EFFECTS OF SALT AND WATER STRESS ON WALNUT OF THE LEAF NUTRIENT ACCUMULATION

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Abstract

This study was conducted to determine the effects of salt and water stress on the accumulation of macro and micro elements in walnut. Two-old year walnut samples from plants grafted onto seedling rootstocks of Juglans regia L. *were used. The experiment was designed by factorial design in randomized blocks and lasted for 2 years in pots. In the research, the plants were exposed to five irrigation water salinity levels (C_0=Control, C_1=1, C_2=2, C_3=3 and C_4=5 dS.m^{-1}) and three irrigation water rates (I_1=1 liter/week, I_2=3 liters/week and I_3=5 liters/week) with four replications. Soil salinity was increased with increasing irrigation water salinity. Plants couldn't survive at 11 dS.m^{-1} soil salinity under I_3 conditions while dead plants were observed at 2.68 dS.m^{-1} soil salinity under I_1 conditions. Higher soil salinity caused increases in leaf Fe, K, Na and Cl accumulations. Leaf Cl content varied between 100 ppm (S_0-I_1) to 970 ppm (C_2-I_2) and Na content ranged from 146.1 ppm (C_0-I_3) to 227.8 (C_2-I_3). K/Na and Ca/Na ratios of leaf were decreased under salinity conditions.*

Key words: *K/Na ratio, salinity stress, walnut*

1. INTRODUCTION

Walnut is among fruit species that are sensitive to soil salinity and salinity of irrigation water. Toxicity symptoms in the leaves proceed towards the edges of the leaf from the tip as necrosis, and cover the whole of the leaf in severe situations. Leaf necrosis deteriorates starting from the middle of summer towards the end of the season, and early losses of leaves are commonly observed. The symptoms are basically the same for excessive chloride sodium and bor.

Soils with rough texture and sandy soil types are not desired for walnut growing (Begg, 1985; Losche and Phares, 1972; Ponder, 1981). This situation mostly stems from low water retention capacity and from the lack of water for long durations or for some periods. However, the low-level plant nutrient element contents in these soil types may also be another negative factor.

The development of the plants grown in saline soil types is limited (Flowers and Yeo, 1981). Insufficient use of water by the plants due to increasing osmotic potential in the soil or excessive Na and Cl ions in salty soil types and the disruptions in ion balance of the plants are shown as the reason for this decrease in growth (Lewitt, 1980).

In recent years, researchers have tried to choose plant types that have tolerance to salt or have tried to breed them with genetic studies. It has been reported that the plant types that absorb less sodium and chlorine ions are more tolerant to salt stress. The salt rate in the soil is one of the most important factors that affect the yield in fruit species. When salinity of the soil is 2.3 dS/m, the walnut yield decreases at a rate of 10%; however, when salinity increases in two folds (4.8 dS/m), the decrease in the fruit yield reaches 50%. This situation shows that walnuts are sensitive to increases in salinity after a certain level.

0.3% chloride, 0.1% sodium and 300 ppm boron contents in the leaf tissue are accepted as excessive amounts. Studies that are conducted under controlled conditions are needed to determine the difference in the absorbance and transport capacity of salt. In order to determine whether there are differences in the reaction levels against osmotic elements of salt stress, absolute results are necessary.

This study was conducted to determine the effects of salt stress on the accumulation of macro and micro elements in walnut.

2. MATERIAL AND METHODS

Two-year old Şebin walnut cv saplings grafted on *Juglans regia* L. seedling were used in the study. The plants were planted in pots with dimensions of 45 cm × 60 cm. Sodium bicarbonate ($NaHCO_3$), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) salts were used as salt sources. Some characteristics of the soils used in the study are given in Table 1.

Table 1. Some characteristics of the soil

Sand (%)	41	Volume weight ($g.cm^{-3}$)	1.42
Silt (%)	31	Field capacity (cm^3/cm^3)	0.24
Clay (%)	28	Wilting point (cm^3/cm^3)	0.14
Soil texture	Clay-loam	Total available water (cm^3/cm^3)	0.10

2.1. Irrigation water salinity level and irrigation water rates

In the study, effects of five irrigation water salinity levels (C_0= 0.3 dS/m-control, C_1= 1 dS/m, C_2= 2 dS/m, C_3= 3 dS/m and C_4= 5 dS/m) and three irrigation water rates (I_1= 1 liter/week, I_2= 3 liters/week and I_3= 5 liters/week) on Şebin walnut were investigated. The plants in the control group were watered using tap water with constant salinity. The experiment that was designed by factorial design in randomized blocks with 15 treatments and lasted for 2 years. Each treatment was replicated 4 times.

Saline irrigation waters were prepared with three different salts ($NaHCO_3$, $MgCl_2$ and $CaCl_2$). SIW Salinity Appraisal Laboratory Set was used for preparing saline waters. To prevent detrimental effect of Na on soil physical conditions, Sodium Adsorption Ratios (SAR) of saline waters were kept between 0.17-0.38. After checking of electrical conductivity of saline irrigation waters, plant irrigations were carried out according to the treatments at each week except early several weeks in first year with tap water to ensure plant stand. The results were analyzed via the SPSS statistics software (Standard version 11.0).

2.2. Leaf mineral content analysis

Leaf analyses were carried out on two leaf samples collected at the beginning of the growth season and end of July to determine mineral accumulation 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 was 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).

3. RESULTS AND DISCUSSION

The salinity levels of soil saturation paste extracts for the treatments were presented in Table 2. The change in the salinity of the soil was found significant at 0.01 level for the saline irrigation water treatments, irrigation water rates and salinity-water rate interactions. Soil salinity increased with increasing irrigation water salinity levels and irrigation rates. The lowest mean soil salinity (0.66 dS/m) was observed under S_0 while the highest mean soil salinity was observed under C_4. The highest mean soil salinity was determined for I_3 irrigation water rates (6.0 dS/m), while the lowest one (2.06 dS/m) for I_1 irrigation water rates (Table 2). The walnut water consumption increased as increased irrigation rates and higher salt accumulation was occurred without leaching.

Table 2. The effects of irrigation water salinity levels and the irrigation water rate levels on the salinity of the soil

Irrigation water salinity levels	Irrigation water rates			Mean
	I_1	I_2	I_3	
C_0	0.68	0.73	0.58	0.66 d
C_1	0.96	1.47	1.72	1.38 d
C_2	2.02	3.80	5.71	3.84 c
C_3	2.68	5.34	10.95	6.32 b
C_4	3.95	8.61	11.03	7.86 a
Mean	2.06 c	3.99 b	6.00 a	

Walnut was classified as sensitive plant according to foliar injury (Grieve et al., 2012). Fulton et al. (1988) have reported that salinity values > 4.8 dS/m according to the root region salinity classification cause excessive damage in walnut cultivation. In our results, these values show that sufficient salinity stress has been obtained in the research material. As a result of Akça and Samsunlu (2012), the salt accumulation in the soil varied between 4.72-10.39 (dS/m).

3.1. The effects of irrigation water salinity levels and the irrigation water rate on the accumulation of mineral substance in leaves

In the second year of the study, all of the plants exposed to C_3 and C_4 irrigation water salinity levels could not survive and died by the end the growth season in second year. For this reason, in the second year of the study, leaf mineral analysis was only carried out for the plants exposed to C_0, C_1 and C_2 irrigation water salinity levels with I_1, I_2 and I_3 irrigation water rates. The macro and micro element contents of the leaf samples for 2nd year of the study were presented in Table 3. According to the statistical analysis, the difference between the macro and micro contents of the leaf samples in the first year of the study was not found significant according to the irrigation water salinity levels and irrigation water rates.

Table 3. The contents of micro and macro elements according to Irrigation water salinity levels and water rates

Minerals		Irrigation water salinity levels and water rates														
		C_0I_1	C_0I_2	C_0I_3	C_1I_1	C_1I_2	C_1I_3	C_2I_1	C_2I_2	C_2I_3	C_3I_1	C_3I_2	C_3I_3	C_4I_1	C_4I_2	C_4I_3
P (%)	+	0.146	0.150	0.122	0.153	0.140	0.128	0.146	0.125	0.148	0.118	0.130	0.144	0.128	0.128	0.172
	++	0.059	0.079	0.067	0.055	0.088	0.095	0.088	0.063	0.112	0.165	0.074	0.075	0.107	0.074	0.067
	+++	0.16	0.15	0.14	0.19	0.16	0.19	0.29	0.27	0.24						
K (%)	+	1.29	1.24	1.10	1.37	0.88	1.29	1.59	1.18	1.56	1.27	1.21	1.34	1.54	1.53	1.40
	++	1.31	1.15	0.76	1.07	0.73	0.91	1.57	0.90	0.87	2.13	1.03	0.79	1.98	1.29	0.93
	+++	1.10	1.12	1.12	1.34	1.02	1.26	1.48	1.29	1.40						
Ca (%)	+	0.95	0.83	0.82	0.91	0.88	0.95	1.00	0.93	1.09	1.02	1.11	0.98	1.10	1.17	0.98
	++	1.27	1.23	1.31	1.32	1.37	1.60	1.45	1.47	1.84	1.35	1.68	1.51	1.22	1.45	1.50
	+++	1.05	1.14	1.02	1.01	1.16	1.20	0.96	1.40	1.09						
Mg (%)	+	0.40	0.35	0.37	0.39	0.42	0.39	0.40	0.40	0.40	0.40	0.41	0.38	0.40	0.40	0.39
	++	0.37	0.36	0.34	0.37	0.40	0.39	0.37	0.39	0.41	0.38	0.41	0.41	0.34	0.38	0.39
	+++	0.27	0.27	0.25	0.26	0.24	0.27	0.21	0.21	0.14						
Fe (ppm)	+	195.7	167.1	264.2	138.1	164.7	193.7	205.4	171.8	174.0	174.7	-	170.1	189.7	185.4	174.1
	++	460.2	755.1	-	510.6	457.3	390.2	387.8	462.0	423.9	556.9	474.4	428.4	348.0	394.9	392.3
	+++	719.0	697.8	656.7	987.0	768.9	792.2	957.6	1131.4	512.9						
Mn (ppm)	+	68.5	50.4	77.7	75.2	97.3	104.6	64.8	93.5	72.1	80.5	117.9	69.8	75.0	83.9	78.7
	++	58.4	51.2	-	70.1	75.5	94.1	57.4	65.9	81.8	85.1	112.2	72.4	49.6	67.5	75.1
	+++	50.2	45.6	48.9	62.4	46.6	62.9	50.3	56.5	40.7						
Zn (ppm)	+	36.1	27.1	31.3	26.7	29.9	22.7	22.0	28.9	22.2	27.9	43.1	26.1	30.5	28.0	33.7
	++	26.2	54.7	285.0	39.9	32.6	24.4	27.0	28.4	34.8	38.7	44.9	30.4	37.7	29.4	28.1
	+++	47.4	41.9	37.8	46.5	27.1	51.7	88.7	69.4	44.2						
Na (ppm)	+	7.06	1.65	1.14	2.82	0.63	2.69	2.49	1.70	4.63	3.39	5.73	1.53	4.98	4.76	2.59
	++	14.2	11.2	13.9	14.9	12.9	17.1	16.1	13.1	17.4	16.1	14.4	13.2	11.0	13.2	13.0
	+++	159.1	151.6	146.1	174.7	186.0	162.3	191.5	154.2	227.8						
Cl (ppm)	+++	100	120	150	160	610	810	560	970	930						

(First year in May+; First year in July ++, Second year in July+++)

3.2. Phosphorus (P)

The mean phosphorus contents over irrigation rates varied between 0.15% for S_0 and 0.27% (C_2), and the mean phosphorus contents over salinity treatments varied between 0.18% for I_3 and 0.21% for I_1. The effect of salinity on phosphor accumulation in leaves was found significant ($p<0.01$) (Table 4). With increasing salinity, the phosphor uptake also increased. When the highest P accumulation was determined in C_2, less P accumulation was determined in S_0 and C_1 irrigation water salinity levels, which were in the same group.

Although the P accumulation increased together with the increasing salinity under different irrigation water rate, salinity×water interaction was not found significant.

3.3. Potassium (K)

Differences in leaf potassium content was found significant only for salinity treatments at 0.05 probability level. The highest K content 1.39% was determined for C_2 and the lowest values of 1.12 and 1.21% for S_0 and C_1, respectively. Increased salinity level caused increases in K content. K^+ is the most important inorganic plant nutrition especially under saline conditions for turgor regulation (Grattan and Grieve, 1999). Generally cortical root cells have K^+ selectivity against Na^+ and this selectiveness is very important under sodic and saline-sodic conditions. The K^+ selectivity may vary among cultivars and enough Ca and O_2 levels in root zone keep plant K^+ selectivity (Gorham, 1990; Carter, 1983; Drew et al. 1988). It is concluded that walnut has an ability to extract K^+ under saline conditions.

3.4. Calcium (Ca)

Leaf calcium content varied between 1.07% and 1.18% for C_0 and C_2 salinity treatments and between 0.96% and 1.23% for I_1 and I_2 irrigation water rate treatments, respectively. But only irrigation water rates were found significant effect on calcium accumulation in the walnut leaves ($p<0.05$). Ca accumulated at the lowest level in I_1 with the effect of water stress. Ca accumulation increased with increasing water rates especially for I_2. However, slight decrease in Mg content was observed for I_3 treatment (Table 4).

Table 4. The contents of micro and macro elements according to irrigation water salinity levels and water rates for the end of the experiment

Minerals	Irrigation water salinity levels	Irrigation water rates			Mean	Minerals	Irrigation water salinity levels	Irrigation water rates			Mean
		I_1	I_2	I_3				I_1	I_2	I_3	
P (%)	C_0	0.16	0.15	0.14	0.15 b	Zn (ppm)	C_0	47.40	41.90	37.80	42.4 b
	C_1	0.19	0.16	0.19	0.18 b		C_1	46.50	27.10	51.70	41.8 b
	C_2	0.29	0.27	0.24	0.27 a		C_2	88.70	69.40	44.20	70.2 a
	Mean	0.21	0.19	0.18			Mean	58.30	46.10	44.60	
K (%)	C_0	1.10	1.12	1.12	1.12 b	Na (ppm)	C_0	159.1	151.6	146.1	152.3 b
	C_1	1.34	1.02	1.26	1.21 b		C_1	174.7	186.0	162.3	174.3 a
	C_2	1.48	1.29	1.40	1.39 a		C_2	191.5	194.2	227.8	183.0 a
	Mean	1.31	1.14	1.26			Mean	173.6	163.9	168.9	
Ca (%)	C_0	1.05	1.14	1.02	1.07 b	Cl (ppm)	C_0	100.00	120.00	150.00	123.33 c
	C_1	1.01	1.16	1.20	1.12 a		C_1	160.00	610.00	810.00	526.67 b
	C_2	0.96	1.40	1.09	1.18 a		C_2	560.00	970.00	930.00	820.00 a
	Mean	1.01	1.23	1.10			Mean	273.33b	566.67a	630.0a	
Mg (%)	C_0	0.27	0.27	0.25	0.26 a	Ca/Na	C_0	66	75	70	70
	C_1	0.26	0.24	0.27	0.26 a		C_1	58	62	74	65
	C_2	0.21	0.21	0.14	0.19 b		C_2	50	72	48	56
	Mean	0.25	0.24	0.23			Mean	58	70	64	
Fe (ppm)	C_0	719.03	697.80	656.74	691.19 b	K/Na	C_0	69	74	77	74
	C_1	987.03	768.94	792.19	836.87 ab		C_1	65	55	78	66
	C_2	957.63	1131.36	512.91	936.02 a		C_2	77	66	61	68
	Mean	871.01 a	866.03 a	682.15 b			Mean	74	65	72	
Mn (ppm)	C_0	50.20	45.60	48.90	48.20 b						
	C_1	62.40	46.60	62.90	57.30 a						
	C_2	50.30	56.50	40.70	52.20 a						
	Mean	54.70	49.60	54.20							

Slight but non-significant increases in leaf Ca were observed with increasing irrigation water salinity may due to $CaCl_2$ contents of the saline waters. Ca is an important nutrient for plant metabolism functions. Ca keeps cell membrane and its functions, stabilize cell wall structure, regulates ion acquisition and cell wall enzyme activities (Hanson, 1984; Demarty et al., 1984). Conservation of enough Ca in soil extract is an important factor against to Na and Cl toxicities especially fruit trees and vines (Grattan and Grieve, 1999). It may be concluded that saline water with enough Ca contributes Ca uptake by walnut.

3.5. Magnesium (Mg)

Leaf magnesium content significantly affected at 0.01 level due to salinity and decreased from 0.26% for S_0 and C_1 to 0.19% for C_2 (Table 4). Mg was required for plant growth and has beneficial effects to soil water and air movement. Only excessive amount of this cation (3000-5000 mg/l) were found toxic to bean (Ayyıldız, 1990).

Although salinity had negative effect on Mg uptake but irrigation water rates did not cause significant differences in leaf Mg content (Table 4). Mean leaf Mg content over irrigation rates is 0.24%.

3.6. Iron (Fe)

Leaf iron content (ppm) varied between 691.2 (C_1) and 936.0 (C_3) according to salinity treatment and between 682.2 (I_3) and 871.0 (I_1) according to irrigation rates (Table 4). Both salinity and irrigation rates caused significant difference in leaf iron content of the walnut ($p<0.01$). Fe accumulation increased due to salinity effect and water stress.

When compared with the S_0 control irrigation water salinity levels, 1.21 and 1.35 times more Fe accumulation was observed in C_1 and C_2, respectively. The highest Fe accumulation was occurred in I_1 and I_2 irrigation water rates and the lowest Fe accumulation in I_3. Salinity and irrigation rates interaction was also found significant effect on lead Fe accumulation of the walnut. Fe uptake increased for C_1 and C_2 treatments under I_1 while the highest accumulation occurred in C_2 under I_2. Fe accumulation initially increased with increasing salinity and then decreased with increasing salinity under I_3.

3.7. Manganese (Mn)

Leaf manganese content varied between 48.2 (C_0) and 57.3 ppm (C_2) due to effects of salinity and between 49.6 (I_2) and 54.7 ppm (I_1) due to irrigation rates (Table 4). Nor salinity effects and irrigation rates affected significantly on leaf Mn accumulation.

3.8. Zinc (Zn)

Leaf zinc content varied between 41.8 (C_1) and 70.2 ppm (C_2) according to irrigation water salinity and between 44.6 (I_3) and 58.3 ppm (I_1) according to irrigation rates (Table 4). Although these changes in leaf Zn content, only irrigation water salinity affected Zn accumulation, significantly ($p<0.05$). The highest Zn accumulation (70.2 ppm) observed for C_2 treatment while the lowest ones (42.4 and 41.8 ppm) observed for S_0 and C_1 treatments, respectively.

3.9. Sodium (Na)

Primary toxic ions to plants are chloride, sodium and boron. If these ions are taken up by the plants from soil extract or water and accumulated in tissue enough to toxic concentration, crop damage and yield loss occur. The perennial crops such as trees are the more sensitive plants (Ayers and Westcot, 1989).

Leaf sodium content varied between 152.3 (C_0) and 183.0 ppm (C_2) for irrigation water salinity and between 163.9 (I_2) and 173.6 (I_1) for irrigation rates (Table 4). Due to effects of irrigation water salinity and salinity-water rates interaction, Na accumulation in the walnut leaves were affected, significantly ($p<0.01$). C_1 and C_2 irrigation water salinity treatment caused more Na accumulation than C_0 did because these saline waters included more Na content. But it should be noticed that although C_2 treatment had higher Na content, leaf Na content was not significantly higher than C_1 (Table 4). It may be concluded that the walnut has an excluding ability for Na under higher salinity conditions. Na and Cl excluding ability was also reported by Küçükyumuk et al. (2015) for “0900 Ziraat” sweet cherry variety grafted on mahaleb rootstock.

Different irrigation water rates caused differences in Na accumulation. While leaf Na intake increased together with increasing salinity under I_1 and I_3 irrigation water rates, no clear relations were detected between salinity change and Na intake under I_2 irrigation water rate.

3.10. Chloride (Cl)

Chloride content varied between 123.3 ppm (C_0) and 820.0 ppm (C_3) for salinity treatment and between 273.3 ppm (I_1) and 630.0 ppm (I_3) for irrigation rates. Because of the effects of salinity, water application rates and salinity-water rate interaction, leaf Cl accumulation was significantly affected ($p<0.01$). Cl accumulation in the leaves for C_0, C_1, C_2, C_3 were determined as 120, 530, 820 ppm, respectively (Table 4). Increased irrigation water salinity caused increases in leaf Cl content of the walnut leaf. As noticed in leaf Na accumulation, leaf Cl accumulation in higher salinity levels were not also increased with accordance with salinity increases perhaps due to the excluding effect of the walnut.

For I_1, I_2 and I_3 irrigation water rates, 310, 640 and 700 ppm leaf Cl contents were determined, respectively. Irrigation rates significantly affected on leaf Cl accumulation. More water application means more water depletion and more Cl accumulation in leaf.

One of the important parameters used in determining the resistance to salinity in plants under salt stress conditions is the Ca/Na and K/Na rate (Yu et al., 1978; Muhammed et al., 1987; Heimler et al., 1995; Lopez and Satti, 1996; Maathuis and Amtmann, 1999). In our study, decreases were observed in Ca/Na and K/Na rates depending on the increasing salinity applications.

In our study, although regular increases were determined in the phosphor, potassium, Na and Cl contents depending on the salt stress in walnut, decreases were determined in the Ca/Na and K/Na rates.

Akça and Samsunlu (2012) conducted a study with four different saline irrigation water levels (C_0= 0.3 dS/m-control, C_1= 1.5 dS/m, C_2= 3 dS/m, C_3= 5 dS/m) and reported that there were regular increases in the K, Na, Cl contents, and there were decreases in the Ca/Na and K/Na rates, which is similar to our results. Under salt stress conditions, it was reported that the K content increased in walnut leaves (Levitt, 1980). The findings of our study overlap with the results of similar studies.

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