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Determination of morphological responses and plant nutrient preferences of some vine rootstocks grown under *in vitro* salt stress conditions

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Abstract

The study was performed to determine mineral nutrition preferences and the morphological response against the salt stress of the rootstocks used in Turkey. 41B, 5BB, 140Ru, Salt Creek, and SO4 were used as rootstocks, and NaCl at concentrations of 0 (control), 0.75, and 1.5 g L^{-1} were applied to the plantlets grown in MS medium. The values of all shoot and root properties examined in this experiment decreased with increasing NaCl concentrations compared to control plants. The highest damage degree was seen on 41B, while there was no damage on Salt Creek plantlets. Shoot and root tolerance ratios of Salt Creek rootstock were found to be the best among the rootstock. These ratios were higher in 0.75 g L^{-1} than 1.5 g L^{-1} concentration. Leaf chlorophyll and nutrient content were negatively affected by the increasing NaCl doses. It has been found that all nutrient elements are positively affected by each other's uptake. The highest N, K, Ca, and Mg levels were detected in Salt Creek, while the lowest level was detected in 41B rootstock. Considering all the parameters examined, rootstocks are ranged from the most sensitive to the most resistant to salinity conditions; 41B, SO4, 5BB, 140Ru, and Salt Creek.

Keywords: Chlorophyll content, Grapevine, NaCl, Plant nutrition, Tissue culture

Introduction

Vine (*Vitis vinifera* L.) is grown latitude between 11-53º in the northern hemisphere and 20-40º in the southern hemisphere (Çelik, 2011). There are some biotic and abiotic factors affecting grapevine cultivation in Turkey, where is among the ideal growing area for viticulture (Mahajan and Tuteja, 2005; Kacar et al., 2006).

Salinity is the most important abiotic stress factor, especially in arid and semi-arid ecologies (Boscaiu et al., 2008; Edriss et al., 2016; Mohammadkhani and Abbaspour, 2018; Haider et al., 2019; Lo'ay and El-Ezz, 2021). The salinity problem in Turkey, as well as in many countries, is growing day by day. It is stated that this is caused mainly by improper irrigation and excessive fertilization, and lack of drainage (Zhani et al., 2012; Patil et al., 2020). Salt stress prevents growth depending on tolerance and can lead to chlorosis and necrotic spots. In addition, weight loss, stunting in both root and stem, and decreasing plant stem and root length can be seen (Fozouni et al., 2012b; Dag et al., 2015).

Salt is an important factor limiting growth in grapevines, as in all plants (Upadhyay et al., 2018; Barakat et al., 2019). Vine can absorb 1-6% of the salt in the soil (Storey et al., 2003; Munns, 2005). Vine development and yield decreased in a salt concentration above 2.5 ds m^{-1} (1.6 g L^{-1}), and when it reaches EC 6.7 ds m^{-1} (4.29 g L^{-1}), deaths can be seen in the vine (Battany, 2004; Bakır, 2012). The sensitivity of American grapevine rootstocks used for combat pests such as phylloxera and nematodes is higher. It is known that rootstocks are more susceptible to adverse soil conditions such as drought and calcareous besides salinity than *V. vinifera* (Patil et al., 2020). The role of grapevine rootstocks in nutrient uptake is also important, and their effects on the growth and yield of cuttings are diverse (Tangolar and Ergenoğlu, 1989).

Various studies have shown differences in salt tolerance between American species and *V. vinifera* varieties (Müftüoğlu et al., 2006). It is stated that some of the rootstocks are tolerant to salinity due to their ability to prevent Na and /or Cl uptake (Troncoso et al., 1999; Storey et al., 2003). There

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are some studies (Desmukh et al., 2003; Xiucai et al., 2004) and *in vitro* (Sivritepe and Eriş, 1999; Troncoso et al., 1999; Hamrouni et al., 2008; Popescu et al., 2015; Barakat et al., 2019; Hao et al., 2021) conducted *in vivo* conditions to determine the physiological and morphological responses of *V. vinifera* varieties and American vine rootstocks against soil salinity. In these studies, stress mechanisms have been studied, and it has been determined that the mechanisms developed by plants within the same species against salt stress are different (Sivritepe and Eriş, 1999). Because the studies carried out under *in vivo* conditions require a long time and cost, *in vitro* studies have started to be shortened of this period, albeit in a limited number, recently. In the study of Fisarakis et al. (2005), salinity increased the phosphorus (P) concentration of the leaf blade, petiole, and shoots; decreased NO_3-N and K concentrations. They reported that Ca and Mg concentrations in the shoots, P and Mg concentrations in the stem, and P, Ca, and Mg concentrations in the root were not affected by salt amount. In certain studies, it has been reported that salinity has a negative effect on mineral element uptake (Singh et al., 2000; Hepaksoy et al., 2006). The way salinity affects plant growth has still not been fully understood. However, it has been reported that the salinity tolerance of plants can be changed by mineral nutrition (Fisarakis et al., 2005).

There are limited studies about the mineral nutrient preferences of vine rootstocks under *in vitro* salt stress conditions. In recent years, how to feed rootstocks in viticulture against abiotic stress conditions has become an important agenda issue. Therefore, this study was planned to determine the morphological responses of some American grape rootstocks grown under different salt (NaCl) stress conditions *in vitro* and determine their mineral nutrition preferences. In addition to this subject, a protocol will be created for early screening of salinity tolerance in breeding new rootstocks using tissue culture technique.

Materials and Methods

Materials

This study was carried out in 2018 at the Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Turkey. In the study, 41B, 5BB, 140Ru, and SO4 rootstocks, which are widely used in Turkey against phylloxera, and Salt Creek rootstock used for nematode problems, were used as plant materials. These materials were obtained from the Viticulture Research and Application area of Cukurova University.

Methods

In the active growth period (April-May), the nodal explants containing a single bud prepared from the 10 cm shoot tip of the rootstocks were disinfected with 5% commercial sodium hypochlorite solution containing few drops Tween 20 for 15 minutes in a sterile laminar flow cabinet. Then, the explants were rinsed three times with sterile distilled water (Meşe and Tangolar, 2019). After surface sterilization, the explants were planted into 25 mm x 150 mm sized test tubes containing 10 mL of MS (Murashige and Skoog, 1962) medium supplemented with 1 mg L^{-1} BAP, 30 g L^{-1} sucrose, and 8 g L^{-1} agar. Explants were cultured in tubes for four weeks. When the shoots had 2-3 leaves, they were cut and transferred to MS medium containing 1 mg L^{-1} IBA for rooting. After 5-6 weeks, the upper parts of the shoots containing three leaves were cut from the plantlets and used for salt applications. To create salt stress, 0, 0.75, and 1.5 g L^{-1} concentrations of NaCl were added to the MS medium containing 1 mg L^{-1} IBA. All the explants cultured in this study were incubated in a growth chamber with a temperature of 25±1 ^oC, a photoperiod of 16 hours, and exposure of 3000-4000 lux (11000-15000 watt. m^{-2}) for 45 days. Lighting was provided by Cool daylight type TLD 36 w/54 fluorescent lamps.

Investigated Characteristics

The plantlets were removed from the tubes at the end of 45 days in the growth room, and the roots were cleaned from the nutrient medium. After that, plant damage degree (0: No salt stress sign, 1: slowing in growth, local yellowing of leaves, 2: yellowing of leaves and 25% necrotic spotting, 3: 25-50% necrotic spots on leaves, 4: 50-75% necrosis on leaves and stems and death, 5: 75-100% severe necrosis on leaves and stems and total death), according to Kıran et al. (2015) were determined. Average shoot length (cm $plant^{-1}$), node number (n $plant^{-1}$), shoot fresh and dry weight (g $plant^{-1}$), root length (cm $plant^{-1}$), root number (n $plant^{-1}$), root fresh and dry weight (g $plant^{-1}$), rooting rate (%) and leaf chlorophyll content (SPAD readings, SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) were detected according to Meşe and Tangolar (2019). Plant vitality rate (%) was determined according to Edriss et al. (2016) and Uyar (2016). In the plant viability calculation, according to the 0-5 scale value taken into account in the plant damage degree, the plants that got 0 and 1 were considered alive, and the others were considered as dead. In addition, shoot and root tolerance rate (TO) and shoot and root tolerance index (TI) were calculated (Dardeniz et al., 2006; Uyar, 2016) as follows:

TO = shoot and root tolerance ratio

$TO = T_x / T_o$

T_x = shoot and root dry weight of the NaCl treated plant (g)

T_o = shoot and root dry weight of the plant without NaCl application (g)

TI = Shoot and root tolerance index

$TI = 100 + \sum^n [x (T_x / T_o)100]$

n = 3 (Number of applications)

x = 0; 0.075 and 0.150 g NaCl 100 mL^{-1}

For mineral nutrient analysis, the shooting part of the plantlets, which were dried for 24 hours at 65 ^oC in the oven, was ground. N concentration was determined according to the Kjeldahl method (AOAC, 1970). To determine the element levels of K, Ca, Mg, Cu, Zn, Fe, Mn, and Na, 0.200 g of ground sample was burned in an ashing furnace at 550 ^oC for 5.5 hours, and then 2 mL of 1/3 HCL

solution and 18 mL of distilled water were added to the ash obtained. This mixture was filtered by the blue band filter paper and taken into a vial. The plant nutrient concentrations were determined by the Atomic Absorption Spectrophotometer (AAS) (Bonomelli and Ruiz, 2010), in which chlorine was also determined. Finally, phosphorus was determined by spectrophotometer according to the Barton method (Barton, 1948).

Experimental Design and Statistical Analysis

The research was carried out according to a randomized factorial design with three replicates. Ten plantlets were used in each replicate. Variance analysis was performed on the data obtained by using JMP statistical package program (v8.00, SAS Institute Inc., USA), and the LSD test was used to determine different groups at a 5% significance level.

Results

In the study, the highest values in terms of shoot length, node number, shoot fresh, and dry weight was determined in Salt Creek rootstock (5.8 cm, 8 (n), 0.446 g, and 0.070 g, respectively) and then 140Ru (4.5 cm, 6.9 (n), 0.205 g and 0.040 g, respectively) rootstock. The lowest values were detected in 41B rootstock (3.4 cm, 5.1 (n), 0.136 g, and 0.029 g, respectively) (Table 1). It was found that the values of shoot properties decreased with increasing salt concentrations. It has been observed that there were significant differences among the rootstocks in terms of plant damage degree. The most severe damage was seen on 41B (3.3 scale degree) followed by SO4 (2.4 scale degree), while there was no damage on Salt Creek (0.0 scale degree) rootstock (Table 1).

The responses of root properties and chlorophyll values (SPAD readings) to different salt applications were parallel to the reactions of shoot properties (Table 2). In terms of these characteristics, Salt Creek had the highest values, followed by 140Ru. Together, 41B and 5BB made up the group with the lowest values. Average root length and number, root fresh and dry weights, and SPAD values were prominent in Salt Creek (7.4 cm, 5.4 (n), 0.429 g, 0.037 g, and 29.6, respectively) compared to other rootstocks. Root growth and SPAD values were determined to be inversely proportional to NaCl concentrations (Table 2).

Regarding plant viability and rooting rate, it was observed that all the plants of Salt Creek rootstock and the control group of applications developed without any problem (Table 3). While viability and rooting rates were over 70% in 140Ru rootstock, these rates remained at the level of 33.3% in 41B rootstock (Table 3).

According to the shoot and root tolerance ratios given in Table 4, the highest values were obtained from Salt Creek, and the lowest data were recorded from SO4 and 41B rootstocks. Shoot and root tolerance ratios of Salt Creek rootstock were found to be 1.118 and 1.097, respectively. Among the rootstock varieties, the order from the best to the lowest root tolerance was determined as Salt Creek, 140Ru, 5BB, SO4, and 41B (Table 4).

Table 1. Effects of different NaCl concentrations on shoot characteristics of different rootstocks.

Sources of Variation	Shoot length (cm)	Node number (n)	Shoot fresh weight (g)	Shoot dry weight (g)	Plant damage degree (0-5 scale)
Rootstock					
5BB	3.9 c [x]	6.5 b	0.163 c	0.024 d	1.6 c
41B	3.4 d	5.1 d	0.136 d	0.029 cd	3.3 a
Salt Creek	5.8 a	8.0 a	0.446 a	0.070 a	0.0 e
140Ru	4.5 b	6.9 b	0.205 b	0.040 b	1.2 d
SO4	3.5 d	6.0 c	0.172 c	0.032 c	2.4 b
LSD 5%	0.1	0.4	0.017	0.004	0.1
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}					
0	5.9 a	8.6 a	0.330 a	0.051 a	0.0 c
0.75	4.2 b	6.5 b	0.214 b	0.039 b	1.5 b
1.5	2.5 c	4.4 c	0.128 c	0.027 c	3.6 a
LSD 5%	0.1	0.3	0.013	0.003	0.1
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
LSD 5%	0.2	0.8	0.030	0.007	0.2
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 2. Effects of different NaCl concentrations on root properties and chlorophyll content of different rootstocks.

Sources of Variation	Root length (cm)	Root number (n)	Root fresh weight (g)	Root dry weight (g)	SPAD Readings
Rootstock					
5BB	4.4 b [x]	1.5 d	0.063 cd	0.006 d	22.5 c
41B	1.8 d	1.4 d	0.054 d	0.007 d	20.7 d
Salt Creek	7.4 a	5.4 a	0.429 a	0.037 a	29.6 a
140Ru	4.9 b	3.3 b	0.189 b	0.020 b	26.8 b
SO4	3.1 c	2.1 c	0.087 c	0.010 c	22.4 c
LSD 5%	0.7	0.4	0.030	0.003	0.8
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}					
0	6.9 a	4.3 a	0.238 a	0.024 a	29.6 a
0.75	4.5 b	2.6 b	0.180 b	0.017 b	26.0 b
1.5	1.5 c	1.2 c	0.075 c	0.007 c	17.5 c
LSD 5%	0.5	0.3	0.023	0.002	0.6
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
LSD 5%	1.2	0.6	0.051	0.005	1.4
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 3. Effects of different NaCl concentrations on plant viability and rooting rates of different rootstocks.

Sources of Variation	Viability rate (%)	Rooting rate (%)
Rootstock		
5BB	66.7 c [x]	58.0 c
41B	33.3 e	33.3 e
Salt Creek	100.0 a	100.0 a
140Ru	72.2 b	73.6 b
SO4	48.9 d	48.9 d
LSD 5%	3.92	6.25
p	<0.0001	<0.0001
NaCl Concentration g L^{-1}		
0	100.0 a	100.0 a
0.75	69.3 b	64.2 b
1.5	23.3 c	24.2 c
LSD 5%	3.04	4.83
p	<0.0001	<0.0001
Interaction		
LSD 5%	6.80	10.79
p	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 4. Effects of different NaCl concentrations on the shoot and root tolerance ratios of different rootstocks.

Sources of Variation	Shoot tolerance ratio	Root tolerance ratio
Rootstock		
5BB	0.539 c [x]	0.268 c
41B	0.352 d	0.000 e
Salt Creek	1.118 a	1.097 a
140Ru	0.630 b	0.551 b
SO4	0.413 d	0.125 d
LSD 5%	0.079	0.108
p	<0.0001	<0.0001
NaCl Concentration g L^{-1}		
0.75	0.720 a	0.605 a
1.5	0.500 b	0.211 b
LSD %5	0.050	0.069
p	<0.0001	<0.0001
Interaction		
LSD 5%	0.112	0.153
p	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

The tolerance rates of shoots and roots were more pronounced at a concentration of 0.75 g NaCl L^{-1}. When the shoot and root tolerance index table was examined (Fig 1), Salt Creek rootstock came first. According to the tolerance index, 140Ru and 5BB were in the same statistical group. Rooting of 5BB rootstock was evaluated to be slightly weaker compared to 140Ru rootstock, and the lowest data were obtained from SO4 and 41B rootstocks (Fig 1).

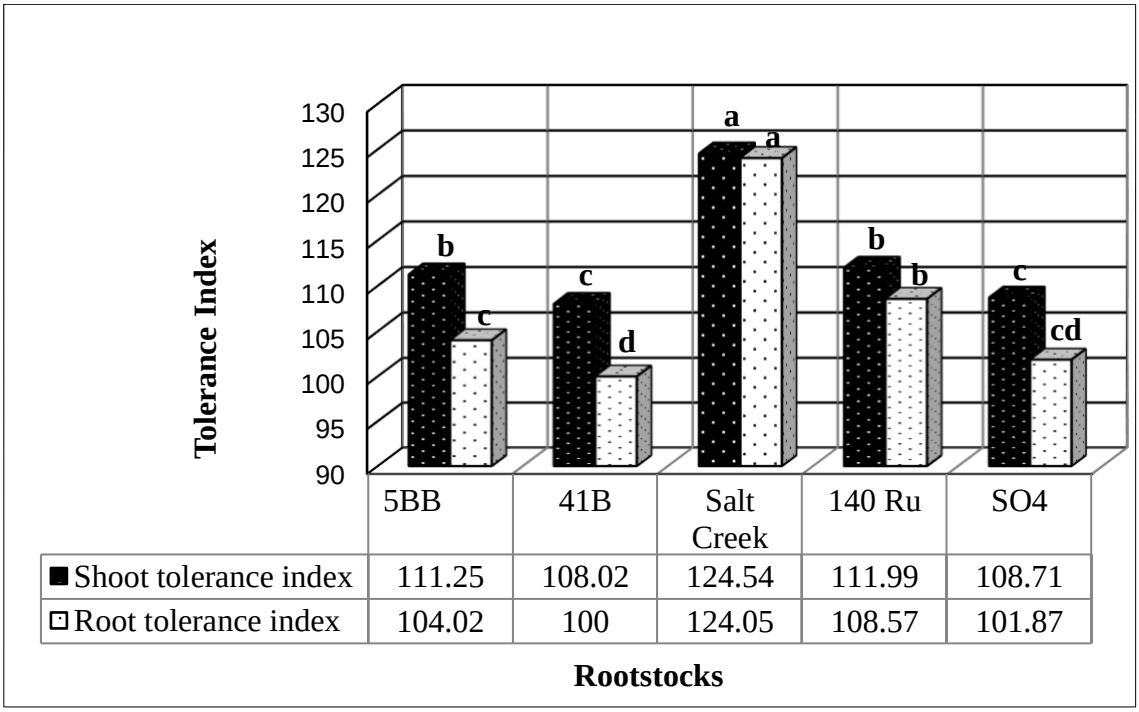

Fig 1. The effect of different NaCl concentrations applied to varying rootstocks on the shoot and root tolerance index.

A significant difference (P≤ 0.05) was found among the means indicated by different letters in the same indicator column.

According to the nutrient analysis performed on rootstock explants under *in vitro* salinity stress (Table 5), it was determined that the rootstock taking up the most N (3.46%), K (2.28%), Ca (0.66%), and Mg (0.34%) elements from the nutrient medium were found to be Salt Creek. The rootstock with the highest P uptake (0.34%) was SO4. It was determined that the rootstock with the lowest N (1.20%), P (0.12%), K (0.83%), Ca (0.23%), and Mg (0.11%) uptake was 41B. The effect of NaCl on macro element uptake of rootstock plantlets was negative. Adding 1.5 mg L^{-1} salt to the nutrient medium reduced the uptake by 17.9% in N, 12.5% in P, 17.7% in K, 18.5% in Ca, and 20.6% in Mg, compared to the control (Table 5).

The effect of different salt applications on the microelement uptake from the nutrient medium of the rootstock plantlets was different at the rootstock level (Table 6). Salt Creek was the rootstock that took up the highest amount of Cu (3.94 ppm), Mn (211.7 ppm), Zn (70.09 ppm), Na (3958.0 ppm), and Cl (30724.7 ppm) elements, except Fe element. In terms of Na and Cl element concentrations, Salt Creek was followed by 5BB. It was determined that the iron element was at the highest level in 140Ru rootstock (189.0 ppm). The rootstock that received the least microelements from the nutrient medium was 41B. Salt Creek rootstock can absorb macro and microelements in the best way is explained as that this rootstock continues to develop without being affected by salt stress. Whereas, it was evaluated that the low element concentrations in the 41B rootstock were caused by the fact that plants did not grow and died under salt stress conditions. In addition, it has been determined that increasing salt dose decreased the microelement uptake of plantlets.

As it can be seen from Table 7, which shows the nutritional correlations of rootstocks from nutrient media containing different salt concentrations, all nutrients are in a positive relationship with each other. The highest values of coefficients were found between Ca-Mg (1.00), N-K (0.99), K-Ca (0.98), K-Mg (0.97), and P-K (0.97).

Table 5. Effects of different NaCl concentrations on the macro element amounts (%) of different rootstocks.

Sources of Variation	N	P	K	Ca	Mg
Rootstock					
5BB	2.79 b [x]	0.24 c	1.77 b	0.41 c	0.22 c
41B	1.20 d	0.12 d	0.83 d	0.23 d	0.11 d
Salt Creek	3.46 a	0.32 b	2.28 a	0.66 a	0.34 a
140Ru	2.08 c	0.24 c	1.47 c	0.42 b	0.24 b
SO4	2.84 b	0.34 a	1.83 b	0.42 b	0.22 c
LSD 5%	0.26	0.01	0.10	0.01	0.01
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}					
0.0	3.91 a	0.40 a	2.54 a	0.65 a	0.34 a
0.75	2.81 b	0.30 b	1.92 b	0.51 b	0.27 b
1.5	0.70 c	0.05 c	0.45 c	0.12 c	0.07 c
LSD 5%	0.20	0.01	0.08	0.01	0.004
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
LSD 5%	0.45	0.03	0.18	0.02	0.01
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 6. Effects of different NaCl concentrations on the microelement amounts (ppm) of different rootstocks.

Sources of Variation	Cu	Mn	Fe	Zn	Na	Cl
Rootstock						
5BB	2.37 b [x]	148.5 c	143.9 b	61.58 b	3049.3 b	31949.6 a
41B	0.69 d	86.2 d	56.6 c	18.58 e	581.3 e	13119.8 b
Salt Creek	3.94 a	211.7 a	153.5 b	70.09 a	3958.0 a	30724.7 a
140Ru	1.90 c	214.5 a	189.0 a	42.53 d	1509.3 d	15330.0 b
SO4	2.42 b	188.0 b	142.9 b	53.72 c	1748.8 c	9170.3 c
LSD 5%	0.47	7.7	10.6	2.90	215.9	2494.9
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}						
0.0	3.41 a	276.7 a	223.6 a	78.05 a	1406.6 b	29175.7 a
0.75	2.69 b	197.2 b	160.5 b	58.28 b	3767.7 a	23200.9 b
1.5	0.70 c	35.4 c	27.5 c	11.56 c	1333.7 b	7800.0 c
LSD 5%	0.36	5.9	8.2	2.24	167.2	1932.6
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction						
LSD 5%	0.81	13.3	18.4	5.02	373.9	4321.3
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 7. Correlation coefficients of uptake characteristics of plant nutrients obtained from different rootstocks grown under different NaCl concentrations.

Element	P	K	Ca	Mg	Cu	Mn	Fe	Zn	Na	Cl
N	0.96*	0.99	0.96	0.95	0.91	0.90	0.87	0.97	0.59	0.78
P		0.97	0.94	0.94	0.88	0.95	0.80	0.93	0.49	0.63
K			0.98	0.97	0.92	0.93	0.90	0.95	0.61	0.79
Ca				1.0	0.92	0.94	0.89	0.93	0.59	0.78
Mg					0.91	0.96	0.92	0.93	0.61	0.77
Cu						0.84	0.79	0.91	0.60	0.71
Mn							0.97	0.87	0.42	0.63
Fe								0.87	0.43	0.65
Zn									0.584	0.77
Na										0.72
Cl										1.00

*: Significant coefficients at P≤ 0.05

Discussion

Salinity has been known as one of the significant problems in the soils of the world. Because the soil salinity is an important factor limiting the growth of vine plants, this study was carried out to determine mineral nutrition preferences and the morphological response against the salt stress of the rootstocks consisting of 41B, 5BB, 140Ru, Salt Creek, and SO4 that are widely used in Turkey viticulture. In the study, shoot and root characteristics and chlorophyll content levels of rootstock plantlets decreased, and the degree of plant damage increased compared to control plantlets (without salt) (Table 1 and 2). According to Troncoso et al. (1999), shoot elongation decreases with an increasing salt concentration *in vitro* conditions similar to the results of our study. They ordered the rootstocks from sensitive to tolerant as 41B, 140Ru, and Salt Creek. Popescu et al. (2015) stated that the first symptom against salt stress was decreased growth in both *in vitro* and *in vivo* conditions. With the increase in salt concentrations, necrotic spots on shoot tips and chlorosis on leaves were also observed. The researchers determined that the SO4 and 5BB rootstock were moderately sensitive, and 140Ru rootstock was resistant. Troncoso et al. (1999) and Popescu et al. (2015) also stated that root growth and rooting rate also changed inversely with the increase in salt concentration in the growth medium. These results were by our findings, except for the SO4 rootstock.

Hamrouni et al. (2008) applied *in vitro* salt stress to 1103P, SO4, and 41B rootstocks for six weeks. They stated that the survival rate, reproduction, and

growth characteristics of vine plantlets decreased with salt concentrations. In addition, necroses were observed depending on the rootstock and NaCl concentrations, and the salt dose of 80 mM (4.7 g L-1) was determined to be the critical threshold. The same research revealed that the formation and development of roots are significantly affected by NaCl concentrations. Edriss et al. (2016), in their *in vitro* salt stress studies on the vine, stated that shoot length, the number of leaves, shoot fresh and dry weight, and plant vitality decreased depending on salinity. It was determined that root formation and chlorophyll contents of plantlets of Dogridge and Richter varieties were severely affected by increasing NaCl applications. At the same time, Salt Creek and Freedom rootstocks were less affected. Similar to our results, the researchers determined that the most tolerant rootstock to salt stress was Salt Creek. Also, they reported that Salt Creek rootstock continued to grow and develop by preserving its vitality at a high rate, even at 75 mM (4.4 g L^{-1}) salt concentration. Alizadeh et al. (2010) determined that the root's fresh and dry weights, number of roots per plantlet, and chlorophyll contents were negatively affected by NaCl in their *in vitro* studies. Stevens et al. (1996) and Uyar (2016) also stated that salt stress reduced root growth *in vivo*, and consequently, plant growth decreased. In addition, they reported that the total chlorophyll content decreased due to increased salinity.

Considering the viability and rooting rate in Salt Creek among the rootstocks and control (without salt stress) and 0.75 g L^{-1} among the concentrations, there was no significant problem (Table 3). Their rates were 100%, and the rates decreased by increasing NaCl doses. Similarly, Edriss et al. (2016), who determined the Salt Creek rootstock as the most resistant rootstock in *vitro* conditions, found that with increasing salt dose, plant vitality and the rooting rate decreased, and roots were more affected. Turhan et al. (2005) also reported that with the increase of salt concentration in vine rootstocks irrigated with salt water, the vitality of the cuttings decreased, and the plant roots were damaged. In addition, Kök (2012) stated that the rooting ability of some rootstocks irrigated with saltwater decreased. In our study, while 140Ru and 5BB rootstocks gave similar results, SO4 was the most affected rootstock. In another study conducted on rootstocks under *in vivo* conditions, Dardeniz et al. (2006) determined that irrigation with salt water gradually reduced plant viability. In their research, unlike our study, 41B was the most resistant rootstock, followed by 140Ru, and the most damaged rootstock was 5BB. In salt stress studies on grapevine rootstocks and varieties, it was concluded that the plants lost their vitality as the stress intensity increased (Salem et al., 2011; Desouky et al., 2015). Salem et al. (2011) and Desouky et al. (2015) found that the most tolerant rootstock was Salt Creek. Hamrouni et al. (2008) studied vine rootstocks in *vitro*, and Uyar (2016) studied grape varieties *in vivo* and stated that root and shoot viability decreased with the increase in salt stress intensity.

According to the shoot and root tolerance ratios given in Table 4, the highest values were in Salt Creek, and the lowest data were in SO4 and 41B rootstocks. The tolerance rates of shoots and roots were pronounced more at 0.75 g NaCl L^{-1} concentration. Regarding the shoot and root tolerance index (Fig 1), Salt Creek rootstock came first. Turhan et al. (2005), studying with 1103P, 420A, and 5BB grapevine rootstocks *in vivo*, determined that shoot and root tolerance rates decreased with the increase of the salt-water dose. In their study, salt concentration on shoot tolerance index was not significant, but the root tolerance index was significant, and 5BB gave the best result. In another salt stress study on 1103P, 41B, 140Ru, and 5BB vine rootstocks, Dardeniz et al. (2006) determined that shoot and root tolerance rates tended to decrease with increasing salt dose. But, the difference between the varieties in terms of root and shoot tolerance rates and tolerance index was not statistically significant. However, the best shoot tolerance index was determined in 140Ru, while the best root tolerance index was obtained from 41B rootstock. Müftüoğlu et al. (2006) and Uyar (2016) observed that shoot and root tolerance rates decreased with the increase in salt concentrations in their salt stress studies on grapevine varieties. Despite the literature mentioned above, the shoot and root tolerance index parameters were found to be significant by Sivritepe and Eriş (1999) and Uyar (2016), while Müftüoğlu et al. (2006) stated that these parameters were insignificant.

The mineral analysis of rootstock explants grown under salinity conditions (Table 5 and 6) showed that the rootstock taking up the most N, K, Ca, and Mg elements from the nutrient medium were found to be Salt Creek. The rootstock receiving the lowest N, P, K, Ca, and Mg was 41B. The effect of NaCl on macro and microelement uptake of rootstock plantlets was negative. Troncoso et al. (1999) reported in their *in vitro* studies that the contents of Na and Cl elements started to increase with salt stress and that the element Cl was taken into the plant more than Na. Although there were slight differences between the rootstock genotypes in their study, it was noticed that all element amounts decreased as the salinity increased. Similar results were obtained in our study. Edriss et al. (2016) found in their *in vitro* studies that the accumulation of N, Mg, K, Ca, P, Fe, Zn, and Mn in rootstocks decreased with increasing salt concentration, and Cl and Na contents increased with stress. They concluded that Salt Creek was the best rootstock taking the highest K and the lowest Cl and Na. In an *in vivo* study on 41B, 5BB, 1103P rootstocks, and Alphonse Lavallée variety, Babalık (2012) stated that Na and Cl uptake increased with salt stress. Still, Na and Cl levels decreased with water application. It was concluded that the amounts of K, N, P, Fe, Mg, and Zn decreased in general, although there were differences between genotypes as the water level decreased and salt doses

increased. Dag et al. (2015) stated that Na and Cl ions increase in the plant as the salinity of irrigation water increases. It has been determined that 140Ru rootstock accumulated Cl ions in the lowest amount and that chlorine ions were taken into the plant body more than Na. Alizadeh et al. (2010) stated that Na, Cl, and K ions increased with increasing salinity *in vitro* conditions. They observed that SO4 rootstock was sensitive, although it was one of the rootstocks accumulating the least toxic ions. Mohammadkhani et al. (2013) observed in their pot culture studies on Iranian varieties that Na and Cl elements were correlated positively, while potassium and nitrate minerals were negatively correlated depending on the severity of salt stress. Also, they stated that the Na element was accumulated more than Cl (2-5 times), but it is more likely that the effect of Cl may cause damages. Except for the excessive accumulation of Na, the results were consistent with our findings. Mohammadkhani et al. (2013) also declared that the decrease in the amount of nitrate caused by Cl antagonism and Cl accumulation was two times higher in the shoots than in the roots. Although it was stated that K is relatively high intolerant varieties, they concluded that Na and Cl elements limit plant growth and development. Uyar (2016) found in his *in vivo* study on Muscat of Hamburg and Isabella grape varieties that increasing NaCl doses increased Na ions in the plant. He concluded that K and Ca contents decreased with increasing salinity, and Mg ions gave similar results against salt stress. Mohammadkhani and Abbaspour (2018) examined the effect of NaCl on two sensitives and two tolerant Iranian varieties *in vivo*. They observed that Na and Cl ions accumulated three times more in the shoots of sensitive genotypes than tolerant varieties. This accumulation was much more in the roots intolerant varieties. In other words, sensitive varieties could not prevent the uptake of toxic ions into their bodies. They also stated that Cl ions accumulated more than Na. Fisarakis et al. (2005) reported that the K concentration of the leaf blade, petiole, and shoots of the plants increased, and NO_3-N and K concentrations decreased with salinity. Salt stress did not affect the concentrations of Ca and Mg in the shoots, P and Mg in the stem, and P, Ca, and Mg concentrations in the roots. Salinity has a negative effect on nutrient uptake (Najafi et al., 2007). Although the way salinity affects plant growth is still not fully understood, it has been reported that the salinity tolerance of plants can be changed by mineral nutrition (Fisarakis et al., 2005).

The nutritional correlations of rootstocks from nutrient media containing different salt concentrations, all nutrients are in a positive relationship with each other (Table 7). Since the conditions in tissue culture were controlled and the pH was initially adjusted to 5.8, it was evaluated that the rootstocks easily took the nutrients. At the same time, the elements affect each other's uptake positively. On the other hand, in *in vivo* cultivation, elements such as Mg, Ca, and K may negatively affect each other's uptake due to various climate and soil characteristics (Fozouni et al., 2012a; Esfandiari and Pourmohammad, 2013).

Conclusions

Considering the rapid increase in world population and the decrease in quality water, it is necessary to obtain sufficient yield and quality in salty soils and salty water. This situation makes it important to determine the growth conditions and nutritional levels of plants under stress. This study determined that plant elongation, node number, and plant vitality decreased compared to control plantlets with increasing salt stress in all rootstocks used. The decline in plant growth determined decreases in shoot fresh and dry weights. 41B and SO4 rootstocks were affected by NaCl more than Salt Creek and 140Ru rootstocks. With the increase in salt concentrations, it was observed that the root length and the number of roots per plantlet and root fresh and dry weights decreased. Chlorophyll and nutrient contents also decreased in plantlets compared to the control.

Parameters such as plant vitality, shoot length, node number, root number, and Na and Cl ions are important parameters in evaluating the tolerance to salinity. As a result of considering all the parameters examined, the rootstocks could be listed as 41B <SO4 <5BB <140Ru <Salt Creek from sensitive to tolerant. Since 41B and SO4 rootstocks are sensitive to salt stress, application of NaCl concentrations below 0.75 g L^{-1} may be proper. In addition, it is recommended to test intermediate concentrations between two doses of NaCl applied in this study for rootstocks that die at a dose of 1.5 g NaCl L^{-1}.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest in the publication.

Author contribution

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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