



## Biochemical and Physiological Responses of Some Grape Rootstocks to Salt Stress



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UTILIZING grape rootstocks is considered an efficient method to enhance the resistance against biotic and abiotic stresses. Therefore, a pot experiment was conducted during Two consecutive seasons 2020 and 2021 to evaluate the responses of 1-year-old rooted cuttings of commercially available grape rootstocks (SO4, Freedom, Ramsey, and Richter) planted in sandy soil, amended with compost; then irrigated with graduated levels of salt mixture solution (control, 1500, 3000, 4500, and 6000 ppm). The results obtained from biochemical and physiological measurements revealed that Ramsey rootstock outperformed the other rootstocks, significantly exhibiting the highest concentrations of nitrogen, potassium, magnesium, and proline in roots, while demonstrating the lowest sodium concentration in petioles. The results offer valuable insights on grape cultivation under salinity conditions with irrigation water concentration ranging from 1,500 to 3,000 ppm.

**Keywords:** Grape rootstocks, Salinity, Mineral content, Physiological properties, Biochemical properties.

### Introduction

Grapes are an important crop globally, serving various purposes like wine, juice, and jam production. In Egypt, table grapes are the second most important fruit after Citrus. In 2020-2021, Egypt ranked twenty-first globally by producing around 1.42 million tons of grapes from 77,000 hectares of cultivated land (United States Department of Agriculture, 2020; OIV, 2022).

Soil salinity is a major global issue affecting millions of hectares of land annually, primarily in hot and arid or semi-arid regions (Ghassemi et al., 1995). About 20% of irrigated land and 50% of arable land worldwide are affected by soil salinization, with the global area of salt-affected soils estimated at 424 million hectares for topsoil and 833 million hectares for subsoil (FAO, 2021). Human activities and natural factors contribute to soil salinization, particularly in arid regions. Egypt's arable land covers 3.3 million hectares, but agricultural productivity is limited by environmental stresses such as salt stress and drought (Shin et al., 2022).

Soil degradation in the North Delta is caused by high salt concentrations in groundwater, resulting in environmental problems and limiting agricultural productivity (Mohamet et al., 2019).

The Nile River provides approximately 93% of the country's conventional water resources, equivalent to approximately 55.5 BCM/yr. However, there is a deficit of 25.8 BCM/yr due to the annual total water demand, which amounts to 81.3 BCM/yr. Out of this demand, 86% is allocated to agriculture (Omar et al., 2021). Egypt utilizes shallow groundwater and the reuse of drainage water and wastewater to compensate for the water gap. The country has six main aquifer systems with different levels of salinity (El Tahlawi et al., 2008).

Salinity in the soil can reduce yield and grape quality, and high salt concentrations in irrigation water can negatively impact grapevines' ability to absorb water and essential elements, and it can reduce photosynthesis. Grapes possess moderate salt tolerance, its show tolerance to salinity up to 1.5 dS m<sup>-1</sup>, but a decrease in plant growth by 10%

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(Received 21/06/2023, accepted 06/08/2023)

DOI: 10.21608/EJOH.2023.218924.1258

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is observed at 2.5 dS m<sup>-1</sup> (Kotuby-Amacher *et al.*, 2000).

To combat high soil salinity, grape cultivators should evaluate their rootstocks' ability to withstand stress by studying root response to salinity (Sabir *et al.*, 2021). Grafting grapevines on rootstocks has been shown to improve plant physiology and nutrient status plants (Upreti, Varalakshmi, and Jayaram 2012). The ideal rootstock for salt tolerance should have high innate vigor and the ability to exclude moderate to high amounts of chloride and sodium (Saritha *et al.*, 2017).

Consequently, grapevine growers are interested in grafting grapevines onto commercial rootstocks with maybe improve plant vigor, nutrient status, and salt tolerance. Therefore, the research aimed to evaluate and compare the salinity tolerance among some commercial rootstocks (SO4, Freedom, Ramsey, Richter) with respect to biochemical and physiological response to identify the most promising salinity-tolerant rootstock.

### **Material and Methods**

The present study was performed over at a pomology greenhouse (one stratum of black shadow cloth and 30% of shade rating) of Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt during the two successive seasons of 2020 and 2021 to investigate the response of four grape rootstocks (SO4, Freedom, Ramsey, and Richter) to saline irrigation with salt mixture solutions in concentration of (control, 1500, 3000, 4500 and 6000 ppm).

#### *Experimental setup*

Pots were arranged in a randomized complete design with two variables, the first was grape rootstocks which included one-year-old rooted cuttings from 4 commercial grape rootstocks (Freedom, SO4, Ramsey, and Richter) and the second variable was salt mixture concentration whereas, transplants were irrigated with salt water at 5 levels (control, 1500, 3000, 4500, and 6000 ppm), with a total of 20 treatments repeated three times and each replicate include one transplant per season, at the beginning of February.

Fifteen transplants of 4 commercial grape rootstocks then each one transplanted inside 50 liters of sandy soil amended with 1/6 compost

in plastic containers. Transplants were irrigated manually with tap water at a rate of 2.5 L per pot every 3 days to maintain 70% of field capacity. Transplants were fertilized every 15 days with commercial water-soluble fertilizers 20-19-19 N P K + Micronutrients. The different salinity levels (control, 1500, 3000, 4500, and 6000 ppm) were initiated in mid-June by adding a salt mixture solution (NaCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub>) prepared according to the method described by El Kobbia and Ibrahim (1986) For all above mentioned treatments, transplants were supplied every 3 days with different salt mixture solutions at a rate of 2.5 L/pot. It should be pointed out that leaching process is done every two weeks with an increase in the volume of saline water at a rate of 25% using tap water to prevent salt accumulation in the soil pots (El-Sayed & Ennab, 2012).

#### *Recorded data and findings*

Plant samples collected in mid-August were analyzed to evaluate the effects of various treatments on their physiological and biochemical properties.

#### *Mineral content*

Petiole samples were taken from the fully expanded leaves, typically starting from the fourth leaf and continuing to the ninth leaf from the shoot tip downwards (Moyer *et al.*, 2018). At the end of each growing season (late August), Fresh samples of the transplants were taken and sorted out into (leaves, stem and root), washed with distilled water, then oven dried at 70°C to calculate the dry weight of each part. Leaf samples were dried at 70°C and finally ground and digested according to (Jackson, 1973). Petioles and roots mineral content of N, P and K & Na<sup>+</sup> were determined using micro Kjeldahl method, spectrophotometer and flame photometry, respectively according to (Cottenie *et al.*, 1982), Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations were determined using EDTA Titration, following the procedure outlined by (Tucker & Kurtz, 1961), Cl<sup>-</sup> content was measured via titration with silver nitrate using the Mohr's method, described by (Belcher & Macdonald, 1957).

#### *Prolin (Pro)*

At the conclusion of every season samples of roots and fully expanded leaves were chosen. The concentration of proline was determined using the ninhydrin reaction method described by (Bates *et al.*, 1973), and calculated as mmol/g-1 FW based on a standard curve using D-proline.

### *Enzyme activity*

The extraction of enzymes and soluble proteins was done by homogenizing 0.5 g of fresh fully expanded leaves (at last August) in a solution of 4ml of 0.1M sodium phosphate buffer (pH 7.0) containing, 1% polyvinylpyrrolidone (PVP), and 0.1mM EDTA. The mixture was then centrifuged at 4°C for 20 min at 10,000×g, and the supernatant was used for testing. The Bradford method was used to evaluate the soluble proteins (Bradford, 1976).

### *Peroxidase (POD)*

The Peroxidase (PPO) activity was determined using the method described by (Oktay et al., 1995). The enzyme activities were expressed as a unit per milligram of protein per minute.

### *Polyphenol oxidase (PPO)*

The Polyphenol oxidase (POX) activity was quantified using the method of of Dias & Costa (1983) with slight modifications. The enzyme activities were expressed as a unit per milligram of protein.

### *Chlorophyll content (mg/g-1 FW)*

Chlorophyll a (Chl a) and chlorophyll b (Chl b), as well as total chlorophyll (a+b), in the juvenile and fully mature leaves were determined in mid-August following the methods described by (Rajalakshmi & Banu, 2015). The amount of chlorophyll was determined as mg per gram of the sample.

### *Leaf relative water content (LRWC)*

LRWC was determined the methods outlined by (Aroca, 2013), by taking five (0.8 cm) leaf discs from fully expanded juvenile leaves in mid-August.

LRWC was calculated as follows:  $LRWC = (FW - DW) / (TW - DW) \times 100$ , where FW is fresh weight, DW is dry weight, and TW is turgid weight.

### *Statistical analysis*

The CoStat Package Program (Version 6.303 by CoHort Software, USA) was utilized to conduct a statistical analysis, while ANOVA was used to examine the data. To compare the differences in data means, Duncan's Multiple Range Test (Hartzell, 1946) was employed. All statistical decisions were based on a significant level of  $P \leq 0.05$ .

## **Result and Discussion**

### *Effect of different rootstocks and water salinity levels on leaf mineral content on mineral content*

#### *Nitrogen content*

Data displayed in Table 1 revealed the effect of rootstocks, salinity level, and their interaction on nitrogen content in petioles, and roots of some grape rootstocks in 2020 and 2021 seasons.

The results indicated that there were no significant differences in N concentration in petioles among rootstocks. In terms of the nitrogen concentration in the roots, both Ramsey and Richter rootstocks consistently recorded the highest significant values in both seasons.

Regarding the salinity levels, there was a significant impact on nitrogen (N) concentration in petioles and roots during both seasons. The N concentration in petioles was reduced with increasing salinity levels, with the highest significant values found in the control. However, the behavior of N in roots displayed diverse responses to salinity levels: decreasing at 1,500 ppm, increasing at 3,000 ppm, and decreasing again between 4,500 and 6,000 ppm in the first season. In the second season, N in roots decreased at 1,500 ppm, increased at 3,000 ppm and 4,500 ppm, and then decreased at 6,000 ppm. Notably, 3,000 ppm exhibited the highest significant difference in nitrogen concentration compared to salinity in both seasons.

The interaction between rootstock and salinity level indicated that there were no significant differences in nitrogen concentration in petioles among the rootstocks at the same salinity level. Generally, at 6000 ppm salinity, the N concentration in petioles decreased by approximately 40-50% compared to the control. Ramsey and Richter rootstocks demonstrated the highest N concentration in the roots, with a significant difference observed at the salinity level of 6000 ppm during the first season, resulting in a decrease of around 25-50% compared to the control.

These findings align with the observations of Wasim (2011), who found that Flame Seedless on Ramsey rootstock exhibited the highest leaf nitrogen content with saline water irrigation (3000 and 4000 PPM). Similarly, Ahmad (2016) demonstrated an increased leaf nitrogen percentage when grafting Superior seedless grape onto Salt Creek rootstock in saline soil (EC 3.4 dS m<sup>-1</sup>). Elaidy et al. (2019) further confirmed these

results, emphasizing the significant improvement in nitrogen uptake with Salt Creek rootstock compared to own roots in Superior seedless grape under soil salinity.

This can be attributed to Salt stress can disrupt N transport in grapevines by affecting water flow through the xylem, which typically carries N from the roots to the leaves and stems (Boersma *et al.*, 1993). High levels of Cl<sup>-</sup> salts in the external medium can reduce nitrate reductase activity (NRA) in the leaves, leading to decreased NO<sub>3</sub><sup>-</sup> concentration (Flores *et al.*, 2004). However, the concentration of nitrate in the roots increases (Flores *et al.*, 2000). The reduced N concentration in the leaves and stems may be due to difficulties in nutrient uptake by the roots under high salinity conditions.

#### *Phosphorus content*

Data in Table 2 showed the effect of rootstocks, salinity level and their interaction on phosphorus (P) and potassium (K<sup>+</sup>) content in petioles and roots of some grape rootstocks in 2020 and 2021 seasons.

Results showed that SO4 rootstock had the highest significant P concentration in petioles throughout all seasons, with Freedom and Ramsey rootstocks closely following in the first season. In terms of P concentration in the roots, both Freedom and Richter rootstocks consistently recorded the highest significant values in both seasons, with SO4 rootstock closely following in the first season.

The results revealed significant variations in phosphorus (P) concentration in the petioles and roots under different salinity levels in both seasons. With increasing salinity levels, there was a decrease in P concentration in the petioles but an increase in the roots. The Control exhibited the highest significant values of P concentration in the petioles but the lowest significant values in the roots in both seasons.

The interaction between rootstock and salinity level demonstrated that there were no significant differences in petioles and roots P concentration among the studied rootstocks at the same salinity level. Overall, at a salinity level of 6000 ppm, there was a reduction of 50-85% in P concentration in petioles across all rootstocks compared to the control. Various studies on plant species have produced conflicting findings on the impact of salt stress on the plants P content.

This result could be supported by Wasim (2011), who found that Flame Seedless on Ramsey rootstock exhibited the highest leaf phosphorus content with saline water irrigation (3000 and 4000 ppm). Similarly, Ahmad (2016) demonstrated an increased leaf phosphorus percentage when grafting Superior seedless grape onto Salt Creek rootstock in saline soil (EC 3.4 dS m<sup>-1</sup>). Elaidy *et al.* (2019) further confirmed these results, emphasizing the significant improvement in phosphorus uptake with Salt Creek rootstock compared to own roots in Superior seedless grape under soil salinity.

This outcome can be attributed to the importance of phosphorus as a crucial nutrient for plant growth. High osmotic pressure, caused by solutes like Na<sup>+</sup> and Cl<sup>-</sup>, can impede P transfer to petioles (Dieter Jeschke & Hartung, 2000). Additionally, salinity can decrease hydraulic conductivity, affecting the transfer of water and nutrients, including P (Steudle, 2000).

#### *Potassium content*

The findings illustrate significant disparities in the levels of potassium (K<sup>+</sup>) in both roots and petioles under different salinity levels throughout both seasons. The study revealed a decrease in K<sup>+</sup> concentration in the roots and petioles by increasing salinity level, with a more pronounced reduction observed in the roots compared to petioles. The control level exhibited the highest significant values of K<sup>+</sup> concentration in both petioles and roots.

Concerning the rootstocks, Ramsey and Richter consistently demonstrated the highest significant values for K<sup>+</sup> concentration in petioles. As for K<sup>+</sup> concentration in the roots, Ramsey rootstock recorded the highest significant values in both seasons.

Regarding the interaction between rootstock and salinity level, K<sup>+</sup> concentration in petioles, no significant difference was observed among the rootstocks at the same salinity level. However, Ramsey and Richter rootstocks demonstrated the highest K<sup>+</sup> concentration during the first season at a salinity level of 4500 ppm, with a significant difference compared to other rootstocks. In general, at a salinity level of 6000 ppm, there was a reduction of approximately 40-70% in petioles and 80% in roots compared to the control group in both seasons.

TABLE 1. Effect of rootstock, salinity level and their interaction on nitrogen content in petioles and roots of some grape rootstocks in both seasons 2020 and 2021.

Rootstock	N (% dw)					Mean	SO <sub>4</sub>		
	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>				
Salinity Level (ppm)	Leaf petioles					Roots			
	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean
<b>2020 Season</b>									
<b>Control</b>	1.41 ab	1.37 ab	1.40 ab	1.42 A	0.48 b-e	0.40 ef	0.63 a-d	0.55 b-e	0.48 B
<b>1500</b>	1.27 a-c	1.26 a-c	1.21 a-d	1.30 A	0.41 ef	0.36 ef	0.43 de	0.45 c-e	0.41 C
<b>3000</b>	1.11 a-e	1.13 a-e	0.96 b-e	1.09 B	0.63 a-d	0.51 b-e	0.75 a	0.67 ab	0.65 A
<b>4500</b>	0.93 b-e	0.97 b-e	0.85 c-e	0.88 C	0.48 b-e	0.44 c-e	0.64 a-c	0.65 a-c	0.55 B
<b>6000</b>	0.87 c-e	0.84 c-e	0.67 e	0.78 C	0.22 fg	0.17 g	0.48 b-e	0.45. c-e	0.35 C
<b>Mean</b>	1.11 A	1.11 A	1.02 A		0.44 B	0.38 B	0.55 A	0.58 A	
<b>2021 Season</b>									
<b>Control</b>	1.12 ab	1.07 a-c	1.08 ab	1.12 A	0.39 c-g	0.33 d-g	0.67 ab	0.56 a-d	0.49 A
<b>1500</b>	0.92 b-d	0.93 b-d	0.93 b-d	0.98 B	0.29 e-g	0.22 fg	0.52 a-e	0.43 b-g	0.37 B
<b>3000</b>	0.84 c-e	0.75 de	0.73 de	0.79 C	0.54 a-e	0.48 a-f	0.7 a	0.63 a-c	0.59 A
<b>4500</b>	0.77 de	0.69 de	0.73 de	0.74 CD	0.43 b-g	0.37 c-g	0.62 a-c	0.59 a-d	0.50 A
<b>6000</b>	0.66 e	0.66 e	0.65 e	0.65 D	0.22 fg	0.16 g	0.25 fg	0.18 g	0.20 C
<b>Mean</b>	0.86 A	0.83 A	0.82 A		0.37 B	0.31 B	0.55 A	0.48 A	

\*Means in each column or row with the same letter(s) are not significantly different at 5% level. Different letters indicate the differences based on Duncan's multiple range test.

†The standard for grape petiole nitrogen (N) concentration (% dry weight) ranges from 0.8 to 1.1 (Reuter & Robinson, 1997).





This result may find support in Hatami & Taimoor's study (2012), which examined the salt tolerance of grape cultivars 'Rishbaba' and 'Sahebi'. They discovered that as salinity levels increased, there was a decline in  $K^+$  concentrations in both roots and leaves, particularly in the 'Rishbaba' cultivar. The decrease in  $K^+$  concentration was more pronounced in roots compared to leaves. Ahmad (2016) reported an elevated leaf potassium percentage in Superior seedless grape when grafted onto Salt Creek rootstock in saline soil (EC 3.4 dS m<sup>-1</sup>). This observation was corroborated by Elaidy et al. (2019), who confirmed a notable increase in potassium uptake with Salt Creek rootstock compared to using own roots in Superior seedless grape under soil salinity.

This outcome can be corroborated by studies indicating that maintaining a proper  $K^+/Na^+$  balance is crucial for adaptation to saline environments (Jamali et al., 2015). High  $Na^+$  concentrations reduce  $K^+$  uptake and photosynthesis by reducing stomatal conductance (Hasanuzzaman et al., 2018). Competition from  $Na^+$  at transport sites for  $K^+$  entry into the simplest may result in  $K^+$  deficiency, and cytoplasmic  $Na^+$  competes for  $K^+$  binding sites and inhibits metabolic processes dependent on  $K^+$  (Henderson et al., 2018).

#### *Calcium content*

Data in Table 3 presented the effect of rootstocks, salinity level and their interaction on calcium ( $Ca^{2+}$ ) and magnesium ( $Mg^{2+}$ ) content in petioles and roots of some grape rootstocks in 2020 and 2021 seasons.

Regarding the rootstocks, no significant differences were found in  $Ca^{2+}$  concentration in petioles and roots among the different rootstocks in both seasons. The results reveal a significant influence of salinity on calcium ( $Ca^{2+}$ ) concentration in petioles and roots in both seasons. While  $Ca^{2+}$  concentration in leaf petioles gradually increased up to 3000 ppm, it decreased in the roots. Conversely,  $Ca^{2+}$  concentration in petioles decreased up to 6000 ppm but increased in roots. The highest significant values for  $Ca^{2+}$  concentration in the petioles were found at the 3000-ppm salinity level. However,  $Ca^{2+}$  concentration in the roots showed the highest significant values in the control, as well as at salinity levels of 3000 and 6000 ppm in both seasons.

With respect to the interaction between rootstock and salinity level, no significant differences were found in  $Ca^{2+}$  concentration in petioles and roots which was observed among the rootstocks at the same salinity level. Overall, at a salinity level of 6000 ppm,  $Ca^{2+}$  concentration in petioles decreased by approximately 35-70% compared to the control group, while  $Ca^{2+}$  concentration in the roots increased by around 15-25% relative to the control group in both seasons.

This outcome could be supported by the findings of Mehanna et al. (2010) who assessed the performance of two grapevine rootstocks, Salt Creek and 1103 Paulsen, under saline water irrigation with various soil treatments. Ramsey rootstock demonstrated superior performance, particularly in terms of root calcium content. In Ahmad (2016), it was observed that as soil salinity increased, the leaf calcium content decreased. Superior seedless grapes grafted on Salt Creek rootstock showed the highest  $Ca^{2+}$  content compared to own roots.

This result could be supported by Hadi & Karimi (2012) who pointed out that  $Ca^{2+}$  is an indispensable inorganic nutrient that maintains the structural and functional integrity of the cell wall and membrane by forming intermolecular linkages. The extent of ion accumulation in salt-treated plant leaves was dependent on cultivar and salinity level (Sivritepe et al., 2010). Fisarakis et al. (2005), found that although salinity decreased elemental absorption, it increased  $Ca^{2+}$  and  $Mg^{2+}$  ion uptake, which balanced for the increased This result could be explained by  $Na^+$  ion concentration. This is of most importance in vulnerable trees and vines that are more susceptible to damage from sodium and chloride ions (Hansen & Munns, 1988).

#### *Magnesium content*

In terms of rootstocks, Ramsey and Richter rootstocks consistently exhibited the highest significant  $Mg^{2+}$  concentration in petioles in both seasons. SO4 and Freedom rootstocks closely followed in the first and second seasons, respectively. Ramsey rootstock showed the highest significant values in roots in both seasons, with SO4 and Richter rootstocks closely following in the second season.

The findings indicate that salinity significantly influenced  $Mg^{2+}$  concentration in petioles and roots across the first and second seasons.  $Mg^{2+}$  concentration increased gradually in petioles

up to 3000 ppm. At 4500 and 6000 ppm  $Mg^{2+}$  concentration decreased in petioles, in contrast, it increased in roots. The petioles exhibited the highest significant  $Mg^{2+}$  concentration values at the salinity level of 3000 ppm, whereas the roots showed the highest significant values at the salinity level of 6000 ppm in both seasons.

Concerning the interaction between rootstock and salinity level, no significant difference was observed among the rootstocks at the same salinity level in term of petiole  $Mg^{2+}$  concentration. However, a general decline of approximately 25-50% in petiole  $Mg^{2+}$  concentration compared to the control was observed at 6000 ppm salinity. Furthermore, Ramsey and Richter rootstocks exhibited the highest  $Mg^{2+}$  concentration in roots, with a significant difference observed at the same salinity level of 6000 ppm during the first season.

This result could be reinforced by Ahmad (2016), which indicated a correlation between increasing soil salinity and a decrease in leaf  $Mg^{2+}$  content. Furthermore, when superior seedless grapes were grafted on Freedom rootstock, they exhibited the lowest  $Mg^{2+}$  content compared to their own roots. Elaidy *et al.* (2019) further confirmed these findings, highlighting the significant improvement in  $Mg^{2+}$  uptake when using Salt Creek rootstock compared to using own roots in superior seedless grape under soil salinity.

This result may be explained by the fact that transport of  $Mg^{2+}$  to leaves is affected by transpiration (Marschner, 2012), and the rate of delivered  $Mg^{2+}$  decreases under the salinity conditions due to the closure of stomata and the decrement of stomatal conductance. This, in turn, leads to an increase in the concentration of  $Mg^{2+}$  in the roots, as demonstrated in research by Koksal *et al.* (2016) Barzana *et al.* (2021) have also found that under stress conditions, there is a higher expression of  $Mg^{2+}$  transporters, which may lead to a greater accumulation of  $Mg^{2+}$  in the roots.

#### *Sodium content*

Data in Table 4 illustrated the effect of rootstock, salinity level and their interaction on Sodium ( $Na^+$ ) and chloride ( $Cl^-$ ) content in petioles and roots of some grape rootstocks in 2020 and 2021 seasons.

Concerning the rootstocks, Ramsey rootstock demonstrated the lowest significant values for  $Na^+$  concentration in petioles on the other hand, no significant differences were observed among the rootstocks in terms of  $Na^+$  concentration in the

roots in both seasons.

The presented data demonstrated a significant influence of salinity on  $Na^+$  ion concentration in petioles and roots in both seasons. Increasing salinity levels resulted in an increase in  $Na^+$  ion concentration in both roots and petioles. The control level exhibited the lowest significant values for  $Na^+$  concentration in both petioles and roots. In terms of the 1500 and 3000 ppm salinity levels, the roots displayed a significantly higher  $Na^+$  ion concentration compared to the petioles. However, this pattern varied for some rootstocks at the 4500 and 6000 ppm levels. Specifically, the Ramsey rootstock maintained a higher concentration of  $Na^+$  ions in the roots than in the petioles at the 6000-ppm salinity level.

With regard to the interaction between rootstock and salinity level, Petiole  $Na^+$  concentration, Ramsey rootstock exhibited the lowest significant value, with a significant difference observed at the salinity level of 6000 ppm during the first season. In contrast, no significant differences were observed among the rootstocks for roots  $Na^+$  concentration at the same salinity level in both seasons.

This outcome could be reinforced by Ahmad (2016) findings, which indicated that the lowest Na accumulation in the leaves of Superior Seedless was observed when it was grafted onto Salt Creek rootstock. Conversely, ungrafted Superior Seedless exhibited the highest  $Na^+$  values in saline soil (EC 3.4 dS  $m^{-1}$ ). A study by Hamrouni & Abdelly (2015) examined grafting combinations of rootstocks and scions under salinity conditions. The findings revealed that grafted varieties generally performed better or similarly to when tested individually in terms of salinity tolerance. Notably, the Muscat d'Italie/SO4 combination exhibited an increase of sodium concentration in leaves and stems, but not in roots, when subjected to NaCl treatment.

This result could be elucidated by the understanding that the exclusion of  $Na^+$  from leaf blades is crucial, especially for perennial species, as their leaves have longer lifespans and transpire for extended periods (Munns & Tester, 2008). When water is absorbed by roots, some ions travel up to the shoot through the transpiration stream. Consequently,  $Cl^-$  and  $Na^+$  accumulate in older leaves and continue to build up with the increasing concentration of these ions in the soil solution (Shani & Ben-Gal, 2005). The accumulation of  $Na^+$  within the root was found to be facilitated by the non-selective cation channels



TABLE 3. Effect of rootstock, salinity level and their interaction on calcium and magnesium content in petioles and roots of some grape rootstocks in both seasons 2020 and 2021.

Rootstocks	Ca <sup>2+</sup> (% dw)										Mg <sup>2+</sup> (% dw)										
	Leaf petioles					Root					Leaf petioles					Root					
	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	
	Season																				
	2020										2021										
<b>Control</b>	0.95 e-g	0.92 e-g	0.85 f-h	1.03 d-g	0.94 C	0.81 e	0.97 a-c	1.00 a-c	0.96 b-d	0.94 A	0.62 c-g	0.57 d-h	0.66 b-e	0.57 d-h	0.61 C	0.10 g	0.10 g	0.10 g	0.18 e-g	0.14 fg	0.13 C
<b>1500</b>	1.12 b-f	1.05 c-g	1.32 ab	1.23 a-d	1.18 B	0.71 c-e	0.71 c-e	0.87 a-d	0.85 a-d	0.78 B	0.64 c-f	0.58 d-h	0.81 ab	0.73 a-d	0.69 B	0.13 fg	0.19 e-g	0.19 e-g	0.28 c-f	0.17 e-g	0.19 BC
<b>3000</b>	1.31 a-c	1.12 a-c	1.39 a	1.37 a	1.31 A	0.64 a-d	0.60 de	0.52 e	0.47 e	0.56 C	0.85 a	0.74 a-c	0.82 ab	0.81 ab	0.81 A	0.15 fg	0.23 d-g	0.18 e-g	0.38 a-d	0.18 e-g	0.23 B
<b>4500</b>	0.8 gh	0.96 e-g	0.96 e-g	1.01 d-g	0.93 C	0.99 a-c	0.89 a-d	1.09 ab	1.17 a	1.04 A	0.47 g-j	0.49 f-j	0.54 e-i	0.63 c-g	0.53 D	0.17 e-g	0.18 e-g	0.46 ab	0.4a bc	0.32 bc	0.30 A
<b>6000</b>	0.63 hi	0.61 hi	0.47 ij	0.31 j	0.50 D	1.07 ab	0.88 a-d	1.16 ab	1.15 ab	1.06 A	0.38 j	0.40 ij	0.44 h-j	0.50 f-j	0.43 E	0.24 d-g	0.15 fg	0.52 a	0.52 a	0.32 b-e	0.31 A
<b>Mean</b>	0.96 A	0.94 A	1.00 A	0.99 A	0.84 A	0.81 A	0.93 A	0.92 A	0.59 AB	0.55 B	0.65 A	0.65 A	0.16 C	0.17 C	0.37 A	0.24 B					
	2021 Season																				
<b>Control</b>	0.93 b-f	0.68 e-h	0.93 b-f	0.76 d-h	0.83 C	0.57 a-c	0.49 a-d	0.61 ab	0.55 a-c	0.56 A	0.51 d-g	0.61 a-e	0.60 b-e	0.55 c-f	0.57 C	0.10 e-g	0.04 g	0.05 fg	0.10 e-g	0.04 g	0.06 D
<b>1500</b>	0.96 b-e	1.01 a-d	1.12 a-c	1.20 ab	1.07 B	0.37 b-d	0.37 b-d	0.40 b-d	0.44 b-d	0.40 B	0.58 b-f	0.65 a-d	0.63 a-d	0.68 a-c	0.63 B	0.12 d-g	0.07 fg	0.10 e-g	0.10 e-g	0.10 e-g	0.10 CD
<b>3000</b>	1.27 a	1.19 ab	1.28 a	1.32 a	1.26 A	0.24 d	0.25 d	0.32 cd	0.37 b-d	0.30 B	0.65 a-d	0.74 a	0.72 ab	0.75 a	0.72 A	0.18 c-f	0.13 d-g	0.12 d-g	0.13 d-g	0.13 d-g	0.14 C
<b>4500</b>	0.75 d-h	0.64 gh	0.87 c-g	0.75 d-h	0.75 C	0.53 a-c	0.57 a-c	0.71 a	0.52 a-c	0.58 A	0.48 e-g	0.44 fg	0.59 b-e	0.54 c-f	0.51 C	0.22 b-e	0.24 b-d	0.22 b-e	0.23 b-e	0.23 b-e	0.23 B
<b>6000</b>	0.48 h	0.67 fgh	0.57 h	0.69 e-h	0.60 D	0.59 ab	0.61 ab	0.71 a	0.73 a	0.66 A	0.24 i	0.38 gh	0.30 hi	0.30 hi	0.31 D	0.30 bc	0.10 e-g	0.32 ab	0.43 a	0.43 a	0.28 A
<b>Mean</b>	0.88 A	0.84 A	0.96 A	0.94 A	0.84 A	0.46 A	0.46 A	0.52 A	0.55 A	0.49 B	0.56 A	0.56 A	0.57 A	0.56 A	0.19 A	0.12 B	0.16 AB	0.19 A	0.19 A	0.19 A	

\*Means in each column or row with the same letter(s) are not significantly different at 5% level. Different letters indicate the differences based on Duncan's multiple range test.

†The standard for grape petiole calcium (Ca<sup>2+</sup>) concentration (% dry weight) ranges from 1.2 to 2.5. Additionally, the standard for grape petiole magnesium (Mg<sup>2+</sup>) concentration (% dry weight) is above 0.40, and deficiency is indicated when the concentration falls below 0.3 (Reuter & Robinson, 1997).

TABLE 4. Effect of rootstock, salinity level and their interaction on sodium, and chloride content in petioles and roots of some grape rootstocks in both seasons 2020 and 2021.

Rootstocks	Na <sup>+</sup> (% dw)										Cl <sup>-</sup> (% dw)									
	Leaf petioles					Root					Leaf petioles					Root				
	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean
	Season																			
	2020										2021									
<b>Control</b>	0.04 g	0.11 g	0.12 g	0.08 g	0.09 D	0.07 h	0.08 h	0.17	0.24	0.14 E	0.09 e	0.09 e	0.09 e	0.09 e	0.09 E	0.14 g	0.14 g	0.14 g	0.16 g	0.16 g
								gh	f-h											
<b>1500</b>	0.76 ef	0.91 d-f	0.67 fg	1.22 b-f	0.90 C	0.67 d-g	0.65 d-g	0.83	0.53	0.67 D	0.69 d	0.97 d	0.83	0.80 d	0.90 D	0.76 f	1.18 ef	1.18 ef	1.40 de	1.13 ef
								c-e	e-h											
<b>3000</b>	1.31 b-f	1.09 d-f	1.17 c-f	1.32 b-f	1.22 B	1.26 bc	1.51 b	1.24 bc	0.76	1.19 C	0.90 d	0.94 d	0.76	1.58 c	1.07 C	0.83 f	1.28 de	1.28 de	1.35 de	1.16 ef
								c-f	c-f											
<b>4500</b>	1.25 b-f	1.10 d-f	1.27 b-f	1.86 b	1.37 B	1.18 b-d	1.63 b	1.54 b	1.66 b	1.50 B	1.54 c	1.80 c	1.61 c	1.77 c	1.68 B	1.16 ef	1.89 a-c	1.89 a-c	2.18 a	1.84 a-c
<b>6000</b>	1.83 bc	2.95 a	1.38 b-c	1.51 b-d	1.92 A	2.69 a	2.55 a	2.84 a	2.80 a	2.72 A	2.27 b	2.72 a	2.41 ab	1.39 c	2.20 A	2.04 ab	1.89 a-c	1.89 a-c	1.63 b-d	1.56 c-e
<b>Mean</b>	1.04 AB	1.23 A	0.92 B	1.20 A	1.17 A	1.17 A	1.28 A	1.34 A	1.18 A	1.10 B	1.10 B	1.30 A	1.20 AB	1.13 AB	0.98 B	1.28 A	1.34 A	1.34 A	1.17 A	1.17 A
	2021 Season																			
<b>Control</b>	0.08 g	0.08 g	0.19 fg	0.28 c-g	0.16 D	0.45 d	0.50 cd	0.73	0.45 cd	0.45 C	0.12 g	0.09 g	0.12 g	0.19 fg	0.13 D	0.14 g	0.14 g	0.14 g	0.12 g	0.19 g
								a-d	a-d											
<b>1500</b>	0.37 d-g	0.67 b-f	0.65 b-f	0.44 c-g	0.53 C	0.31 d	0.80 a-d	0.82	0.31 d	0.56 B	0.38 d-g	0.38 d-g	0.26	0.85 b-e	0.47 C	0.5 fg	0.78 c-f	0.78 c-f	0.52 fg	0.59 e-g
								a-d	a-d											
<b>3000</b>	0.57 b-g	0.79 b-e	0.72 b-e	0.83 b-d	0.73 BC	0.74 a-d	0.69 b-d	0.73	0.75 ad	0.73 B	0.64 c-g	0.71 c-g	0.85	0.57 c-g	BC	0.64 d-g	1.25 a-c	1.25 a-c	1.11 b-e	1.33 ab
								a-d	a-d											
<b>4500</b>	0.7b c-f	1.07 b	0.66 b-f	0.96 bc	0.85 B	0.73 a-d	1.43 a	1.31 ab	1.30 ab	1.19 A	0.97 b-d	1.11 a-c	0.83	0.78 c-f	0.92 B	0.64 d-g	1.12 b-d	1.12 b-d	1.39 ab	1.25 a-c
<b>6000</b>	2.06 a	2.39 a	0.86 b-d	1.94 a	1.81 A	1.37 ab	1.17 a-c	1.35 ab	1.42 a	1.33 A	0.78 c-f	1.58 a	1.09	1.42 ab	1.22 A	1.06 b-e	1.42 ab	1.42 ab	1.70 a	1.14 b-d
								a-c	a-c											
<b>Mean</b>	0.75 BC	1.00 A	0.62 C	0.90 AB	0.72 A	0.72 A	0.93 A	0.99 A	0.85 A	0.58 A	0.58 A	0.77 A	0.63 A	0.76 A	0.60 B	0.60 B	0.95 A	0.95 A	0.97 A	0.9 A

\*Means in each column or row with the same letter(s) are not significantly different at 5% level. Different letters indicate the differences based on Duncan's multiple range test.

†Grape petiole sodium (Na<sup>+</sup>) concentration (% dry weight) is considered toxic when it exceeds 0.5, while chloride (Cl<sup>-</sup>) concentration is considered toxic when it exceeds 0.1 (Reuter & Robinson, 1997).

(NSCC) were identified as the major route for  $\text{Na}^+$  uptake into the root. In addition, a bypass flow pathway was observed, which occurs when  $\text{Na}^+$  leaks into the root via the apoplast, the space between the plasma membrane and the cell wall (Taiz & Zeiger, 2002).

#### *Chloride content*

Concerning the rootstocks, no significant difference was observed among the rootstocks in terms of petiole  $\text{Cl}^-$  concentration at the same salinity level across all seasons. However, the SO4 rootstock consistently exhibited the lowest significant values for  $\text{Cl}^-$  concentration in roots in both seasons.

The data indicated a significant impact of salinity on  $\text{Cl}^-$  ion concentration in both seasons. Increasing salinity levels resulted in an increase in  $\text{Cl}^-$  concentration in both petioles and roots. The control level exhibited the lowest significant values for  $\text{Cl}^-$  concentration in both petioles and roots. At the 1500, 3000, and 4500 ppm salinity levels, the roots displayed a significantly higher  $\text{Cl}^-$  concentration compared to the petioles, while this pattern varied at the 6000-ppm level. Specifically, the Ramsey rootstock maintained a higher concentration of  $\text{Cl}^-$  in the roots than in the petioles at the 6000-ppm salinity level.

In respect of the interaction between rootstock and salinity level, petiole  $\text{Cl}^-$  concentration, no significant difference was observed among the rootstocks at the same salinity level in all seasons. In contrast, the SO4 rootstock displayed the lowest  $\text{Cl}^-$  concentration in roots, with a significant difference observed at the salinity level of 6000 ppm during the first season.

This result could be corroborated by Martin et al. (2020), who evaluated the physiology of Malbec vines by comparing four *V. vinifera* rootstocks under varying levels of NaCl exposure for 65 days. It was observed that both mild and high salinity levels resulted in an accumulation of  $\text{Cl}^-$  in both petioles and roots. In a study conducted by Sharma et al. (2011), the susceptibility of Thompson Seedless (*Vitis vinifera* L.) vines grafted on four different rootstocks (DogRidge, Salt Creek, B2-56, and 1613C) to saline irrigation water with high sodium ( $\text{Na}^+$ ) content was evaluated. It was observed that the Ramsey rootstock exhibited effective chloride exclusion based on the concentration in the leaf blade.

This result could be clarified by the role of  $\text{Cl}^-$  ions in regulating turgor pressure, pH,

and enzyme activities in the cytoplasm as they are considered an essential micronutrient that regulates enzyme activities as well as an essential co-factor in photosynthesis (Dang et al., 2008). However, the accumulation of both  $\text{Na}^+$  and  $\text{Cl}^-$  at high concentrations in the cytoplasm can be metabolically toxic to plants (White & Broadley, 2001). Furthermore, some studies have shown that  $\text{Cl}^-$  is more dangerous than  $\text{Na}^+$  (Tavakkoli et al., 2011).

#### *Proline (Pro)*

Data in Table 5 showed the effect of rootstock, salinity level and their interaction on enzyme activity, and proline content of some grape rootstocks in 2020 and 2021 seasons.

Regarding rootstocks, Ramsey and SO4 exhibited the highest significant Pro content values during the first season, while Ramsey rootstock showed the highest significant values in roots in both seasons.

The results indicated a significant influence of salinity on Pro content in leaves and roots in both seasons. Increasing salinity levels led to an increase in Pro content in both leaves and roots. The increase was more pronounced gradually in roots up to 3000 ppm, but the pattern differed at salinity levels of 4500 and 6000 ppm. The highest significant values for Pro content in leaves were observed at the 6000-ppm salinity level.

The interaction between rootstock and salinity level, no significant differences observed among the rootstocks in terms of leaves Pro content at the same salinity level across all seasons. However, in roots Pro content, Ramsey rootstock displayed the highest values of Pro content with significant differences noted at salinity levels of 1500 and 4500 ppm in the first season, and at the salinity level of 3000 ppm in the second season.

This result could be substantiated by the study conducted by Loay et al. (2021), who evaluated the sensitivity of 'Flame Seedless' vines to soil salinity while being grown on four different rootstocks ('Self-grafted', 'Freedom', '140 Ruggeri', and '1103 Paulsen') throughout the berry development stages. They discovered that the lowest proline content was observed in 'Self-grafted' vines, followed by the Freedom rootstock. Additionally, Ahmad (2016) indicated that there is a positive correlation between the capacity of grape rootstocks to accumulate proline and salt levels. However, there may be differences between Ahmad's results and our own findings where it was

found that Superior Seedless grapes grafted on Salt Creek rootstock had the lowest proline percentage compared to Superior Seedless grapes grafted on Freedom rootstock, which recorded the highest proline percentage. This result could be explained by the versatile amino acid proline, which serves multiple functions such as osmolyte activity, Reactive oxygen species scavenging, and protein structure stabilization. These functions help protect cells from stress-induced damage (Abdul Qados, 2011). The levels of proline are regulated by the equilibrium between biosynthesis and catabolism, with abiotic stress conditions promoting proline biosynthesis, while its breakdown is stimulated during the recovery phase from stress (Krasensky & Jonak, 2012). In grapevine tissues, salinity-induced ROS initiate processes that lead to specific Glutamate dehydrogenase (GDH) subunit expression and promote glutamate production for proline synthesis (Skopelitis *et al.*, 2006).

#### *Peroxidase (POD)*

Regarding rootstocks, Ramsey rootstock consistently displayed the highest significant values of Peroxidase activity in both seasons, followed closely by Richter without any significant difference in the second season.

The results indicate a significant influence of salinity on Peroxidase activity in both seasons of the study. Increasing salinity levels resulted in an increase in Peroxidase activity. The highest significant values for Peroxidase activity were observed at 1500-ppm in both seasons, followed closely by 3000-ppm in the second season only.

The interaction between rootstock and salinity level revealed that in the first season, Ramsey rootstock exhibited the highest peroxidase activity, with significant differences observed at salinity levels of 1500 and 4500 ppm compared to other rootstock at the same salinity level. Richter rootstock closely followed Ramsey, although without any significant difference. In the second season, Ramsey rootstock showed the highest peroxidase activity at the 1500-ppm salinity level only.

The aforementioned outcome could find support in the work of Ahmad (2016), who found a positive correlation between grape rootstocks' capacity (1103 Paulsen, Salt Creek and Freedom) to enhance peroxidase activity under saline conditions, but no significant variations were observed among the different rootstocks. Loay *et al.* (2021) evaluated the sensitivity of 'Flame Seedless' vines to soil

salinity on four different rootstocks ('Self-grafted', 'Freedom', '140 Ruggeri', and '1103 Paulsen') during berry development. The lowest peroxidase activity was observed in 'Self-grafted' vines, followed by the Freedom rootstock.

This result could be elucidated by the fact that under salinity stress conditions, peroxidases play a role in detoxifying reactive oxygen species (ROS) produced by the plant as a response to stress (Basu *et al.*, 2017). They act by catalyzing the conversion of harmful hydrogen peroxide into water and oxygen, thereby reducing oxidative damage to cells (Skopelitis *et al.*, 2006).

#### *Polyphenol oxidase (PPO)*

Regarding rootstocks, Ramsey consistently exhibited the highest significant values of PPO activity in both seasons.

The results demonstrate a significant impact of salinity on Polyphenol oxidase (PPO) activity in both seasons of the study. Increasing salinity levels led to an increase in PPO activity compared to the control. The highest significant values for PPO activity were observed at the 1500-ppm salinity level.

The interaction between rootstock and salinity level showed that Ramsey rootstock displayed the highest PPO activity, with significant differences noted at salinity levels of 3000 and 6000 ppm in the first season compared to other rootstock at the same salinity level, and at the 4500-ppm salinity level in the second season.

This finding could be reinforced by Ahmad (2016), who discovered that there is a positive connection between the ability of grape rootstocks to increase polyphenol oxidase activity in the presence of salinity. However, there were no significant differences observed among the various rootstocks. Somkuwar *et al.* (2021) studied stress-relieving enzymatic activities during bud sprouting in 'Red Globe' grapevines on different rootstocks in arid conditions. The results showed that Richter 110 rootstock exhibited an increase in polyphenol oxidase (PPO) activity, resulting in early and increased bud sprouting.

This result could be clarified by the fact that the increase in PPO activity can be seen as a defense mechanism of the plant to counteract the harmful effects of ROS, where PPO helps to detoxify the ROS and reduce oxidative damage to plant cells (Hasanuzzaman *et al.*, 2021). Furthermore, the increase in PPO activity may also be associated

with the plants attempt to strengthen its cell walls. Phenolic compounds produced by PPO can participate in the cross-linking of cell wall components, providing structural support and increasing the rigidity of the cell walls. This reinforcement of cell walls can enhance the plant's resistance to osmotic stress caused by high salt concentrations (Boeckx et al., 2015).

#### *Chlorophyll content*

Data in Table 6 showed the effect of rootstock, salinity level and their interaction on chlorophyll content and leaf relative water content (LRWC) of some grape rootstocks in the 2020 and 2021 .

Regarding the rootstocks, no significant differences were found in Chlorophyll a (Chl a) and chlorophyll b (Chl b) in both seasons.

The results demonstrate significant variations in salinity levels on Chlorophyll (Chl a) and (Chl b) in both seasons. As salinity levels increased, there was a decrease in Chlorophyll (Chl a) and (Chl b). The control level exhibited the highest significant values of Chlorophyll (Chl a) and (Chl b). Regarding the interaction between rootstock and salinity level, there are no significant differences observed among the rootstocks at the same salinity levels in both seasons.

This finding aligns with the results obtained by Rezazad Bari et al. (2021) who investigated 21 grapevine rootstocks under salinity conditions. They measured 19 parameters and employed chemometrics methods, including principal component analysis (PCA) and quadratic discriminant analysis (QDA), to determine the significant characteristics for distinguishing the rootstocks. It was observed that characteristics associated with chlorophyll a and chlorophyll b achieved a perfect 100% discrimination rate. Loay et al. (2021) investigated the impact of soil salinity on 'Flame Seedless' vines during berry development, utilizing four different rootstocks ('Self-grafted', 'Freedom', '140 Ruggeri', and '1103 Paulsen'). The analysis revealed that 'Self-grafted' vines exhibited the lowest chlorophyll a and b content, with a subsequent increase observed in the Freedom rootstock.

This result could be explained by understanding that Several factors contribute to the decrease in chlorophyll A and B concentration in grapes under salinity conditions. High salinity disrupts ion balance and osmotic potential in plant cells, damaging cellular structures and impairing chlorophyll synthesis

(Mbarki et al., 2018). Salinity-induced oxidative stress leads to the accumulation of reactive oxygen species, which directly damage chlorophyll molecules and inhibit their production (Hasanuzzaman et al., 2021).

#### *Leaf relative water content (LRWC)*

Regarding the rootstocks, no significant differences were found in LRWC in both seasons. The results demonstrated significant variations in LRWC due to salinity levels during both seasons. As salinity levels increased, there was a decrease in LRWC. The control exhibited the highest significant values of LRWC.

The interaction between rootstock and salinity level indicated that insignificant differences were observed among the rootstocks at the same salinity level in both seasons.

This finding is consistent with the results obtained by Edriss et al. (2016) who studied the response of grape rootstocks (Salt Creek, Freedom, Dogridge, and Richter) to increasing salt stress in a culture medium. The study observed a decrease in relative water content (RWC) in all rootstocks under stress. Salt Creek rootstock exhibited the highest RWC values, followed by Freedom, compared to the other two rootstocks. Jamali et al. (2015) conducted experiments exposing ten grape rootstocks to separate moisture and salinity stresses. Physiological and biochemical parameters were monitored throughout the stress cycle. Relative water content (RWC) decreased in all rootstocks under stress, and the highest RWC was observed in the Richter rootstock.

The clarification of this result can be attributed to the fact that under saline conditions, the decrease in relative water content, as observed in our investigation, can be attributed to various factors. Firstly, the presence of high salt concentration in the soil disrupts the water potential gradient, leading to reduced water uptake by the plant roots and hampering overall water absorption and availability to the plant, thus resulting in decreased relative water content (Dajic, 2006). Additionally, salt stress induces water loss through transpiration as the plant strives to maintain its water balance (Taiz & Zeiger, 2002).

#### **Conclusion**

Ramsey rootstock surpassed other rootstocks under investigation in terms of its ability to withstand irrigation with saline water. This superior capability can be attributed to a range of mechanisms, notably the roots exhibiting significantly higher concentrations of nitrogen,



TABLE 5. Effect of rootstock, salinity level and their interaction on proline content and enzyme activity of some grape rootstocks in both seasons 2020 and 2021.

Rootstock Salinity Levels (ppm)	Proline content (mmol. g <sup>-1</sup> FW)										Enzyme activity										
	Leaf					Roots					Peroxidase (POD) (Unit.mg <sup>-1</sup> protein min <sup>-1</sup> )					Polyphenol oxidase (PPO) (Unit.mg <sup>-1</sup> protein)					
	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsey	Richter	Mean	
<b>2020 Season</b>																					
Control	1.20 ef	1.46 ef	1.40 ef	0.99 f	1.46 E	0.79 hi	1.11 hi	1.11 hi	1.84 g-i	1.21 C	5.34 lm	3.36 m	6.79 k-m	12.91 jk	7.10 D	2.21 k	6.48 h-j	3.76 jk	4.64 i-k	4.27 E	
1500	3.39 d-f	3.36 d-f	4.90 b-e	1.91 ef	3.39 C	5.02 c-e	8.16 ab	8.16 ab	4.06 c-g	5.73 A	25.14 e-h	38.94 bc	44.56 ab	49.36 a	39.50 A	19.60 b	24.15 a	23.01 a	15.58 cd	20.59 A	
3000	6.89 a-c	4.52 c-e	6.39 a-d	2.45 ef	5.06 B	9.42 a	6.57 bc	6.57 bc	2.87 e-h	6.29 A	33.11 cd	20.68 g-i	37.37 c	21.6f g-i	28.19 B	12.34 ef	14.33 de	18.43 bc	9.62 f-h	13.68 B	
4500	7.13 a-c	6.01 a-d	8.72 a	8.08 ab	7.48 A	4.52 c-f	5.87 bc	5.87 bc	0.75 hi	3.84 B	17.49 ij	16.88 ij	29.94 de	19.20 h-j	20.88 C	7.69 g-i	9.51 f-h	11.15 e-g	8.11 g-i	9.12 C	
6000	7.57 a-c	7.31 a-c	9.38 a	8.19 ab	8.11 A	3.24 d-h	3.08 e-h	3.08 e-h	0.11 i	2.17 C	28.16 d-f	12.32 j-l	27.55 d-g	12.13 j-l	20.04 C	5.72 ij	5.42 i-k	10.96 fg	5.48 i-k	6.90 D	
Mean	5.40 AB	4.53 B	6.16 A	4.32 B	7.48 A	4.60 AB	4.96 A	4.96 A	1.93 C	21.85 B	18.44 C	29.24 A	23.04 B	9.51 C	11.98 B	13.46 A	8.69 C	7.04 n	2.38 n	4.29 E	
<b>2021 Season</b>																					
Control	1.29 fg	1.23 fg	1.61 fg	1.07 g	1.30 D	1.09 k	0.43 k	1.38 jk	0.60 k	0.87 E	11.72 ij	10.57 ij	4.52 j	11.55 ij	9.59 D	3.41 mn	4.32 m	7.04 l	2.38 n	4.29 E	
1500	3.78 e-g	4.16 d-g	6.53 c-e	5.78 c-g	5.06 C	9.37 c-e	11.04 bc	12.24 b	7.73 d-g	10.10 B	43.80 c-e	50.86 c	80.25 a	52.85 bc	56.94 A	23.21 b	19.16 c	26.67 a	17.17 d	21.55 A	
3000	6.48 c-e	6.00 c-f	10.43 bc	5.63 c-g	7.14 C	8.52 c-g	12.06 b	15.06 a	9.63 cd	11.32 A	52.13 bc	45.83 cd	46.77 cd	63.49 b	52.06 A	15.72 ef	13.24 g-i	16.41 d	14.44 f-h	14.95 B	
4500	8.672 b-d	9.02 b-d	12.53 ab	8.35 b-e	9.64 B	8.8 c-f	6.42 f-h	7.34 dg	8.11 dg	7.67 C	35.47 d-f	28.86 f-h	30.20 f-h	33.34 e-g	31.97 B	13.87 g-i	9.33 k	14.58 fg	11.09 j	12.21 C	
6000	15.85 a	10.35 bc	15.55 a	11.98 ab	13.43 A	6.80 e-g	3.56 ij	6.04 gh	4.17 hi	5.14 D	5.34 lm	3.36 m	6.79 k-m	12.91 jk	7.10 D	2.21 k	6.48 h-j	3.76 jk	4.64 i-k	4.27 E	
Mean	7.21 B	6.21 B	9.30 A	6.56 B	9.30 A	6.92 B	6.70 B	8.41 A	6.05 B	33.21 AB	30.92 B	36.71 A	36.82 A	13.86 B	11.11 C	15.44 A	10.42 D	7.04 n	2.38 n	4.29 E	

\*Means in each column or row with the same letter(s) are not significantly different at 5% level. Different letters indicate the differences based on Duncan's multiple range test.

TABLE 6. Effect of rootstock, salinity level and their interaction on Chlorophyll content and leaf relative water content (LRWC) of some grape rootstocks in both seasons 2020 and 2021.

Rootstock	Leaf photosynthetic pigments										Leaf relative water content (LRWC) (%)									
	Chlorophyll a (mg/g <sup>-1</sup> FW)					Chlorophyll b (mg/g <sup>-1</sup> FW)					Chlorophyll a+b (mg/g <sup>-1</sup> FW)									
	SO <sub>4</sub>	Freedom	Ramsay	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsay	Richter	Mean	SO <sub>4</sub>	Freedom	Ramsay	Richter	Mean					
	<b>2020 Season</b>																			
<b>Control</b>	6.03 a	5.58 ab	5.50 ab	4.55 a-d	5.40 A	1.97 a	1.99 a	1.70 ab	1.33 a-d	1.75 A	8.00 a	7.57 ab	7.20 ab	5.87 b-d	7.16 A	92.5 ab	95.3 a	95.6 a	96.4 a	94.9 A
<b>1500</b>	4.58 a-d	4.05 a-e	4.86 a-c	4.08 a-e	4.40 B	1.24 a-e	1.06 b-e	1.49 a-c	0.92 b-e	1.18 B	5.82 b-d	5.11 c-e	6.35 a-c	5.01 c-e	5.57 B	76.8 cd	77.3 cd	67.6 de	83.1 bc	76.2 B
<b>3000</b>	3.60 b-f	3.38 b-g	4.29 a-e	3.46 b-g	3.68 C	1.07 b-e	0.91 b-e	0.67 c-e	0.78 c-e	0.86 BC	4.67 c-f	4.29 c-g	4.96 c-e	4.24 d-g	4.54 C	71.3 de	72.3 c-e	64.8 ef	64.2 e-g	68.2 C
<b>4500</b>	1.92 e-g	3.06 c-g	2.28 d-g	2.59 c-g	2.46 D	0.87 c-e	0.67 c-d	0.97 b-e	0.60 de	0.78 C	2.79 f-h	3.73 e-h	3.25 e-h	3.20 e-h	3.24 D	55.5 f-h	47.3 h-k	53.8 g-i	52.3 hi	52.2 D
<b>6000</b>	1.45 fg	1.94 e-g	1.96 e-g	1.26 g	1.65 E	0.60 de	0.51 de	0.61 de	0.46 e	0.54 C	2.05 h	2.45 gh	2.57 gh	1.72 h	2.20 E	42.7 i-k	40.3 jk	36.1 k	48.1 h-j	41.8 E
<b>Mean</b>	3.52 A	3.60 A	3.78 A	3.19 A	3.19 A	1.15 A	1.03 A	1.09 A	0.82 A	0.82 A	4.66 A	4.63 A	4.86 A	4.01 A	4.01 A	67.7 AB	66.5 AB	63.6 B	68.8 A	68.8 A
	<b>2021 Season</b>																			
<b>Control</b>	3.10 b	2.78 bc	2.87 bc	3.59 a	3.08 A	2.62 b	2.23 bc	3.67 a	2.47 bc	2.74 A	5.72 bc	5.01 de	6.53 a	6.05 ab	5.83 A	96.8 a	94.5 ab	96.5 a	95.9 a	95.9 A
<b>1500</b>	2.73 bc	2.40 c-e	2.80 bc	2.47 cd	2.60 B	2.17 bc	2.09 c	2.50 bc	2.09 c	2.21 B	4.90 de	4.48 e	5.30 cd	4.56 e	4.81 B	83.3 bc	79.3 cd	83.2 bc	80.2 cd	81.5 B
<b>3000</b>	1.99 d-g	2.15 d-f	1.82 e-h	1.94 d-g	1.97 C	1.13 de	1.46 d	1.40 d	1.03 d-f	1.26 C	3.12 fg	3.61 f	3.22 fg	2.97 gh	3.23 C	75.5 c-e	73.7 e-e	60.4 fg	79.3 cd	72.2 C
<b>4500</b>	1.35 g-j	1.70 f-i	1.56 f-j	1.01 j	1.41 D	0.80 e-g	1.04 d-f	0.89 e-g	0.72 e-g	0.86 D	2.12 ij	2.75 gh	2.45 hi	1.73 jk	2.27 D	64.2 e-g	65.2 e-g	65.6 e-g	68.7 d-f	65.9 D
<b>6000</b>	1.10 ij	1.20 h-j	1.33 g-j	0.96 j	1.15 E	0.51 g	0.66 e-g	0.59 fg	0.58 fg	0.58 E	1.61 jk	1.86 jk	1.92 i-k	1.56 k	1.74 E	52.9 gh	45.3 h	42.2 h	52.9 gh	48.3 E
<b>Mean</b>	2.08 A	2.05 A	2.05 A	2.00 A	2.00 A	1.40 B	1.50 B	1.81 A	1.38 B	1.38 B	3.50 B	3.54 B	3.88 A	3.37 B	3.37 B	74.5 AB	71.6 AB	69.6 B	75.4 A	75.4 A

\*Means in each column or row with the same letter(s) are not significantly different at 5% level. Different letters indicate the differences based on Duncan's multiple range test.

potassium, magnesium, and proline. Furthermore, Ramsey rootstock effectively restricts the movement of harmful ions towards the leaves, as evidenced by low sodium concentration in the petioles and a high chloride concentration in the roots. Moreover, Ramsey rootstock exhibits the most significant activity of peroxidase and polyphenol oxidase enzymes, thereby greatly enhancing its tolerance to elevated levels of salinity. Based on these findings, it is recommended that when cultivating grapes in regions where the irrigation water contains salts ranged from 1500 to 3000 ppm, utilizing a symbolic root for grafting different varieties onto Ramsey rootstock is advisable for optimal results.

#### Acknowledgement

The authors express their gratitude to all individuals who contributed to the completion of this paper.

#### Funding statement

The authors declare that no external funding was received for this study.

#### Conflict of interest

The authors have stated that they have no conflicts of interest to disclose.

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## الاستجابات الفسيولوجية والبيوكيميائية لبعض أصول العنب للإجهاد الملحي

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يعد استخدام أصول العنب الجذرية من الطرق الفعالة لتعزيز المقاومة للإجهادات الحيوية وغير الحيوية. أجريت تجربة أصص خلال مواسم 2020 و2021 لدراسة مدى تحمل عقل متجنزة لأصول العنب المتاحة تجاريًا والنامية في تربة رملية مدعومة بإضافة الكمبوست للإجهاد الملحي مع دراسة بعض الاستجابات الفسيولوجية والبيوكيميائية المرتبطة بتحمل الإجهاد الملحي. وشملت التجربة خمس مستويات من الري الملحي (كنترول، 1500، 3000، 4500، و6000 جزء في المليون) وأربع أصول (فريدوم، رمزي، ريختر، SO<sub>4</sub>) في تجربة عاملية. أظهرت النتائج أن أصل رمزي تفوق على الأصول الأخرى، حيث أظهر أعلى تركيز معنوي للنيتروجين والبوتاسيوم والمغنيسيوم والبرولين في الجذور، مع أقل تركيز معنوي للصدويوم في الأعناق. تقدم النتائج رؤى قيمة حول زراعة العنب في ظروف الملوحة مع تركيز مياه الري الذي يتراوح من 1500 إلى 3000 جزء في المليون.