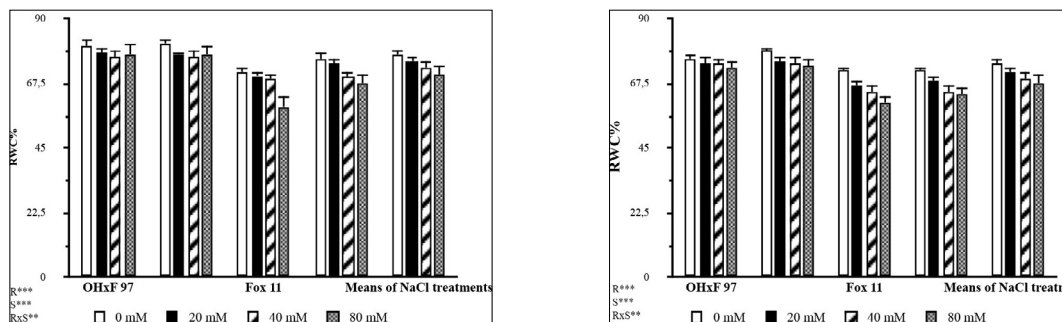


Figure 1. The effect of NaCl stress on the RWC% of four pear/quince rootstocks (A: first year, B: second year). Each value indicates the mean $\pm$ standard error (SE) of three replicates ($n=9$). R: rootstock; S: NaCl treatment. Lowercase letters represent the rootstock $\times$ NaCl interaction, and uppercase letters indicate the differences between the means of NaCl treatment. ***, ** and * denote the difference in the significance level of $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$, respectively.

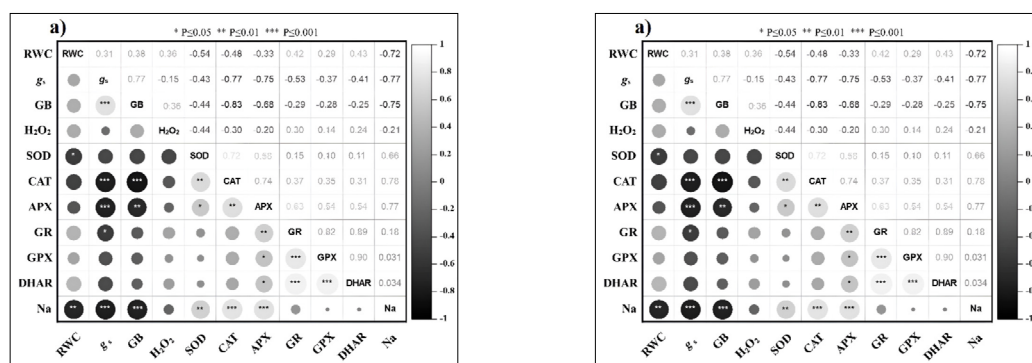


Figure 2. Correlations between enzymatic and non-enzymatic antioxidant activities and biochemical contents generated by Heat map using a) 1st year and b) 2nd year mean values from OH×F 97, OH×F 333, Fox 11 and BA 29 pear rootstocks under salt stress. The color scale displays the intensity of correlation coefficient values of different parameters [RWC, Relative water content; g_s , Stoma conductivity; GB, Glycine betaine; H₂O₂, Hydrogen peroxidase; SOD, Superoxide dismutase; CAT, Catalase; APX, Ascorbate peroxidase; GR, Glutathione reductase; GPX, Glutathione peroxidase; DHAR, Dehydroascorbate reductase; Na, Sodium].

Stomatal conductance was significantly affected by NaCl treatments in all rootstocks across both years, showing a marked decrease compared to the control (Fig. 3). This reduction followed a notably linear pattern in the second year. As salinity levels increased, g_s values declined significantly or proportionally in both years (Fig. 3). Additionally, negative correlations were observed between g_s and catalase (CAT), ascorbate peroxidase (APX), and Na⁺ content (Fig. 2).

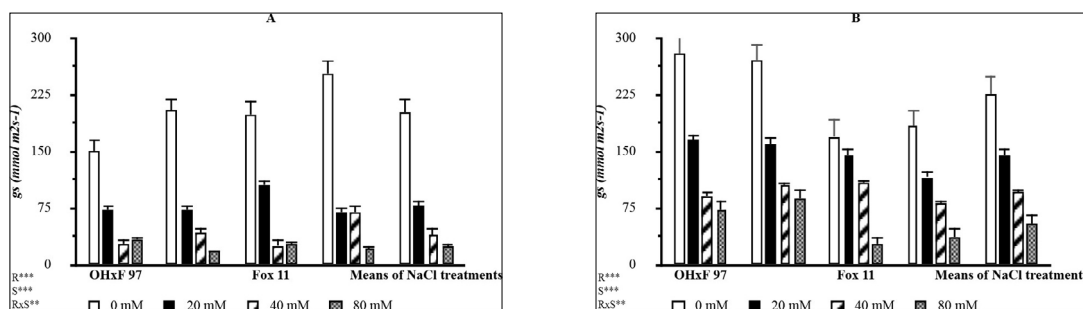


Figure 3. The effect of NaCl stress on the g_s in four pear/quince rootstocks (A: first year, B: second year). Each value indicates the mean $\pm$ standard error (SE) of three replicates ($n=6$). R: rootstock; S: NaCl treatment. Lowercase letters represent the rootstock $\times$ NaCl interaction, and uppercase letters indicate the differences between the means of NaCl treatment. ***, **, * and ns (not significant) denote the difference in the significance level of $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$, respectively.

Biochemical changes in the rootstocks under NaCl stress

In both years of the study, GB content was not significantly affected by different NaCl levels in any of the rootstocks (Table 1). However, OH×F 97 and OH×F 333 exhibited significantly higher GB contents compared to Fox 11 and BA 29. No significant correlation was found between NaCl stress intensity and GB content in either year. Nonetheless, GB was positively correlated with GPX and DHAR, and negatively correlated with TAC (Fig. 2).

Table 1. Effect of NaCl stress on glycine betaine ($\mu\text{mol g}^{-1}$ dry weight) amount in mature leaves of four pear/quince rootstocks.

	NaCl (mM)	OH×F 97	OH×F 333	Fox 11	BA 29	Means of treatments
1st year	0	1.23 ^{ns}	1.04	1.09	0.87	1.05 ^{ns}
	20	1.03	0.98	1.01	0.57	0.90
	40	1.35	1.02	0.94	0.67	1.00
	80	1.34	0.98	1.00	0.64	0.99
Means of rootstocks		1.24 ^{a***}	1.00 ^b	1.01 ^b	0.69 ^c	-
S	ns					
R	***					
S × R	ns					
2nd year	0	1.74 ^{ns}	1.59	1.40	0.93	1.42 ^{ns}
	20	1.86	1.77	1.16	0.83	1.41
	40	1.93	2.05	1.31	1.04	1.58
	80	1.54	1.81	1.01	0.86	1.31
Means of rootstocks		1.77 ^{a***}	1.81 ^a	1.22 ^b	0.92 ^c	
S	ns					
R	***					
S × R	ns					

Each value indicates the mean $\pm$ standard error (SE) of three replicates ($n=3$). NaCl: sodium chloride; OH×F: Old Home $\times$ Farmingdale; R: rootstock; S: NaCl treatment. Means of treatments and means of rootstocks (four rootstocks/treatments and three replications per rootstocks/treatments). Lower case letters indicate the difference between the rootstock means ($n=12$) in each row. *** and ns denote the difference in significance level of $p \leq 0.001$ and $p \leq 0.05$, respectively.

The H_2O_2 content of the leaves was significantly affected by NaCl treatments in both years (Fig. 4). In the first year, H_2O_2 content decreased linearly with increasing salt stress, while in the second year the decrease was present but not strictly linear. A negative correlation was observed between H_2O_2 and CAT, APX, and Na^+ content (Fig. 2).

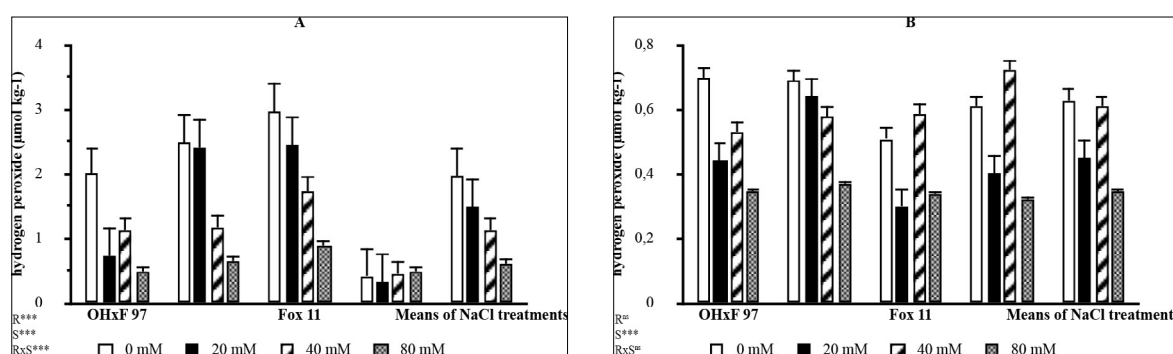
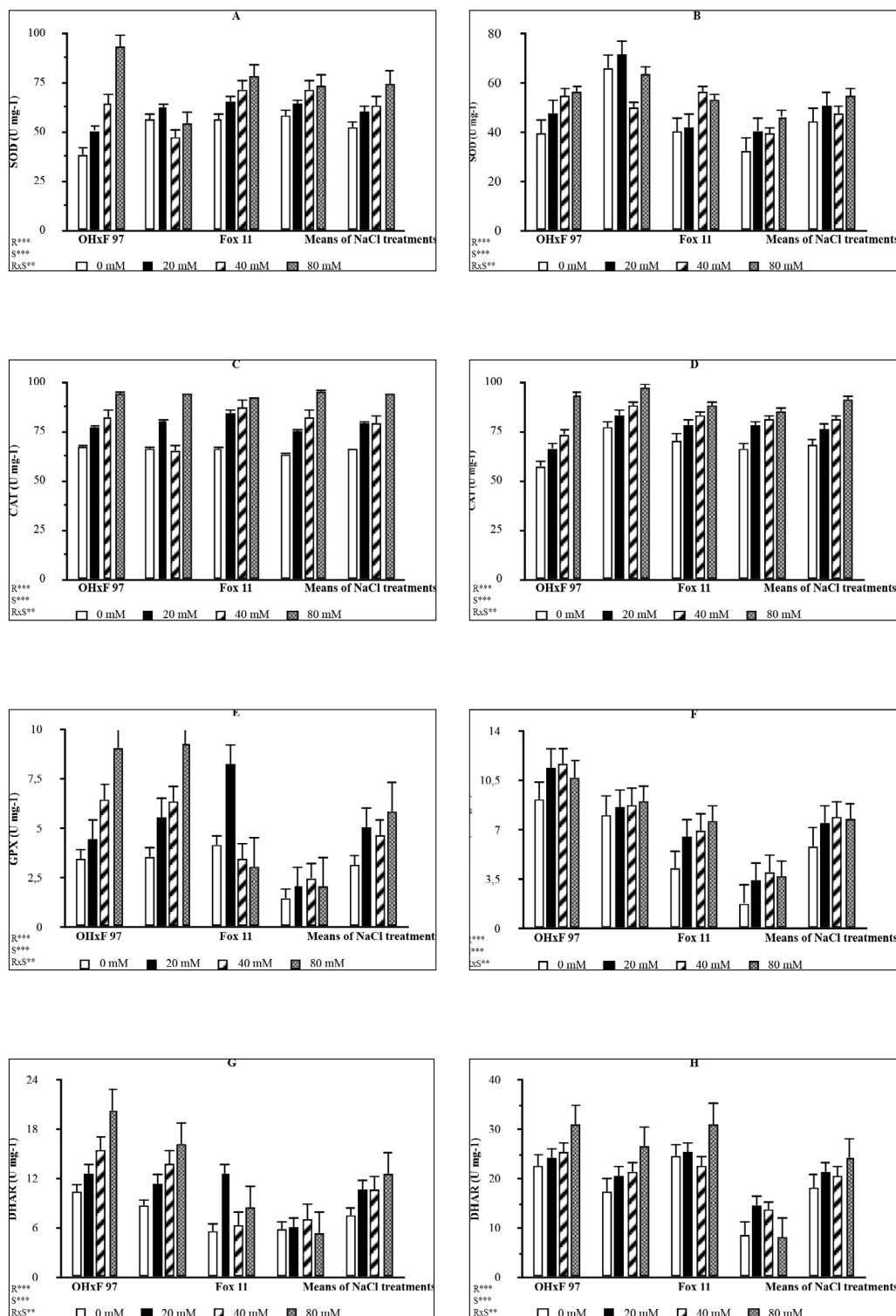


Figure 4. The effect of NaCl stress on the H_2O_2 in four pear/quince rootstocks (A: first year, B: second year). Each value indicates the mean $\pm$ standard error (SE) of three replicates ($n=12$). R: rootstock; S: NaCl treatment. Lowercase letters represent the rootstock $\times$ NaCl interaction in the first year, NaCl treatments within each rootstock in the second year; uppercase letters indicate the differences between the means of NaCl treatment. *** and ns denote the difference in significance of $p \leq 0.001$ and $p \leq 0.05$, respectively.

Antioxidant enzyme activities in all rootstocks were influenced by NaCl levels (Fig. 5). In OH×F 97, enzyme activities generally increased with rising stress, with the highest values observed under 80 mM NaCl, except for GPX in the second year (Fig. 5H). A similar trend was observed in OH×F 333 for GPX, DHAR, APX, and GR (Figs. 5E–5L), whereas SOD and CAT activities in OH×F 333 showed variable responses. In Fox 11 and BA 29, SOD, CAT, and APX activities generally increased with higher NaCl levels (Figs. 5A–5D and 5I–5J). However, GPX, DHAR, and GR activities in Fox 11 displayed inconsistent

patterns across years (Figs. 5E–5F, 5I–5L). In BA 29, elevated GPX (Figs. 5E–5F) and DHAR (Fig. 5G) activities were observed particularly at 20 mM and 40 mM NaCl. GR activity in BA 29 increased at 20 mM and 40 mM in the first year (Fig. 5K), and at 40 mM in the second year (Fig. 5L). In general, GPX, DHAR, and GR activities in BA 29 were lower than those in the *Pyrus* rootstocks. Positive correlations were found between several antioxidant enzyme pairs: SOD and CAT, CAT and APX, GR and Na⁺, APX and Na⁺, GR and GPX, and GPX and DHAR (Fig. 2).



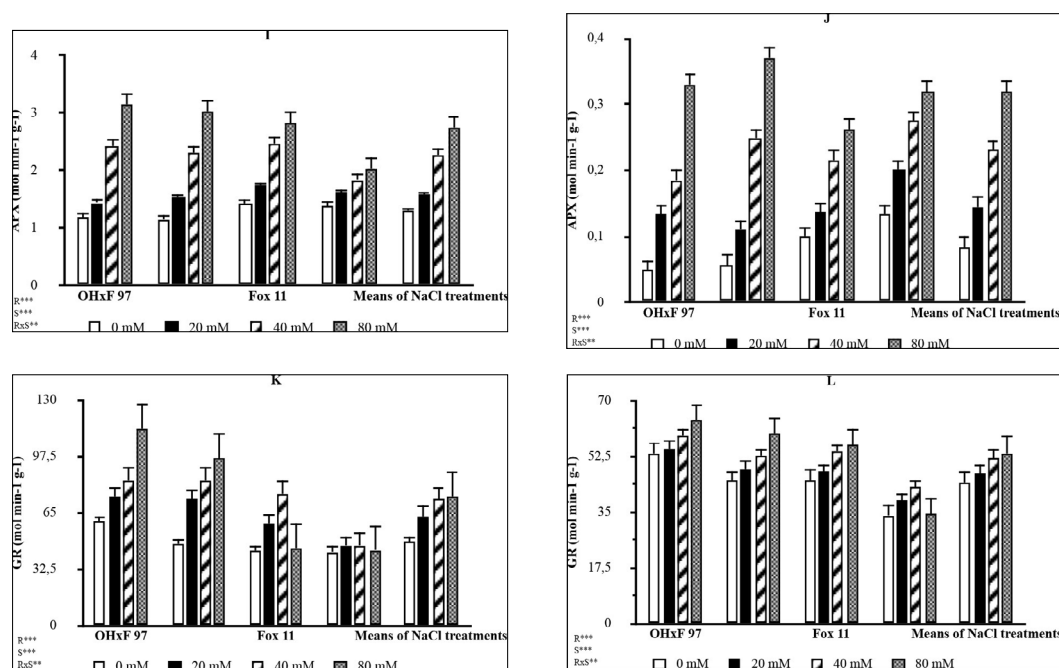


Figure 5. The effect of NaCl stress on the antioxidant enzyme activities in four pear/quince clonal rootstocks (A, C, E, G, I and K: first year, B, D, F, H, J and L: second year). Each value indicates the mean $\pm$ standard error (SE) of three replicates ($n=12$). R: rootstock; S: NaCl treatment. Means of NaCl indicate the average ($n=12$) of the treatments (four rootstocks and three replications per rootstock). Lowercase letters represent the rootstock $\times$ NaCl interaction. Uppercase letters indicate the differences between the NaCl treatment means. ***; represents the difference in significance of $p \leq 0.001$.

The Na^+ content of all rootstocks significantly increased with higher NaCl levels in both years (Table 2). Fox 11 rootstock showed the highest Na^+ accumulation under 80 mM NaCl, with levels 7.02 times higher than the control. Similarly, Na^+ content increased 5.72 times in Fox 11 under 40 mM, and 8.40 and 4.24 times in BA 29 under 80 mM NaCl across the two years. For OHxF 97 and OHxF 333, increases were 3.63 and 1.98 times, and 4.78 and 1.81 times, respectively, depending on year and rootstock. Statistically significant differences in Na^+ content was observed among rootstocks in both years. Fox 11 accumulated the highest Na^+ content, followed by BA 29. Differences between OHxF 97 and OHxF 333 were not significant (Table 2). In both years, a linear and significant increase in Na^+ content was observed with increasing NaCl concentration (Fig. 2).

Table 2. Effect of NaCl stress on sodium (mg g^{-1} dry weight) contents in mature leaves of four pear/quince clonal rootstocks.

	NaCl (mM)	OHxF 97	OHxF 333	Fox 11	BA 29	Means of treatments
1st year	0	0.49 ^{gh*}	0.33 ^h	0.44 ^{gh}	0.25 ^{gh}	0.45 ^{D***}
	20	0.66 ^{f-h}	1.09 ^{c-g}	0.83 ^{f-h}	1.01 ^{c-g}	0.90 ^C
	40	1.29 ^{d-f}	1.26 ^{d-f}	2.52 ^{ab}	1.62 ^{c-c}	1.67 ^B
	80	1.78 ^d	1.58 ^{c-c}	3.09 ^a	2.10 ^{bc}	2.14 ^A
	Means of rootstocks	1.05 ^{C***}	1.06 ^C	1.72 ^A	1.24 ^B	
S	***					
R	**					
S $\times$ R	*					
2nd year	0	0.52 ^{fg***}	0.47 ^g	0.42 ^g	0.50 ^{fg}	0.48 ^{C***}
	20	0.59 ^{c-g}	0.67 ^{c-g}	0.61 ^{c-g}	0.61 ^{c-g}	0.62 ^C
	40	0.93 ^{c-c}	0.66 ^{c-g}	1.25 ^c	0.66 ^{c-g}	0.90 ^B
	80	1.03 ^d	0.85 ^{d-f}	3.19 ^a	2.12 ^b	1.78 ^A
	Means of rootstocks	0.77 ^{C***}	0.66 ^C	1.37 ^A	0.97 ^B	
S	***					
R	***					
S $\times$ R	***					

Each value indicates the mean $\pm$ standard error (SE) of three replicates ($n=3$). NaCl: sodium chloride; OHxF: Old Home $\times$ Farmingdale; R: rootstock; S: NaCl treatment. Means of NaCl and means of rootstocks (four rootstocks/treatments and three replications per rootstocks/treatments) show the mean ($n=12$). The letters represent the treatments according to the rootstocks and the variation between the treatments and the means of the rootstocks. ***, ** and * denote the difference in significance level of $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$, respectively.

Principal component analysis of enzymatic and non-enzymatic antioxidant activities and biochemical parameters for pear and quince rootstocks under NaCl stress is presented in Fig. 6. Three eigenvalues (PCA axes) greater than 1 were obtained based on the PCA for the 1st and 2nd years. The two main principal components, PC1 and PC2, explained 77.27% of the total variation for the 1st year and 73.60% for the 2nd year. In the 1st year, the control group of rootstocks clustered to the left of the PCA1 axis, while in the 2nd year, they clustered to the right of the PC1 axis. Eigenvector values greater than 0.25 were considered (Erbaş & Mutlucan, 2023). In the 1st year, SOD, CAT, APX, GR, GPX, DHAR, and Na^+ were more than 3/2 of the total variation in the PCA1 axis, and RWC, H_2O_2 , GR, GPX, and DHAR were more than 3/2 of the total variation in the PCA2 axis. In the 2nd year, RWC, g_s , GB, and H_2O_2 were more than 3/2 of the total variation in the PCA1 axis, and GB, SOD, GR, GPX, and DHAR were more than 3/2 of the total variation in the PCA2 axis.

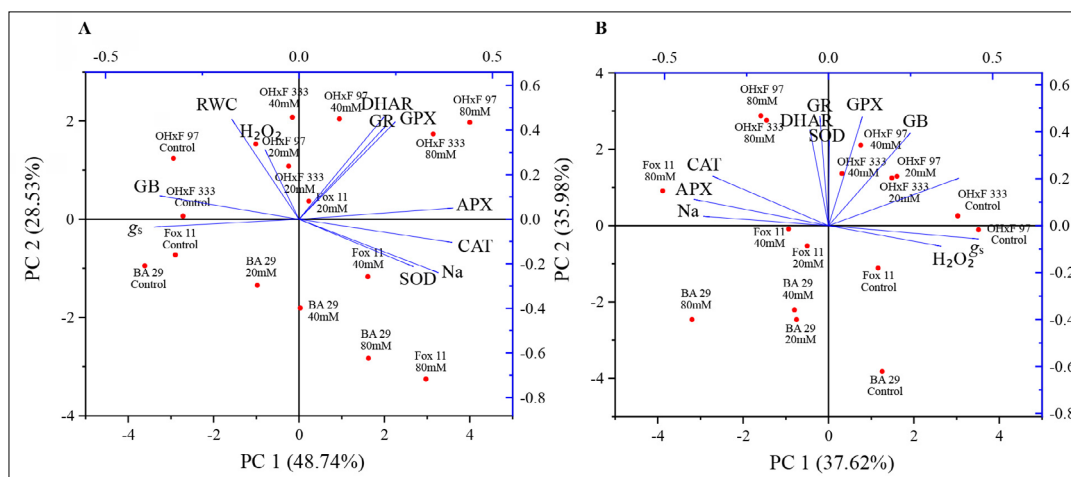


Figure 6. Principal component analysis (PCA) between enzymatic and non-enzymatic antioxidant activities and biochemical contents generated a) 1st year and b) 2nd year mean values from OH×F 97, OH×F 333, Fox 11 and BA 29 pear rootstocks under salt stress. [RWC, Relative water content; g_s , Stoma conductivity; GB, Glycine betaine; H_2O_2 , Hydrogen peroxidase; SOD, Superoxide dismutase; CAT, Catalase; APX, Ascorbate peroxidase; GR, Glutathione reductase; GPX, Glutathione peroxidase; DHAR, Dehydroascorbate reductase; Na, Sodium.

Discussion

NaCl stress adversely affects key plant traits such as growth, development, and ultimately yield by inducing morphological, physiological, and biochemical changes, particularly in glycophytic species. These negative effects have been shown to vary depending on genotype, as demonstrated in previous studies on fruit crops (Simpson et al., 2015; Fu et al., 2019). In the present study, the physiological and biochemical responses of OH×F 97, OH×F 333, and Fox 11 (*P. communis*), as well as BA 29 (*C. oblonga*), varied under NaCl stress, indicating genotype-dependent differences across the measured parameters.

The RWC% is a key physiological parameter that reflects the overall water status and hydration capacity of plants. NaCl stress is known to disrupt water balance in many higher plants (Flowers & Yeo, 1986). In this study, RWC% generally declined under NaCl stress. Notably, significant reductions were observed in the Fox 11 and BA 29 rootstocks. Similarly, Abid et al. (2020) found a decrease in RWC% in kiwi genotypes under NaCl stress. However, in our study, RWC% changes in OH×F 97 under NaCl treatments were not significant. These results observed in OH×F 97 might be related to its tolerance to NaCl stress. Indeed, different researchers have reported that RWC% remains unaffected by salt stress in tolerant plants (Khan et al., 2009).

Stomatal movements, including g_s , are significantly affected by salinity and are more effective in glycophyte species (Kiani-Pouya et al., 2020). We found that g_s significantly decreased linearly with increasing NaCl stress in all rootstocks. That findings are consistent with the results of the study conducted by Wu & Zou (2009) in *Pyrus betulaefolia*. OH×F 97 had a higher g_s among rootstocks. Physiological processes, such as faster stomatal movements involved in gas exchange, are related to a plant's ability to cope with stress under NaCl stress (Kiani-Pouya et al., 2020). Accordingly, it was observed that OH×F 97 was less affected by the investigated physiological traits under NaCl stress.

GB accumulation plays a protective role under salt and osmotic stress in developed plants (Arif et al., 2020). Moreover, an association has been reported between the increase in GB content in plant tissues and salt stress tolerance (Sohrabi et al., 2017). In contrast, Ashraf & Foolad (2007) have shown that this correlation is not always valid. Indeed, Larher et al. (2009) stated that GB could not be found among the substances contributing to osmotic adjustment in Asian and European pears. In the study, the GB content of the leaves was not affected by NaCl stress. However, a genotypic effect was observed in the study, and BA 29 had the lowest GB content, while OH×F 97 had a higher GB content in both years. This difference is believed to arise from the variations in GB synthesis metabolism among genotypes. On the other hand, the lower GB content in BA 29 compared to other rootstocks can be considered as a factor reducing its tolerance to salt stress.

Plants exposed to oxidative stress often produce high levels of ROS, among which H_2O_2 is one of the most abundant. Excessive accumulation of H_2O_2 can lead to increased cellular membrane leakage (Xu et al., 2016). Yahmed et al. (2015) suggested that changes in H_2O_2 levels can serve as a reliable marker for assessing oxidative stress damage. Indeed, several studies have reported that H_2O_2 levels tend to increase under salt stress, particularly in salt-sensitive genotypes (Niu et al., 2018; Sairam et al., 2002). However, contrasting results have also been reported such as decreases in H_2O_2 content under salinity stress in rice (Cunha et al., 2016) and mandarin (Yahmed et al., 2015) indicating that H_2O_2 responses may vary depending on species and tolerance level. In our study, the H_2O_2 content in the rootstocks generally decreased under NaCl stress. Additionally, the H_2O_2 content was significantly reduced in OH×F, especially under 80 mM NaCl. According to the results, it is believed that both pear and quince species activate their mechanisms for removing H_2O_2 under oxidative stress. Particularly, the quantitative and proportional reductions observed in OH×F rootstocks indicate that the enzymatic antioxidative defense systems for eliminating H_2O_2 work more effectively in these rootstocks. CAT is the main enzyme responsible for converting the highly harmful metabolic product H_2O_2 into water and oxygen, thus detoxifying cells (Sabra et al., 2012). We also found a negative correlation between CAT activity and H_2O_2 content, and these results were consistent with the literature.

In studies conducted on different species, under salt stress and at different stress levels, the activities of enzymes such as SOD, CAT, APX, GR, DHAR, and GPX increase, and this increase is higher in tolerant plants compared to sensitive species/varieties/genotypes (Demiral & Türkan, 2004; Yıldız et al., 2015; Khan et al., 2019). SOD enzyme activity was found to be highest in the OH×F 97, which we considered more tolerant to NaCl stress, under 80 mM NaCl treatments in both years. Additionally, the greatest percentage increases were observed in the OH×F 97 compared to control. The results of SOD activity are similar to the findings of Balal et al. (2010), who reported that the highest increase in SOD activity under salt stress in tolerant rootstocks of *Citrus* plants. Regarding CAT activity, it was generally observed that the activity in the leaves of all rootstocks (except OH×F 333 in the first year) increased with increasing stress severity. When the increases in activity levels were examined in rootstocks after 80 mM NaCl treatments compared to control in each year, BA 29 stood out in the first-year treatments, while OH×F 97 was in the second year. The results of the second year are consistent with the research of Yaşar *et al.* (2008), who found that CAT activity increased in all four different watermelon varieties under salt stress, but the increase in enzyme activity was much higher in tolerant varieties and lower in sensitive varieties. The first-year results support Gupta and Huang's (2014) assertion that "sometimes tolerant genotypes exhibit higher activity under salt stress, while the opposite can also be observed." GPX activities in the leaves of rootstocks generally increased under NaCl stress. In the first year of the study, the enzyme activity in the Fox 11 at 40 mM and 80 mM NaCl was lower than in control plants. A similar situation was also observed in the BA 29. In the second year, the GPX activity in OH×F 97 and BA 29 rootstocks decreased at 80 mM NaCl levels compared to other NaCl treatments. Additionally, OH×F rootstocks had the highest GPX activity in both years. Carrasco-Ríos & Pinto (2014) stated that GPX activity decreased in the tolerant maize variety under stress, while it significantly increased in the sensitive variety. However, they also emphasized that despite the decrease in enzyme activity in the tolerant variety, it remained at higher levels than in the sensitive variety. When considering the APX activities of the leaves under NaCl stress, the activity increased with the severity of stress in all rootstocks, and this increase was correlated with stress intensity. Percentage changes in activity between control and NaCl stress (especially 80 mM NaCl) also highlighted the OH×F 97. The DHAR activity in the rootstocks increased with the increase in stress level in OH×F rootstocks. In BA 29, 80 mM NaCl reduced this enzyme activity. Although differences were observed between years, it was found that the DHAR enzyme in the Fox 11 did not work as effectively as in the OH×F rootstocks. The GR activity increased with stress level in OH×F rootstocks according to the first-year results, while NaCl stress did not affect the enzyme activity in the BA 29. It was determined that the enzyme activity did not change between 80 mM NaCl and control in the Fox 11. In the second year, in addition to OH×F rootstocks, GR activity also increased with stress intensity in the Fox 11, while at 80 mM NaCl level, the activity did not change in the BA 29. The highest GR activity was observed in OH×F 97 in both years.

Tolerance to salt stress is generally associated with a more effective antioxidant system (Bor et al., 2003). For instance, in *Citrus* plants, APX activity has been found to be higher in tolerant varieties under salt stress (Shahid et al., 2019), and in rice, similar to APX, GR, and DHAR activities are triggered (Demiral & Türkan, 2004). Khan et al. (2009) determined that the APX activity increased in mustard plants under salt stress because of the rapid conversion of produced H_2O_2 . López-Gómez et al. (2007) stated that in mandarin varieties grafted on two different rootstocks, DHAR activity was higher in those rootstocks classified as tolerant under salt stress. According to Sekmen et al. (2007), generally, tolerant plants under salt stress have higher GR activity compared to sensitive ones.

In many plant species, specific ion damage is mainly attributed to Na^+ (Türkan & Demiral, 2009). Plant salt tolerance is generally associated with the limitation of salt ion uptake by roots and/or transport to the leaves (Fernández-García et al., 2004). Indeed, in mandarin (García-Sánchez et al., 2002), loquat (López-Gómez et al., 2007), and almond (Zrig et al., 2011) exposed to salt stress, the Na^+ concentration in the leaves was found to vary depending on the rootstock, with sensitive rootstocks accumulating more Na^+ in their leaves. In our study, the Na^+ content in the leaves of all rootstocks relatively or significantly increased with stress treatments. Especially in both years, the Fox 11 showed a significant increase in Na^+ content with 40 mM and 80 mM NaCl treatments. In OH×F rootstocks, significant Na^+ accumulation was observed in the leaves with 80 mM NaCl compared to the control plants. However, the differences between treatments varied depending on the rootstock and year. When examining the overall Na^+ content in the rootstocks, in both years, the leaves of the Fox 11 accumulated more of this ion. Additionally, in

both years, this rootstock was followed by the BA 29. In the salt tolerance of glycophyte plants, limiting Na⁺ uptake by roots and maintaining low levels of Na⁺ in the leaves play a crucial role (López-Aguilar et al., 2003). Considering this view, OH×F rootstocks had less Na⁺ related damage (burning, chlorosis, wilting) in their leaves compared to the leaves of Fox 11 and BA 29 rootstocks. In OH×F rootstocks, it is believed that mechanisms such as restricting the transport of Na⁺ ions from the root system to the upper parts or accumulating these ions in the stem may contribute to their capacity for salt stress tolerance.

Conclusion

This study underscores the importance of rootstock selection in managing salinity stress in pear cultivation. The clear genotypic differences observed highlight the potential of certain rootstocks, particularly OH×F 97, to maintain physiological stability and activate antioxidant defenses under NaCl stress. Key indicators of salinity tolerance identified in this study include RWC%, g_s , Na⁺ accumulation, and the activities of antioxidant enzymes. These findings contribute to a better understanding of salt tolerance mechanisms in *Pyrus* species and offer practical guidance for selecting resilient rootstocks for salt-affected growing conditions.

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