International Journal of Horticultural Science and Technology

Journal homepage: http://ijhst.ut.ac.ir

Responses of 'Chandler' Walnut Variety Grafted onto Different Rootstocks to Salt Stress

Yasar Akca* and Umut Sahin

Department of Horticulture, Faculty of Agriculture, University of Tokat Gaziosmanpaşa, Tokat, Turkey

ARTICLE INFO

Article history:

Received: 17 February 2021,
Received in revised form: 10 June 2021,
Accepted: 2 July 2021

Article type:

Research paper

Keywords:

salinity,
walnut rootstocks,
plant growth,
abiotic stress

ABSTRACT

To investigate effects of salinity stress on growth, nutrient status, chlorophyll and water consumption, four different saline irrigation waters (S0= 0.3 dS/m-1 control, S1=1.5 dS/m-1, S2=3 dS/m-1, S3=5 dS/m-1) were applied to two-year Chandler saplings which were grafted on Juglans nigra L. (JN), Paradox (PR) and Juglans sieoboldiana L. (JS) walnut rootstocks. Three different salts including NaCl, MgSO4, and CaCl2 were used to prepare saline irrigations. Shoot length change rate (%) was varied between 77.57 (JN) to 81.83 (PR) for S0 salinity treatments and between 55.60 (JS) to 56.84 (PR) for S3 treatments. The plant diameter change rate (%) varied between 75.10 (JN) to 99.22 (PR) for S0 treatments and 60.63 (JN) to 80.97 (PR) for S3 treatments. Average of root length (cm) was between 30.75 cm (JN) to 37.50 cm (PR) for S0 treatments, and 8.91 cm (JN) to 21.50 cm (PR) for S3. Number of roots changed between 19.00 (JS) to 22.16 (PR) for S0 treatments and between 6.41 (JN) and 8.08 (PR) for S3 treatments. Sodium (Na) content (%) in S3 was 1.41, 1.97 and 3.41 in JN, PR, and JS, respectively. Chloride (Cl-) content (ppm) for S3 was 0.88, 0.99 and 0.91 in JN, PR, and JS, respectively. Ca/Na ratios of 0.43, 0.27 and 0.14 and K/Na ratios of 0.24, 0.12 and 0.10 were detected in JN, PR, and JS, respectively. Depending on leaf Na+, Cl- and K contents, K/Na and Ca/Na ratios, the ranking of salinity tolerance of rootstocks were determined as JN$^{>}$ PR $^{>}$ JS.

Introduction[*]

Due to the great economic and food value, walnut (*Juglans regia* L.) has received increasing attention during recent years worldwide (Vahdati, 2014). However, water stress and salinity limit walnut growth, production, and quality (Yadollahi et al., 2010; Baoqing et al., 2020). Salinity is a major abiotic stress negatively affect the growth and production of crops in terrestrial systems. Salinity represents the physiological dryness and low water potential that leads to osmotic stress (Li et al., 2016). Excess salt concentrations may cause toxic effects (Polle and Chen, 2015). Global climate change is causing higher temperature and less irrigation in larger areas, which could result in more salinization (Vahdati et al., 2019).

* Corresponding author's email: akca66@gmail.com

The growth of walnut is strongly affected by salt stress. Walnut is sensitive to salt stress resulted from soil and irrigation water (Lotfi et al., 2009). Therefore, the availability of salt-resistant walnut rootstocks is important for walnut cultivation (Rezaei Qusheh Bolagh et al., 2021). Vast differences in the tolerance of walnut rootstocks to salt stress has been reported (Behrooz et al., 2019). Comparisons between rootstocks are primarily based on salt accumulation in scion and signs of leaf toxicity. In the base of leaf content, 0.3% chloride, 0.1% sodium and 300 ppm boron are considered to be excessive levels in walnut (Caprile and Grattan, 2006; Batchelor et al., 1945; Hendricks et al., 1977).

There are wealth of information on the tolerance of *J. regia, J. nigra* and *Paradox* rootstocks against salt and boron stresses (Fulton and et al., 1988). However, there is not enough information and research about the effect of rootstocks on salt stress of the grafted variety. In this study, the effect of salt stress on Chandler variety, which was grafted on three walnut rootstocks, was investigated.

The reactions of plants to salt varies according to the effective development period of the plant, the concentration of salt, which is the salt factor, and the time that the salt affects the plant. In addition, the effect of salt stress on the plant can vary depending on the climate and soil characteristics (Greenway and Munns, 1980). Studies on the effects of salinity stress on plants have primarily focused on growth, proline accumulation, chlorophyll content, K/Na, Ca/Na ratio, Na^+ and Cl^- accumulation (Behzadi Rad et al., 2021). It has been stated that genotypes with a high chlorophyll content, high K/Na ratio and low Na^+ and Cl^- accumulation are more tolerant to salt stress (Mane et al., 2011).

The salt stress negatively affects osmotic stress and plant nutrient uptake in plants (Levitt, 1980). Salty soils restrict the development of plants (Sheikh Beig Goharrizi et al., 2016). As a result of this growth retardation, due to the decreasing osmotic potential in the soil, the plant cannot use water adequately or the toxic effects caused by ions such as Na^+ and Cl^- in excessive amounts in saline soils and deterioration in plant ion balance (Lewitt, 1980). Based on our observation, we hypothesized that *J. sieboldiana* species in response to salt stress will exhibit superior root performance under salt stress. The reaction of *J. sieoboldiana* rootstock was investigated for the first time in this study. The aim of this study was to determine the responses of two-year old 'Chandler' plants grafted on *J. nigra L., Paradox* and *J. sieoboldiana* L. rootstocks to salt stress.

Material and Methods

Plant material and growth conditions

Two-year old Chandler walnut cultivar that were grafted on *Juglans nigra* L. (*JN*), Paradox (*PR*) and *Juglans sieoboldiana* L. (*JS*) walnut rootstocks according to the routine methods of grafting (Ebrahimi et al., 2007; Rezaee et al., 2008; Dehghan et al., 2010). Early April, 2016, the saplings were planted in pots in 45 L plastic pots (one seedlings per pot). Pots were filled with, homogenized soil, air-dry clay loam soil (45% sand, 34% silt, and 30% clay). The values of field capacity (cm^3/cm^3), wilting point (cm^3/cm^3); and total available water (cm^3/cm^3) were 0.26, 0.18 and 0.08, respectively. All saplings included in the experiment were approximately of the same size and height range. After two month of growth with normal management to adapt to the environment, all saplings were exposed to salinity treatments.

Salt treatments and plant water consumption

The salt applications were carried out in 2016 and 2017. This study was conducted in greenhouse conditions, by a completely randomized block design with four treatments

and three replications (S_0= 0.3 dS/m (control), S_1=1.5 dS/m, S_2= 3.0 dS/m, S_3= 5.0 dS/m). Sodium chloride (NaCl), magnesium sulfate ($MgSO_4$) and calcium chloride ($CaCl_2$) salts were used to prepare saline irrigation waters. The amount of salt to be used in each mixture was adjusted to provide a sodium adsorption ratio (SAR) value of 5, Ca/Mg ratio of 1/1 (mmol/L). The EC values of prepared mixtures were checked in the laboratory and corrections were made if needed. Tap water with $EC_{i=}$ 0.30 dS m^{-1} was used as a control treatment. Salt mixtures were stored in 100-l plastic containers. The irrigation water amounts were determined by adding the leaching amount to the water consumed by the plants (Ünlükara et al., 2008). The saplings were irrigated during the months of June 1 to August 15. To calculate the amount of irrigation water, the pots were weighed just before each irrigation and amount of applied irrigation water was calculated as:

$$I = \frac{\frac{W_{fc} - W}{\rho_w}}{1 - LF}$$

Where, I is amount of applied irrigation water (lt), LF is the leaching fraction (0.20 was chosen as suggested by Ayers & Westcot (1985), W*fc* is the pot weight at field capacity (kg), W is the pot weight (kg) before irrigation was implemented, and ρ_w is density for water (1.0 kg/L). The amount of water drained was measured after irrigation.

Total evapotranspiration (ET; L) was calculated by water balance equation as follows:

$$ET = \frac{(Wb - We)}{\rho_w} + (I - R)$$

Where *Wb* and *We* are the pot weights (kg) at the beginning and at the end of the experiment, respectively, ρ_w is water density (1.0 kg/L), I and R are total amounts of water applied and drained (l), respectively (Düzdemier al., 2009).

Plant growth characteristics

After exposing the saplings to different saline waters along their growing season, plant length, plant diameter, rootstock diameter, root length, lateral root number, the number of new fresh root, and the number of leaf were measured at the end. All these parameters were also recorded at the beginning of the experiment.

Macro and micro nutrition and leaf chlorophyll content analyses

Leaf analyses were carried out on two leaf samples collected at the beginning of the growth season and at August 15 to determine mineral contents such as phosphorus (P), potassium (K^+), sodium (Na^+), chloride (Cl^-), magnesium (Mg^{++}), manganese (Mn), calcium (Ca^{++}), zinc (Zn) and iron (Fe). The leaf samples were washed in pure water and dried at 65 °C. After drying and grinding, wet decomposition method was used to 0.3 g samples with sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) then filtered through blue band filter paper. The volumes of the samples were completed to 50 mL with pure water for ICP analysis. Analyses for K^+ and Ca^{++} content were carried out via flame photometry while atomic absorption spectrophotometer was used for Mg^{++}. P content was analyzed using calorimetric method. Dry leaf samples were extracted in a 0.1 N acid concentration for chloride analyses and chloride content was read in Sherwood MK II chloride Analyzer 926, chloride contents were calculated according to Taleisnik and Grunberg (1994). Chlorophyll analysis was determined according to Withan et al., (1971).

Statistical analyses

The salt treatments were carried out as 5 repetitions for each salt treatments and as 4 plants for each repetition. The results were analyzed via the SPSS statistics software (Standard version 11.0).

Results

Effect of irrigation water salinity on the soil salinity

Electrical conductivities of soil saturation paste extracts (EC_e) for each treatment were presented in Table 1. Soil salinity (EC_e) increased gradually as a function of increasing electrical conductivities of irrigation waters (EC_i) (Table 1). Soil salinities were determined to be 2.8 $dS.m^{-1}$, 6.71 $dS.m^{-1}$, 8.72 $dS.m^{-1}$, and 11.7 $dS.m^{-1}$ for S_0, S_1, S_2 and S_3 treatments. Statistically significant differences were observed between the salt treatments in soil salinity.

Table 1. Impact of irrigation water salinity on soil salinity in the studied treatments.

Treatments (dS m^{-1})	Soil salinity (EC_e)
S_0 (0.3)	2.8 d
S_1 (1.5)	6.71c
S_2 (3.0)	8.72 b
S_3 (5.0)	11.7 a*

*Means followed by the same letter are not significantly different according to Duncan's multiple range test at 0.05 significance level

Plant growth characteristics

Significant growth changes compared to ones at the beginning of growth for plant length, plant diameter, root length, fresh root number and leaf number are described in Table 2.

Table 2. Effects of salt stress on the development parameters of two-old year grafted Chandler cv. onto seedling rootstocks of *Juglans nigra*, Paradox and *J. sieboldiana*.

	Rootstocks	S_0	S_1	S_2	S_3
Rootstocks diameter change rate (%)	*Juglans nigra*	66.88a* B**	64.55ab B	60.12ab B	55.68b B
	Paradox	88.13a A	79.12a A	78.86a A	77.70a A
	J. sieboldiana	89.30a A	85.95a A	85.88a A	84.78a A
Plant length change rate (%)	*Juglans nigra*	77.57a A	69.90ab B	60.70ab B	56.18b A
	Paradox	81.83a A	72.44ab AB	67.40ab B	56.84b A
	J. sieboldiana	82.51a A	80.91a A	77.02ab A	56.60b A
Plant diameter change rate (%)	*Juglans nigra*	75.10a B	72.98ab A	65.92cb B	60.63c B
	Paradox	99.22a A	84.91a A	83.83a A	80.97a A
	J. sieboldiana	84.23a A	78.41a A	76.68a A	76.00a A
Root length (cm)	*Juglans nigra*	30.75aB	22.66b B	17.08c B	12.83d B
	Paradox	37.50a A	31.08b A	26.58c A	21.50d A
	J. sieboldiana	20.58a C	13.83b C	11.58c C	8.91d C
Number of lateral root	*Juglans nigra*	20.33aA	12.75ab A	6.75b AB	6.41b A
	Paradox	22.16a A	16.16ab A	12.41ab A	8.08b A
	J. sieboldiana	19.00a A	15.66ab A	12.66ab A	7.58b A
Number of fresh root	*Juglans nigra*	14.00a AB	11.00b A	8.00c A	5.00d A
	Paradox	20.91a A	13.75b A	9.91c A	7.08d A
	J. sieboldiana	15.50a AB	11.83b A	9.08c A	5.00d A
Number of leaf	*Juglans nigra*	69.95a B	68.29a B	66.14a B	63.06a A
	Paradox	85.13a A	83.57a A	75.18a A	63.45ab A
	J. sieboldiana	74.52a A	72.15a A	68.33ab B	65.80ab A

Means followed by the same letter are not significantly different between salt treatments (*), and rootstocks (**) according to Duncan's multiple range test at 0.05 significance level

The shoot length change rate (%) varied between 77.57 (*JN*) to 82.51 (*JS*) for S_0 and between 55.18 (*JN*) to56.84 (*PR*) for S_3. The plant diameter change rate (%) varied between 75.10 (*JN*) to 99.22 (*PR*) for S_0 and 60.63 (*JN*) to 80.97 (*PR*) for S_3. The average root length (cm) was between 20.58 cm (*JS*) to 37.50 (*PR*) for S_0 treatments, and 8.91 cm (*JN*) to 21.50 cm (*PR*) for S_3 (Table 2).

The average fresh root number changed between 19.00 (*JS*) to 22.16 (*PR*) for S_0 treatments and, 6.41 (*JN*) to 8.08 (*PR*) for S_3 treatments. The average number of leaves was between 69.95 (*JN*) to 85.13 (*PR*) for S_0 treatments, and 63.06 (*JN*) to 65.80 (*PR*) for S_3 (Table 2).

Salinity and plant water consumption

Plant water consumption decreased significantly with gradual increase in soil salinity for the rootstocks (Fig. 1). The greatest water consumption was observed in the control treatments, the lowest was observed in S_3 treatments. The lowest water consumption of S_3 treatments was calculated in the *JN* rootstock and the greatest water consumption was determined in *JS* rootstock.

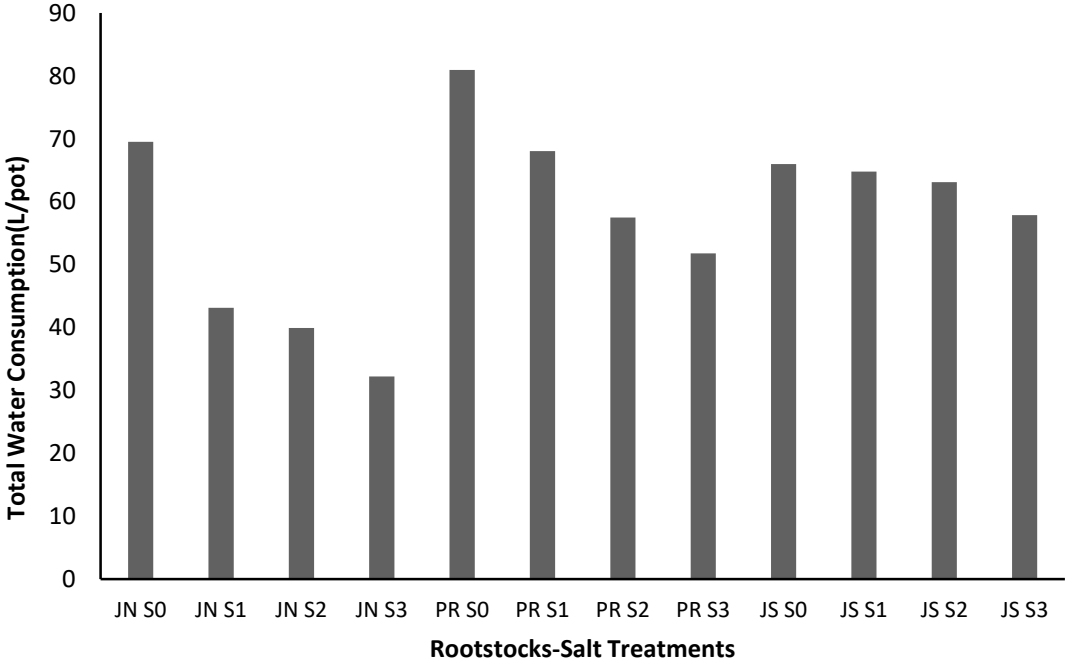

Fig. 1. Effect of irrigation water salinity on the total water consumption of walnut (L/pot). *Juglans nigra* L. (*JN*), Paradox (*PR*) and *Juglans sieoboldiana* L. (*JS*) walnut rootstocks were used. This study was conducted in greenhouse conditions, by a completely randomized block design with four treatments and three replications (S_0 = 0.3 dS/m (control), S_1 = 1.5 dS/m, S_2 = 3.0 dS/m, S_3 = 5.0 dS/m).

Macro and micro nutrition and leaf chlorophyll constituents

The nutrition contents of leaves obtained for different irrigation water salinity treatments are given in Table 3.

Table 3. Effect of salinity on the macro and micro nutrients content and Ca/Na and K/Na ratios of two-year-old grafted Chandler walnuts onto walnut seedling rootstocks

	Rootstocks	**S_0**	**S_1**	**S_2**	**S_3**
N (%)	*Juglans nigra*	2.85a*A**	2.11b A	2.00b A	1.95b A
	Paradox	2.54a A	2.19b A	2.07b A	1.95b A
	J. sieboldiana	2.66a A	2.18b A	2.07cb A	1.83c A
P (%)	*Juglans nigra*	0.37a A	0.21b A	0.21b A	0.23b A
	Paradox	0.28a A	0.26a A	0.23a A	0.24a A
	J. sieboldiana	0.33a A	0.22b A	0.24b A	0.23b A
K (%)	*Juglans nigra*	2.35b A	2.52b A	2.92ab A	3.44a A
	Paradox	2.01b A	2.75b A	3.32ab A	3.44a A
	J. sieboldiana	2.24b A	2.68b A	2.99ab A	3.42a A
Na (%)	*Juglans nigra*	0,49b B	0,61b B	0,88b B	1,41a C
	Paradox	0,74c A	0,89cb A	0,95b B	1,97a B
	J. sieboldiana	0,84c A	1,03c A	2,10b A	3,41a A
Cl (%)	*Juglans nigra*	0.17c C	0.62b B	0.86a A	0.88a A
	Paradox	0.41c A	0.71b A	0.75b B	0.99a A
	J. sieboldiana	0.28c B	0.57b B	0.88a A	0.91a A
Ca (%)	*Juglans nigra*	0.45a A	0.45a A	0.53a A	0.60a A
	Paradox	0.43a A	0.46a A	0.46a A	0.53a A
	J. sieboldiana	0.30b A	0.44ab A	0.47a A	0.49a A
Mg (%)	*Juglans nigra*	0.54a A	0.60a A	0.68a A	0.69a A
	Paradox	0.50a A	0.58a A	0.66a A	0.67a A
	J. sieboldiana	0.51a A	0.52a A	0.55a A	0.59a A
Cu (ppm)	*Juglans nigra*	19.38a B	16.79b A	17.20ab A	16.76b B
	Paradox	18.91a B	19.88a A	16.32a A	18.89a A
	J. sieboldiana	23.33a A	15.96b A	17.76b A	17.28b AB
Fe (ppm)	*Juglans nigra*	409.20a A	343.53a A	286.14a A	266.32a A
	Paradox	356.44a A	347.13a A	335.67a A	308.08a A
	J. sieboldiana	341.65a A	251.96a A	246.58a A	239.36a A
Mn (ppm)	*Juglans nigra*	84.56a A	90.92a A	114.84a A	117.30a A
	Paradox	143.42a A	177.52a A	140.46a A	103.49a A
	J. sieboldiana	70.58a A	84.44a A	80.05a A	89.40a A
Zn (ppm)	*Juglans nigra*	25.15a A	23.05a A	23.26a A	22.88a AB
	Paradox	24.00a A	25.38a A	21.10a A	25.50a A
	J. sieboldiana	28.66a A	21.32b A	22.53b A	20.82b B
Ca/Na	*Juglans nigra*	0.93a A	0.75a A	0.74a A	0.43a A
	Paradox	0.60a AB	0.52ab AB	0.49ab AB	0.27b AB
	J. sieboldiana	0.47a B	0.37ab B	0.21cb C	0.14c B
K/Na	*Juglans nigra*	0.48a A	0.41ab A	0.38ab A	0.24b A
	Paradox	0.27a C	0.25a AB	0.19ab AB	0.12b B
	J. sieboldiana	0.35a B	0.26b AB	0.14c B	0.10c B
Chlorophyll a (mg/g)	*Juglans nigra*	4.59a A	3.32b A	2.83b A	2.39b A
	Paradox	4.56a A	3.78a A	2.84b A	2.37b A
	J. sieboldiana	4.72a A	3.63ab A	2.48cb A	2.17c A
Chlorophyll b (mg/g)	*Juglans nigra*	1.30a A	1.08b A	0.86c A	0.78c A
	Paradox	1.24a A	0.94ab A	0.81b A	0.61b B
	J. sieboldiana	1.15a A	0.97b A	0.73c A	0.64c B

	Rootstocks	S_0	S_1	S_2	S_3
Chlorophyll total (mg/g)	*Juglans nigra*	5.89a A	4.40b A	3.69cb A	3.17c A
	Paradox	5.80a A	4.72ab A	3.65cb A	2.98c A
	J. sieboldiana	5.87a A	4.60b A	3.21c A	2.81c A

Means followed by the same letter are not significantly different between salt treatments (*), and rootstocks (**) according to Duncan's multiple range test at 0.05 significance level

Under salt stress conditions, Na^+ and Cl^- toxic ions accumulating in plant tissues compete with other nutrients and disrupt the plant's feeding mechanism. In our study, the enough stress was created to determine the reactions of different walnut rootstocks to salt stress.

Leaf N content varied between 2.54% (*PR*) to 2.85% (*JN*) for S_0 and between 1.83% (*JS*) to 1.95% (*JN* and *PR*) for S_3 treatments. Leaf P content varied between 0.28% (*PR*) to 0.37% for S_0 salinity treatments and between 0.23% (*JS*, *JN*) to 0.24% (*PR*) for S_3 treatments (Table 2). Leaf K^+ content ranged from 2.01% (PR) to 2.35% (JN) for S_0 treatments, and between 3.42% (*JS*) to 3.44% (*PR*, *JN*) for S_3 treatments. The K^+ contents were determined as 3.44 %, 3.42% and 3.42% for the *JN*, *PR* and *JS* rootstocks, respectively (Table 3).

The Na^+ ion content of leaf increased in salt application. The average Na^+ content was determined as 1.41 for the *JN*, 1.97 for the *PR* and 3.41 for the *JS* under S_3 treatments. *JN* displayed less Na^+ ion accumulation in comparison with the *PR* and *JS* (Table 3). At S_3 salt application, *JN*, *PR* and *JS* rootstocks accumulated significantly higher ($P < 0.05$) sodium when compared to the controls. At S_3, the highest sodium concentration was measured in *JS* rootstocks (3.41%) and *PR* (1.97%), while the lowest concentration was found in *JN* (1.41%). In our study, significant difference was found between rootstocks in S_3 treatments (Table 3). The average leaf Cl^- content was determined to be 0.88 for *JN*, 0.99 for the *PR* and 0.91 for the *JS* in S_3 treatment. The lowest Cl^- ion accumulation was determined in the *JN* (Table 3). No difference was found between rootstocks in S_3 application in leaf Cl^- content. The highest Cl^- accumulation was determined in *PR* rootstock.

The K/Na ratio in young leaves decreased with increasing salt concentrations in all rootstocks. The leaf K/Na values changed between 0.24 (*PR*) to 0.10 (*JS*) for S_3 salinity treatments and the Ca/Na ratio varied between 0.43 (*JN*) to 0.4 (*JS*) for S_3 treatments. The K/Na and Ca/Na ratios decreased in general depending on salinity stress. At higher levels of salinity, *JN* rootstocks was able to maintain a higher K/Na ratio than the other rootstocks. According to S_0 application, the decrease of Ca/Na ratio in S_3 application was calculated as 2.16 times in *JN* rootstock, 2.22 times in *PR* rootstocks and 3.35 times in *JS* rootstocks compared to control. Significant differences were determined between *JN* and *JS* rootstocks in S_3 application Ca/Na ratios (Table 3).

Discussion

Low levels of salinity did not reduce the diameter of rootstocks and shoot, shoot length, the number of leaf and lateral root of the walnut rootstocks during the course of experiment (Table 2). At S_2 salt treatment, the negative effect of salinity started to appear on the three rootstocks. At the end of the experiment the shoot lengths of *JN*, *PR* and *JS* were impacted the most at S_3 application where lengths were 56.18, 56.84 and 56.60%, respectively, of those in the non-saline control. It has been determined that depending on the salt concentration, development of rootstocks would be influenced. Decrease in the rootstock and shoot diameter was observed in *JN* rootstocks, and decrease in the root length was observed in *JS* rootstocks under S_3 salt

treatments. In S_3 salt treatment, a significant difference was found among rootstocks in the case of plant diameter, rootstock diameter, and root length values. Shoot lenght, lateral root number, fresh root number, and leaf number were not significantly affected (Table 2). Non-significant differences were observed among the three rootstocks in the case of indicated traits for S_3 treatments. Caprile and Grattan (2006) found that the most susceptible species to salt stress is *J. regia*, the moderately sensitive species is *Paradox*, and the most resistant species *is J. nigra*. When the effect of salt stress on rootstock diameter, shoot diameter and root length was examined, statistical differences were found among the rootstocks. When the effect of salt stress on shoot length and root length was examined, *Paradox* rootstock was more tolerant than the the other two rootstocks. The negative effect of increased salt stress on shoot and rootstock development can be caused by excessive toxic ions accumulating in the tissues. Imbalances in plant ion uptake and impaired cell function of plants negatively affect photosynthesis and respiration (Leopold and Willing, 1984). Akça and Samsunlu (2012) stated that plant growth decreased due to increased salinity stress in the seedlings obtained from the seeds of Kaman 1, Kaman 5 and Bilecik walnut varieties. The similar results was obtained by Lotfi et al. (2009 and 2019) in reducing germination of walnut seeds under salinity stress. In the research conducted to determine the performance of pear, quince and wild pears plants under salt stress conditions, the number of shoots, shoot length and shoot diameter were negatively affected in salt stress exposed plants (Javadisaber et al., 2016).

In a study examining the performance of almond rootstocks under salt stress conditions (0, 25, 50 and 75 mM NaCI salt) it was reported that serious decreases in shoot growth were detected under salt stress (Zrig et al., 2016). In the application of salt to the pomegranate plants, salinity negatively affected the number of lateral shoots, mainly in the highest salt concentrations, and the stem diameter at the end of the experiment was affected negatively only in the 120 mM KCl (2.24 mm), compared to control (2.69 mm) (Mastrogiannidou et al., 2016).

In pistachio plant (Ilcıl et al., 2016), root length decreases were determined due to increased salt application. Determination of tolerance levels of salt stress of wheat, tomato, bean and corn plants was tested following exposure to 0, 75, 150 and 250 mM NaCI salt. Based on the obtained result, root lengths of plants were negatively affected in all plant species, especially at 150 and 250 mM salt level, and a significant difference was found among salt concentrations (Aydın and Atıcı 2015). Salt stress in Şebin walnut variety negatively affected root development and significant differences were found between salt applications. In another study, the number of new roots was determined between 5.3 (S_0) and 0.2 (S_2) (Şimşek et al., 2017). The regular growth and development of the plant was prevented, and new root development and shoot growth were negatively affected by salt stress conditions (Soldatini and Giannini, 1985). We found similar results in our research as well.

In our study, decreases in plant growth parameters were observed due to increased salt stress in Chandler variety, which was grafted on the three rootstocks. Salt stress prevented the growth in a salt stress level dependent manner. Under increasing salt stress conditions, the stomata close and photosynthesis slows down. If stress conditions persist, plant development may totally stop (Ashraf, 1994). In our study, it was not possible to reach clear results to differentiate salt stress tolerance levels among the rootstocks in terms of growth parameters of plants. Longer-term studies are required for yield-aged trees to see differences among the rootstocks. Despite all these results, according to the plant growth parameters salinity

tolerance of the rootstocks may be arranged as *Paradox* >*J. sieboldiana* >*J. nigra.*

Correlation coefficients between salt doses and water consumption were found as -0.725 in *PR*, -0.224 in *JS* and -0.637 in *JN*. As salinity increased, water consumption of Chandler plant that is grafted onto three rootstocks decreased (Fig 1.). Plant water consumption decreased significantly with gradually increasing soil salinity, as shown by a medium negative linear relation (r =-0.66) between the two lettuce (*Lactuca sativa var. Crispa*) variables (Ünlükara et al., 2010).

Due to the increasing salt applications in all rootstocks, an increase in Na^+, Cl^-, Ca^{++}, Mg^{++}, K^+, Mn content, and a decrease in N, P, Fe and Ca/Na and K/Na ratios were detected. As a matter of fact, in the salt application on S_3, leaf Na^+ and Cl^- contents were found to be much higher than the values predicted by Caprile and Grattan (2006) for salt stress. Depending on the increased salt doses, a decrease in leaf N content was observed. Akça and Samsunlu (2012) stated that the leaf N content decreased in Bilecik, Kaman 1 and Kaman 5 cultivars, similar to our results, due to increased salt stress. In order to determine the reactions of Gemlik olives (*Olea europaea* L.) cultivar against salt stress, an irregular change in leaf N content was observed due to the increase in salt stress (Kasırga, 2009).

In the research conducted to determine the resistance of Camarosa strawberry variety to salt stress, leaf P content decreased with increasing salt application (Turhan and Eriş, 2004). Our research results were found to be compatible with the decreases in leaf P content of walnuts due to increased salt applications (Akça and Samsunlu, 2012; Akça et al., 2017).

Depending on the increased salt doses, increase in leaf K^+ content was observed (Table 2). Under high saline conditions, the high K^+ uptake may be the reason for avoiding salt stress. A significant difference was found between control and S_3 applications in content of K^+. However, the effect of salt treatments on leaf K^+ content was found insignificant among rootstocks. Our previous studies also showed increase in K^+ content of walnuts due to increased salt applications (Akça and Samsunlu, 2012; Akça et al., 2017).

Na^+ accumulation rate in S_3 application was 65% in *JN* rootstocks and 62% in *PR* rootstocks and 75% in *JS* rootstocks higher than the control. The highest Na^+ accumulation rate was observed in *JS* rootstock (Table 3). Na^+ accumulating in excessive amounts in plants under salt stress prevents the intake of K^+ (Siegel et al., 1980). Cl^- accumulation also prevents NO_3 uptake (Güneş and al. 1994; İnal and al. 1995). Excessive Na^+ and Cl^- accumulation disrupts the ion balance of plants. In salt stress conditions, Na^+ accumulates in the leaves and is replaced with Mg^{++}. In walnuts, the content of 0.3% chloride, 0.1% sodium and 300 ppm boron in leaf tissue is considered to be very extreme levels (Caprile and Grattan, 2006). Baoqing et al. (2020) reported that salinity and combined stress significantly increased Cl^- contents of leaves, stems, and roots in both walnuts. The Cl^- contents of leaves and stems were significantly affected by the irrigation × salinity interaction, and the root Cl^- content was significantly affected by the genotype × salinity interaction.

In the research on salt resistant species, it was found that the distribution of Na^+ and Cl^- ions in the green parts of the plant is important in the varieties removing salt. Salt-resistant plants impede Na^+ and Cl^- ions in the old leaves and prevent their passage to young leaves (Wolf et al 1991). In our study, although there was no difference among the rootstocks in terms of Cl^- leaf content in S_3 salt application, significant differences were found in the case of Na^+ content. Our results were found to be more complicated compared to the findings of Wolf et al. (1991).

K/Na drop rate in S_3 application was determined as 2 times in *JN* rootstock, 2.25 times in *PR* rootstocks and 3.5 times in *JS*

rootstocks compared to S_0 application. Despite the increase in Na content as a result of salinity, the decrease in Ca/Na and K/Na ratio are common results in the literature (Akça and Samsunlu 2012). Due to the increased salt application, regular decreases in K/Na and Ca/Na ratios were found to be consistent with the results of Akça and Samsunlu (2012) and Akça et al., (2017). Depending on the increase in salt stress in strawberry plants, leaf Ca/Na contents decreased and K/Na contents increased (Torun et al., 2007). Shirazi et al., (2005) stated that giving K^+ to the plant increases the K/Na ratio. Yıldız and Üzal (2014) found that strawberry varieties had an increase in leaf Na^+ and Cl^- contents and a decrease in K/Na ratio due to increased salt stress. The Istarska bjelica and Oblica cultivars had the lower Na^+ concentrations, the higher K/Na ratios, and the higher relative shoot growth at the 100 mM NaCl. These results are in accordance with the previous studies (Benlloch et al., 1991; Tatini et al., 1992; Chartzoulakis et al., 2002) who reported that the more tolerant olive cultivars are able to reduce Na^+ accumulation in the leaves and maintain high level of K^+, thus maintaining a higher K/Na ratio. Based on this experiment, Istarska bjelica and Oblica cultivars appear to be more tolerant to salinity than the others tested cultivars. However more research is needed to test their performance, particularly on mature bearing trees, under filed conditions.

A significant difference was determined between *PR* and *JS* rootstocks in leaf Zn content of S_3 application. In terms of leaf Cu content, a difference was found between *JN* and *PR* in S_3 application. In S_3 application, there was no difference among rootstocks in Ca leaf content. Increases in leaf Ca content were observed due to increased salt application, but there was no statistical difference among rootstocks and salt treatments. A statistically significant difference was found between *JN* and *PR* rootstocks in Cu content of S_3 treatments (Table 3). Our results are consistent with the other studies (Akça and Samsunlu, 2012; Akça et al., 2017).

The chlorophyll a and chlorophyll b content decreased by 38.27% and 32. 32% respectively as a result of the 5 dS/m salt application in comparison with the control application. Decrease in chlorophyll content was determined as a result of increasing salt applications. The greatest leaf chlorophyll a content was determined in *JN* and *PR* rootstocks whereas the greatest chlorophyll b content was determined in *JN* rootstocks. The chlorophyll a and total chlorophyll content of rootstocks was not statistically significant in S_3 (Table 3). Decrease in chlorophyll content under salinity stress is observed more in salt sensitive genotypes in comparison with cultivars with low salt tolerance (Khan et al., 2009).

Conclusion

J. regia is considered to be the most susceptible species to salt stress, *Paradox* as the moderately sensitive species, and the most resistant species is *J. nigra* (Caprile and Grattan, 2006). The results of our study were similar to those of Caprile and Grattan (2006). The rootstocks showed the difference in the plant water consumption, plant growth, content of macro and micro nutrients and chlorophyll content under salinity stress condition. Depending on the increased salinity stresses, shoot length, plant diameter, average root length and average root number and the number of leaf decreased. Depending on the salinity levels; K^+, Ca^{++}, Mg^{++}, Na^+, and Cl^- contents of leaf increased. N and Fe contents of leaf, Ca/Na and K/Na ratios of leaf decreased by salt applications. When Na^+, Cl^-, K^+, chlorophyll content, K/Na, and Ca/Na ratios were taken into consideration, the tolerance levels of rootstocks to salt stress can be arranged as *JN*$^{>}$ *PR* $^{>}$ *JS*.

Acknowledgement

We acknowledge Prof. Dr. Kourosh Vahdati for scientific editing of the manuscript.

Conflict of interest

The authors indicate no conflict of interest for this work

References

Akça Y, Samsunlu E. 2012. The effect of salt stress on growth, chlorophyll content, proline and nutrient accumulation, and K/Na ratio in walnut. Pakistanian Journal of Botany 44(5), 1513-1520.

Akça Y, Şimşek H, Çekiç Ç, Ünlükara A, Muradoğlu F. 2017. Effects of salt and water stress on walnut of the leaf nutrıent accumulatıon. Journal of International Scientific Publications 5, 1314-8591.

Ashraf M. 1994. Breeding for salinity tolerance in plants. Critical Reviews in Plant Sciences 13, 17-42.

Awang Y.B., Atherton J.G. 1994. Salinity And Shading Effects On Leaf Water Relations And Ionic Composition Of Strawberry Plants Grow On Rockwool. Journal of Horticultural Science 69, 377-383.

Aydın İ, Atıcı Ö. 2015. Tuz Stresinin Bazı Kültür Bitkilerinde Çimlenme ve Fide Gelişimi Üzerine Etkileri. Muş Alparslan Üniversitesi Fen Bilimleri Dergisi 3(2), 2149-6455.

Ayers R.S., Westcot, D.W. 1985. Water quality for agriculture. FAO Irrigation and Drainage Paper 29. Rev. 1. FAO United Nations, Rome. 174 pp.

Baoqing W., Junpei Z., Dong P., Lei Y. 2020. Combined effects of water stress and salinity on growth,physiological, and biochemical traits in two walnut genotypes. Physiologia Plantarum 172(1), 1–12.

Batchelor L.D, Braucher O.L, Serr E.F. 1945. Walnut production in California, Univ. of California Agricultural Experiment Station 364, 34.

Behrooz A., Vahdati K., Rejali F., Lotfi M., Sarikhani S., Leslie C.A. 2019. Arbuscular mycorrhiza and plant growth-promoting bacteria alleviate drought stress in walnut. HortScience 54(6), 1087–1092.

Behzadi Rad P., Roozban M.R., Karimi S., Ghahremani R., Vahdati K. 2021. Osmolyte accumulation and sodium compartmentation has a key role in salinity tolerance of pistachios rootstocks. Agriculture. 11(8), 708.

Benlloch M.F., Arboleda Barranco D., Fernandez-Escobar R.1991. Response of young olive trees to sodium and boron excess in irrigation water. HortScience 26(7), 867-870.

Caprile J., Grattan S. 2006. Evaluation of the Salt Tolerance of Walnut Rootstocks. Hortscience, 41, 1040-1040.

Chartzoulakis K., Loupassaki M., Bertaki M., Androulakis I. 2002. Effects of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars. Scientia Horticulturae 96, 235-247.

Dehghan B., Vahdati K., Rezaee R., Hassani D. 2010. Walnut grafting success as affected by different grafting methods, cultivars and forcing treatments. Acta Horticulturae 861, 345-352.

Düzdemir O., Kurunc A., Ünlükara A. 2009. Response of Pea (*Pisum sativum*) to salinity and irrigation water regime. Bulgarian Journal of Agricultural Science 15(5), 400-4009.

Ebrahimi, A., Vahdati, K. 2007. Improved success of Persian walnut grafting under environmentally controlled conditions. International Journal of Fruit Science. 6(4), 3-12.

Fulton A.E, Oster J, Nanson B. 1988. Salinity Management of Walnut. In: Walnut Production Manual, Eds. D.E. Ramos, University of California, Division of Agriculture and Naturel Resources, 54-65.

Greenway H., Munns R. 1980. Mechanism of salt tolerance in nonhalophytes. Annual Review of Plant Physiology 31, 149-190.

Güneş A., Post W.H.K., Kirkby E.A., Aktaş M. 1994. Influence of partial replacement on nitrate by amino acid nitrogen or urea in the nutrient medium on nitrate accumulation in NFT grown winter lettuce. Journal of Plant Nutrition 17, 1929-1938.

Hendricks L.C., Gripp R.H., Ramos D.E. 1977. Walnut production in California, Univ. California Div. Agricultural Science Leaflet.

Ilcil F., Acar I., Ak B.E. 2016. Salt resistance of 'Uzun' and 'Siirt' pistachio seedling. Acta Horticulturae 1139, 68.

İnal A., Güneş A., Aktaş M. 1995. Effects of chloride and partial substitution of reduced forms of nitrogen for nitrate in nutrient solution of the nitrate total nitrogen and chloride contents of onion. Journal of Plant Nutrition 18, 2219-2227.

Javadisaber J. 2016. Bazi armut anaçlarında in vitro koşullarda tuzluluk stresi altında gen ifade analizi üzerinde çalışmalar. Doktora tezi, Ankara Üniversitesi, Türkiye.

Kasırga E. 2009. Tuzluluğun Gemlik Zeytin (*Olea Europaea L.*) Çeşidine Etkilerinin İncelenmesi. Master tezi, Adnan Menderes Üniversitesi , Türkiye.

Khan M.A., Shirazi M.U., Muhammad Ali Khan S.M., Mujtaba E., Islam S. Mumtaz A., Shereen R.U., Yasın Ashraf M. 2009. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Trıtıcum Aestıvum* L.). Pakistan Journal of Botany 41(2), 633-638.

Leopold A.C., Willing R.P. 1984. Evidence for toxicity effects of salt on membranes. In: R. C. Staples, and G. H. Toenniessen (Eds) Salinity Tolerance in Plants, Strategies for Crop improvement. A Wiley Inter Science Publication, Toronto, Singapore, 67-76.

Levitt J. 1980. Responses of Plants to Environmenta l Stresses. Vol. II Water, Radiation, Salt and other Stresses. Academic Press Inc. New York, pp. 607.

Li Y, Duan B.L., Chen J., Korpelainen H., Niinemets Ü., Li C. 2016. Males exhibit competitive advantages over females of *Populus deltoides* under salinity stress. Tree Physiology 36, 1573–1584.

Lotfi N., Soleimani A., Vahdati K., Çakmakçı R. 2019. Comprehensive biochemical insights into the seed germination of walnut under drought stress. Scientia Horticulturae 250, 329-43.

Lotfi N., Vahdati K., Kholdebarin B., Najafian Ashrafi E. 2009. Germination, mineral composition and ion uptake in walnut under salinity conditions. HortScience. 44(5), 1352–1357.

Lotfi N., Vahdati K., Kholdebarin B., Hassani D., Amiri R. 2009. Mineral composition of some walnut cultivars (*Juglans regia* L.) for evaluation of ionome and ionomics under salt stress condition. Acta Horticulturae 839, 293-300.

Mane A.V., Deshpande T.V., Wagh V.B., Karadge B.A., Samant J.S. 2011. A critical review on physiological changes associated with reference to salinity. International Journal of Environmental Sciences 6, 1192-1216.

Mastrogiannidou E, Chatzissavvidis C., Antonopoulou C., Tsabardoukas V., Giannakoula A., Therios I. 2016. Response of pomegranate cv. wonderful plants to salinity. Journal of Soil Science and Plant Nutrition 16 (3), 621-636.

Polle A., Chen S.L. 2015. On the salty side of life: molecular, physiological and anatomical adaptation and acclimation of trees to extreme habitats. Plant, Cell and Environment 38, 1794–1816.

Rezaee R., Vahdati K., Grigoorian W., Valizadeh M. 2008. Walnut grafting success and bleeding rate as affected by different grafting methods and seedling vigor. The Journal of Horticultural Science & Biotechnology. 83(1), 94-99.

Rezaei Qusheh Bolagh F., Solouki A., Tohidfar M., Zare Mehrjerdi M., Izadi-Darbandi A., Vahdati K. 2020. *Agrobacterium*-mediated transformation of Persian walnut using BADH gene for salt and drought tolerance, The Journal of Horticultural Science and Biotechnology, 95(4), 1-10.

Sheikh Beig Goharrizi M.A., Dejahang A., Tohidfar M., Izadi Darbandi A., Carillo N., Hajirezaei M.R., Vahdati K. 2016. Agrobacterium mediated transformation of somatic embryos of Persian walnut using fld gene for osmotic stress tolerance. Journal of Agricultural Science and Technology. 18, 423-435.

Shirazi M.U., Ashraf M.Y., Khan M.A., Naqvi M.H. 2005. Potassium induced salinity tolerance in wheat (*Triticum aestivum* L.). International Journal of Environmental Science & Technology 2(3), 233-236.

Şimşek H., Akça Y., Çekiç Ç., Ünlükara A. 2017. Şebin ceviz çeşidinin stres koşullarına dayanımının belirlenmesi. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi 34, 143-15.

Soldatini G.F, Giannini A.1985. The effect of water and salt stress on the fixation of 14CO2 and on amino acid metabolism in seedlings of *Zea mays* L. Agrochimica 29, 74.

Taleisnik E, Grunberg K. 1994. Ion balance in tomato cultivars differing in salt tolerance, I. Sodium and potassium accumulation and fluxes under moderate salinity. Physiologia Plantarum 92, 528-534.

Tatini M., Gucc R., Coradeschi M.A., Ponzio C., Everard J.D. 1995. Growth, gas exchange and ion content in Olea europea plants during salinity stress and subsequent relief. Physiolia Plantarum 95, 203-210.

Torun A, Kaçar Y., Kıllı O., Erdem H., Yardım P., Serçe S., İnan S. 2007. Bazı Fragaria chıloensıs genotiplerinin değişik naci konsantrasyonlarına in vitro çoğaltma sırasındaki tepkileri. Batı Akdeniz Tarımsal Araştırma Enstitüsü Derim Dergisi 24(2), 27-33.

Turhan E., Eris A. 2004. Effects of sodium chloride applications and different growth media on ionic composition in strawberry plant. Journal of Plant Nutrition 27(9), 1653–1665.

Ünlükara A., Cemek B., Karaman S., Erşahin S. 2010. Response of lettuce (*Lactuca sativa var.* Crispa) to salinity of irrigation water, New Zealand Journal of Crop and Horticultural Science 36(4), 265-273.

Ünlükara A., Kurunç A., Kesmez G.D., Yurtseven E. 2008. Growth and evapotranspiration of okra (*Abelmoschus esculentus* L.) as influenced by salinity of irrigation water. Journal of Irrigatıon and Drainage Engineering-ASCE 134,160-166.

Ünlükara A., Kurunç A., Kesmez G.D., Yurtseven E. 2008. Growth and evapotranspiration of *Abelmoschus esculentus* L. as influenced by salinity of irrigation water. Journal of Irrigation and Drainage 134, 160-166.

Vahdati K. (2014) Traditions and folks for walnut growing around the silk road. Acta Horticulturae 1032, 19-24.

Vahdati K., Bavani A.R., Khosh-Khui M., Fakour P., Sarikhani S. 2019. Applying the AOGCM-AR5 models to the assessments of land suitability for walnut cultivation in response to climate change: A case study of Iran. PloS ONE. 14(6), e0218725.

Witham F.H., Blaydes D.F., Devlin R.M. 1971. Experiments in plant physiology. New York, Van Nostrand Reinhold.

Wolf O., Munns R., Tonnet M., Jeschke W.D. 1991. The Role of the Stem in the Partitioning of Na+ and K+ in Salt-Treated Barley. Journal of Experimental Botany 42, 278-282.

Yadollahi A., Lotfi N., Vahdati K., Hassani D., Kholdebarin B., Amiri R. 2010. Morphological and physiological responses to water stress for seedlings of different walnut genotypes. Acta Horticulturae 861, 253-262.

Yıldız K., Üzal Ö. 2014. Bazı çilek (*Fragaria ×ananassa* L.) çeşitlerinin tuz stresine tepkileri. YYÜ Tarım Bilimleri Dergisi 24(2), 159- 167.

Zrig H., Mohammed H.B. Tounekti T. Khemira H. Serrano M. Valero D. Vadel A.M. 2016. Effect of rootstock on salinity tolerance of sweet almond (cv. Mazzetto). South African Journal of Botany 102, 50–59.

Siegel S.M.,Siegel B. Z. Massey J. Lahne P. and Chen J. 1980. Growth of Corn in Saline Waters. Physiol. Plant, 50, 71-73.Combined effects of water stress and salinity on growth,

physiological, and biochemical traits in two walnut genotypes

ombined effects of water stress and salinity on growth,

physiological, and biochemical traits in two walnut genotypes

Combined effects of water stress and salinity on growth,

physiological, and biochemical traits in two walnut genotypes