Pak. J. Bot., 44(5): 1513-1520, 2012.

THE EFFECT OF SALT STRESS ON GROWTH, CHLOROPHYLL CONTENT, PROLINE AND NUTRIENT ACCUMULATION, AND K/NA RATIO IN WALNUT

YAŞAR AKÇA* AND ESRA SAMSUNLU

Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture, Tokat, Turkey
**Correspondence author,s e-mail: akca66@gmail.com*

Abstract

The effects of irrigation water salinity on growth, chlorophyll contents, proline and nutrients accumulation and K/Na ratio in three walnut cultivars was investigated. Three irrigation water salinity levels with electrical conductivities of 1,5, 3, and 5.0 dS/m and tap water as a control treatment were used in a randomized design with four replications. Irrigation practices were realized by considering the weight of each pot. Sodium, clor, prolin, K/Na and Ca/Na ratio of leaf were increased under salinity conditions. But growth of plant and chlorophyll a, chlorophyll b content were decreased under saline condition. There were significant differences between in irrigation water salinity levels in proline and chlorophyll a, chlorophyll b, Na content. But there were not any significant differences in LRWC (%). Results showed that, regarding fresh shoot weight, dry shoot and root weight, there were significant differences between cultivars, but chlorophyll a, chlorophyll b, total chlorophyll, proline accumulation and leaf relative water content (LRWC) there weren't any significant differences between cultivars. Kaman 1 and Bilecik walnut cultivars showed higher accumulation of proline than Kaman 5 but was not observed significant difference between them.

Introduction

Salinity stress has become an important problem regarding agricultural production in many regions of the world especially in arid and semi-arid regions. In Turkey, salinity and sodicity poses a problem for almost 1,5 million hectares of agricultural area. The negative effects of salinity stress on plants are explained as ion toxicity (Na^+, Cl^-); osmotic stress and nutritional disorders (Greenway & Munns, 1980; Lewitt, 1980a; Munns & Termaat, 1986; Yeo *et al*., 1991; Marschner, 1995).

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. It has been stated that genotypes with a high proline accumulation and chlorophyll content, high K/NA ratio and low Na^+ and Cl^- accumulation are more tolerant to salt (Mane *et al.,* 2011). Walnuts are accepted to be highly sensitive to salt in soil and irrigation waters. Thus, genotypes tolerant to salt are important for walnut growing. The fact that there are cultivars regarding salt tolerant even for the same species shows that salt tolerance is determined by a genetic system. Researches indicates that for each 1,0 dS/m increase in average root zone salinity above 1,5 dS/m, the tree growth rate and yield declines by 18 to 21 percent (Fulton *et al*., 1988). Paradox rootstocks are widely used in walnut production in recent years. In regions where black line disease is observed, *Juglans regia* rootstocks are sometimes preferred over paradox rootstock. However, there is a limited supply of documentation regarding the salt tolerance of *J. regia* rootstocks. *J. regia* is more sensitive to salt in comparison with *J. hindsii* and Paradox rootstocks. Comparisons between rootstocks are primarily based on salt accumulation in scion and signs of leaf toxicity. In leaf content 0, 3% chlorine, 0, 1% sodium and 300 ppm boron are accepted to be excessive levels in walnut leaf (Caprile & Grattan, 2011).

The objective of this study is to determine the salt tolerances for three walnut cultivars (*J. regia)* and to examine the changes in parameters (vegetative growth, proline accumulation, chlorophyll content Na^+, Cl^- accumulation, K/Na ratio, Ca/Na ratio) that may be used for salt tolerance in walnuts.

Materials and Methods

The one year old seedlings of Bilecik, Kaman 1 and Kaman 5 walnut cultivars were used. The plants were planted in pots with dimensions of 45 cm x 60 cm. Sodium chloride (NaCl), magnesium sulfate ($MgSO_4$) and calcium chloride ($CaCl_2$) were used in salt application. The plants in the control group were watered using tap water with constant salinity. In the experiment 4 different irrigation water salinities were used (EC_w= 0, 3 dS/m (control), EC_w=1, 5 dS/m, EC_w = 3 dS/m E_w= 5 dS/m). The irrigation water amounts were determined by adding the leaching amount to the water consumed by the plants (Ünlükara *et al*., 2008). The plants were irrigated for a total of 11 times during the months of May-July. Leaf analyses were carried out at the end of July.

The determination of the effects of salt on growth: Different salt concentrations were applied to the plants in the pots and fresh & dry root weights along with fresh & dry shoot weights of plants were measured both at the end and the beginning of the experiments.

Determination of the effects of salt on chemical constituents: Chlorophyll analysis was determined according to Withan *et al*., (1971), proline analysis according to Bates & Walderen, (1973) and leaf relative water content (LRCW %) according to Barr & Weatherley (1962). Analyses for K, Ca and Na content was carried out via flame photometry, for Mg atomic absorption spectrophotometer was used, P was analyzed using calorimetric method whereas N was analyzed via the Kjeldahl method. The results were given as (%) in dry matter. Dry leaf samples were extracted in a 0,1 N acid concentration for chlorine analyses and chlorine content was read in Sherwood MK II chloride Analyzer 926, calculations were carried out according to Taleisnik & Grunberg (1994).

Statistical analyses: The applications were carried out as 4 repetitions for each cv. and as 5 plants for each repetition. The results were analyzed via the SPSS statistics software (Standard version 11.0).

Results

Development parameters: It has been determined that depending on the increasing salt concentration, the fresh & dry root and fresh & dry shoot weights of cultivars decrease. The fresh & dry root and fresh & dry shoot weight losses observed in Kaman 1 cv. depending on salt concentrations have been determined to be less than those of Kaman 5 and Bilecik cultivars. The effects of salt applications on the fresh & dry shoot and root weights of Kaman 1 and Kaman 5 cultivars were determined to be statistically significant. A statistical significant was determined the fresh and dry weights of plants in between the control group and the plants on which salt doses of 1, 5 dS/m, 3 dS/m and 5 dS/m were applied (Table 1). Depending on the 5 dS/m salt application, weight losses of 59,41%, 42,49%, 28, 88% and 51,95% were determined in the fresh root weight, dry root weight, fresh shoot weight and dry sooth weight respectively according to control (Table 1).

Table 1. Effects of salt on fresh and dry weight of shoot and root (g/plant) of different walnut culivars.

Çeşit	Treatments (dS/m)	Fresh root weight (g)	%	Fresh shoot weight (g)	%	Dry root weight (g)	%	Dry shoot weight (g)	%
Bilecik	Control	80,00	0	235,67	0,0	179,61	0,0	56,07a	0,0
	1,5	55,86	-30,20	170,48	-27,7	135,15	-24,8	35,33cde	-37,0
	3	51,57	-35,5	155,26	-34,11	121,71	-32,23	33,55b	-40,10
	5	25,71	-67,87	133,16	-43,48	99,09	-44,82	15,41e	-72,54
Kaman 1	Control	42,86	0	121,05	0	94,95	0	26,10cde	0
	1,5	40,86	-4,66	95,66	-20,97	69,77	-26,52	19,52e	-25,28
	3	30,43	-29,17	83,21	-31,29	63,69	-32,94	25,89cde	-0,76
	5	23,07	-46,15	81,60	-32,61	63,05	-33,57	18,55e	-28,73
Kaman 5	Control	66,57	0	192,29	0	154,64	0	37,65bc	0
	1,5	56,14	-15,76	188,48	-1,97	146,88	-4,98	41,60b	10,34
	3	50,57	-24,02	172,55	-10,24	140,06	-9,37	32,48bcd	-13,79
	5	37,86	-43,09	108,30	-43,68	82,78	-46,44	25,52de	-32,36
LSD		ns		Ns		ns		12,42	
Cultivars	Bilecik	259,89	-	53,28 a	-	133,88 a	-	35,08 a	-
	Kaman 1	238,02	-	34,30 b	-	72,86 b	-	22,51 b	-
	Kaman 5	273,96	-	52,78 a	-	131,09 a	-	34,31 a	-
LSD		ns		9,30		26,89		6,21	-
Treatments (dS/m)	Control	372,99 a	0	63,14 a	0	143,06 a	0	41,25 a	0
	1,5	268,42 b	-28,04	59,52 b	-5,73	117,26 ab	-18,03	30,83 b	-25,26
	3	236,38 b	-36,62	44,19 b	-30,01	108,48 bc	-24,17	30,64 b	-25,72
	5	151,38 c	-59,41	28,88 c	-54,26	81,63 c	-42,94	19,82 c	-51,95
LSD		49,889		9,159	-	26,03		6,83	

Different letters indicate a significant at 0.005 level of probability as evaluatde by ANOVA(LSD) test

Chlorophyll content and proline accumulation: Decrease in chlorophyll content was determined as a result of depending on the increasing salt applications. The greatest leaf chlorophyll a content was determined in Kaman 1 cv whereas the greatest chlorophyll b content was determined in Kaman 5 cv. The chlorophyll a and chlorophyll b content of cultivars was not statistically significant (Table 2). The chlorophyll a and chlorophyll b content decreased 38, 27% and 32, 32% respectively as a result of the 5 dS/m salt application in accordance with the control application.

The proline content depending on salt applications was determined to be between 6, 14 mg/kg (control)–10, 19 mg/kg (5 dS/m). The proline content increased with increasing salt application. Statistically significant differences regarding proline accumulation were determined between the control, 1, 5 dS/m salt application and 3 dS/m and 5 dS/m application. The highest proline accumulation was determined in Kaman 1 cv (8, 96 mg/kg), whereas the lowest proline content was determined in Kaman 5 cv (7, 74 mg/kg). A statistically significant difference in terms of proline accumulation was not determined among cultivars (Table 2).

Table 2. Effects of salinity on chlorophyll content, prolin accumulation and relative water content of different walnut culivars.

Cultivars	Treatments (dS/m)	Chlorophyll a (mg/g)	%	Chlorophyll b (mg/g)	%	Total Chlorophyll (mg/g)	%	Prolin (mg/kg)	%	% LRWC	%
Bilecik	Control	6,43ab	0	3,20	0	9,63a	0	4,75	0	82,14	0
	1,5	4,65cd	-27,68	2,67	-16,56	7,33de	-23,88	7,95	67,36	81,47	-0,81
	3	4,11de	-36,08	2,61	-18,43	6,73e	-30,11	10,06	111,78	75,41	-8,19
	5	3,28e	-48,98	1,85	-42,18	5,13f	-46,72	10,97	130,94	-	
Kaman 1	Control	6,65a	0	2,83	0	9,49ab	0	07,36	0	71,43	0
	1,5	6,39ab	-3,90	2,56	-9,54	8,96abc	-5,58	07,54	2,44	71,22	-0,29
	3	4,34cde	-34,73	2,19	-22,61	6,53ef	-31,19	09,33	26,76	60,10	-15,86
	5	3,44e	-48,27	1,75	-38,16	5,20f	-45,20	10,51	42,79	-	
Kaman 5	Control	5,34bc	0	2,88	0	8,22bcd	0	06,31	0	69,72	0
	1,5	5,06cd	-5,24	2,57	-10,76	7,63de	-7,17	07,45	18,06	66,95	-3,97
	3	4,95cd	-7,30	2,55	-11,45	7,50de	-8,76	08,11	28,52	65,25	-6,41
	5	4,64cd	-13,10	2,42	-15,97	7,07de	-13,99	09,09	44,05	-	
	LSD	1,20	-	**ns**	-	1,39	-	**ns**	-	**ns**	-
Average of cultivars	Bilecik	4,62	-	2,58	-	7,21	-	8,43	-	79,67	-
	Kaman 1	5,21	-	2,33	-	7,54	-	8,68	-	67,58	-
	Kaman 5	5,00	-	2,60	-	7,61	-	7,74	-	67,30	-
	LSD	ns		ns		ns		ns		ns	
Treatments (dS/m)	Control	6,14 a	0	2,97 a	0	9,11 a	0	6,14b	0	74,43	0
	1,5	5,37 b	-12,54	2,60 b	-12,46	7,97 b	-12,51	7,65b	24,59	73,21	-1,64
	3	4,47 c	-27,19	2,45 b	-17,50	6,92 c	-24,03	9,17a	49,34	66,92	-10,09
	5	3,79 c	-38,27	2,01 c	-32,32	5,80 d	-36,33	10,19a	65,96	ns	-
	LSD	0,69	-	0,32	-	0,81	-	1,69	-	-	-

Different letters indicate a significant at 0.05 level of probability as evaluatde by ANOVA(LSD) test

The effects of different salt concentrations on the leaf relative water content (LRWC %) of plants: Depending on the increasing salt applications, the decreasing LRWC% value was determined to be between 74,432% (Control) – 66,921 (3 dS/m). The lowest LRCW (%) value was determined in the Kaman 1 cv (% 67, 30) whereas the highest LRCW (%) value was determined in Bilecik cv. (% 79, 67) (Table 2).

The effects of different salt concentrations on the nutrition accumulation: The nutrition contents of leaves obtained for different irrigation water salinity concentrations are given in Table 3. The Na^+ ion content of leaf increased in salt application. The average Na^+ content was determined to be 15450 ppm for the Bilecik cv., 15125 ppm for the Kaman-1 cv. and 12750 ppm for the Kaman-5 cv. The Kaman 5 cv. displayed less Na^+ ion accumulation in comparison to the Bilecik and Kaman 1 cultivars. The average leaf Cl^- content was determined to be 15493 ppm for the Bilecik cv., 10385 ppm for the Kaman 1 cv. and 11357 for the Kaman 5 cv. The lowest Cl^- ion accumulation was determined in the Kaman 1 cv. (Table 3). The leaf K^+ increased as salt concentrations increased (except 1, 5 dS/m dose). The K^+ contents were determined to be 0, 78%, 1, 22% and 1, 64% for the Kaman 1, Kaman 5 and Bilecik cultivars respectively. The K/Na values of the examined cultivars were determined to be between 0, 66 (Kaman 1)-1, 21 (Bilecik) and the Ca/Na ratio to be between 2, 02 (Bilecik)-2, 28 (Kaman 5). The K/Na and Ca/Na ratios decreased in general depending on salinity stress. The soil salinity values were determined to be 2,77 (dS/m), 4,72 (dS/m), 7,64 (dS/m) and 10,39 (dS/m) respectively depending on the control 1,5(dS/m), 3(dS/m) and 5(dS/m) salt applications. Statistically significant differences were observed between the salt applications in terms of the soil salinity value.

Discussion

This study was carried out in order to determine the tolerance to salinity stress of three different walnut cultivars, to examine the changes that occur in the plants depending on the salinity stress and to test on walnuts the criteria that are accepted as salt tolerance indicators. In plants there are differences between the limit values damaged by salt. Walnuts are accepted to be highly sensitive to salt in soil and irrigation waters. As a result of our study, the salt accumulation in the soil varied between 4, 72 (dS/m) - 10, 39 (dS/m). These values show that sufficient salinity stress has been obtained in the research material. As a matter of fact, **Fulton *et al*., (1988)** have stated that salinity values >4, 8 dS/m according to the root region salinity classification cause excessive damage in walnut cultivation. The unfavorable conditions that are frequently observed in environments with salinity stress are accepted to be the decrease of water consumption due to the low water potential at the root region, the accumulation of Na^+ and Cl^- ions enough to cause toxicity, the imbalances that arise during nutrition intake and transfer and especially the K^+ and partially Ca^{++} deficiencies (Munns & Termaat, 1986; Marschner, 1995). The reactions of plants to salt vary depending upon the time of exposure to salt, the growth period of the plant, salt concentration, climate and soil properties (Greenway & Munns., 1980). Salinity stress may cause the death of the plant as well as hinder growth depending on tolerance, may cause chlorosis and necrotic stains and also decrease yield and quality (Hasegawa *et al*., 1986; Mer *et al*., 2000).

The first reaction to salt in glycophyte plants occurs in leaves. The intensity of the necrosis observed under conditions that hinder growth and development in salinity stress conditions can be used as a parameter for the salt tolerance of plants. Defoliation is frequently observed under increasing salinity stress conditions (Hasegawa *et al*., 1986; Okubo & Saturatarini, 2000). In the cultivars examined in our study, the first necrosis related with increasing salinity stress has been observed in mature leaves. Chlorosis starting from the leaf tips and moving up to the leaf stalk has later become necrosis. Defoliation has been observed after August in plants in especially the 5dS/m salt application. Similar results regarding necrosis intensities have been obtained for all three walnut cultivars.

It has been stated that depending on increasing salinity levels, decrease in vegetative growth parameters have been observed in plants (Sixto *et al*., 2005; Pessarakli & Touchane, 2006). Decrease in root, stem and shoot developments, fresh & dry stem and root weights; leaf area and number; chlorophyll amount and yield have been observed in plants subject to salinity stress. It is stated that when a plant is subject to salinity stress for a long period, ion toxicity and water deficiency in mature leaves and carbohydrate deficiency and related signs are observed in young leaves (Greenway & Munns, 1980; Munns & Termaat, 1986; Cramer *et al*., 1988; Gasim, 1998; Shannon & Grieve 1999; Chookhampaeng, 2011).

The decrease in vegetative development and plant weight under salinity stress is explained by the stasis in cell division and cell elongation depending on the decrease of osmotic potential in plant cells due to the increasing salt concentrations in the soil and the decreasing water potential. Under salinity stress conditions the stomas close and photosynthesis slows down. Plant growth may completely come to a stop in case stress conditions continue (Ashraf, 1994). According to the findings of our study, significant decreases have been observed in the fresh & dry total plant weights depending on the increasing irrigation water salinity. For instance, according to the control group application decreases of 42, 94% and 51, 95% have been observed respectively in the dry root and dry shoot weights in the 5dS/m salt application.

When plant development is taken into account, it has been determined that Kaman 1 cv. is affected from salinity stress less than Bilecik and Kaman 5 cultivars. Decreases in plant development depending on increasing salt concentrations were determined in the Mateur pistachio cv. grafted on *Pistacia vera* L. and *Pistacia atlantica* L. rootstocks at salt concentrations of ECw=5 dS/m and ECw=12 dS/m and the reactions of different rootstocks to salinity stress have been determined (Mehdi *et al.,* 2010).

Table 3. Effects of salinty on macro and micro nutritients content and Ca/Na and K/Na raito of different walnut culivars.

Çeşit	Treatments (dS/m)	N (%)	P (%)	K (%)	%	Ca (%)	%	Mg (%)	%	Na (ppm)	%	Cl (ppm)	%	Ca /Na	%	K/Na	%
Bilecik	Control	2,79	0,11	1,32	0	2,66	0	0,29	0	6600	0	7705	0	3,45	0	2,00	0
	1,5	2,34	0,10	1,45	9,84	2,36	-11,27	0,29	0,00	15500	134,84	8040	4,34	1,52	-55,94	0,94	-53,00
	3	2,49	0,11	1,85	40,15	2,11	-20,67	0,26	-10,34	20500	210,60	18090	134,78	1,02	-70,43	0,90	-55,00
	5	2,27	0,10	1,92	45,45	2,85	7,14	0,32	10,34	19200	190,90	28140	265,21	1,48	-57,10	1,00	-50,00
Kaman 1	Control	3,05	0,09	0,94	0	2,37	0	0,34	0	6700	0	6365	0	3,53	0	1,40	0
	1,5	3,01	0,13	0,41	-56,38	2,48	4,64	0,38	11,76	10000	49,25	8710	36,84	2,48	-29,74	0,41	-70,71
	3	2,23	0,08	0,56	-41,57	2,57	8,43	0,37	8,82	23000	243,28	11725	84,21	1,11	-68,55	0,24	-82,85
	5	2,17	0,14	1,19	26,59	3,11	31,22	0,41	20,58	20800	210,44	14740	131,57	2,07	-41,35	0,57	-59,28
Kaman 5	Control	3,08	0,03	0,98	0	2,26	0	0,33	0	6800	0	4355	0	3,32	0	1,44	0
	1,5	3,02	0,10	1,21	23,46	2,89	27,87	0,37	12,12	12700	86,76	16750	284,61	2,27	-31,62	0,95	-34,02
	3	3,08	0,12	1,32	34,69	2,36	4,42	0,36	9,09	16500	142,64	11055	153,84	1,43	-56,92	0,80	-44,44
	5	2,18	0,09	1,38	40,81	3,12	38,05	0,36	9,09	15000	120,58	14070	223,07	2,08	-37,34	0,92	-36,11
Average of Cultivars	Bilecik	2,47	0,11	1,64	-	2,49	0	0,29	0	15450		15493	0	2,02		1,21	
	Kaman 1	2,61	0,11	0,78	-	2,63		0,37		15125		10385		2,16		0,66	
	Kaman 5	2,84	0,09	1,22	-	2,66		0,35		12750		11557		2,28		1,03	
Treatments (dS/m)	Control	2,97	0,08	1,08	0	2,43	0	0,32		6 700	0	6 141	0	3,63	0	1,62	0
	1,5	2,79	0,12	1,03	-4,62	2,58	6,17	0,35	9,37	12 733	90,04	11 166	81,82	2,09	-42,42	0,77	-52,46
	3	2,60	0,11	1,24	14,81	2,35	-3,29	0,33	3,12	18 333	173,62	18 983	209,12	1,68	-53,72	0,83	-48,76
	5	2,20	0,11	1,50	38,88	3,03	24,69	0,36	-12,5	20 000	198,50	13 623	121,83	1,19	-67,21	0,65	-95,38

Different letters indicate a significant at 0.05 level of probability as evaluatde by ANOVA(LSD) test

There are differences in the salt exclusion methods of plant species and cultivars. Plants may keep salt away by salt exclusion mechanism and may pump Na^+ out of their cells. Another exclusion indicator is the dilution of salt with rapid growth (Levitt, 1980 b; Lauchli, 1986). In our study the leaf Na^+ content increased depending on the increasing salt concentrations. According to the control application increase of 90, 04% - 198, 50% were observed in the Na^+ ratios for 1, 5 dS/m and 5 dS/m salt applications respectively. The Na^+ content of the cultivars were determined to be between 12750 ppm (Kaman 5) – 15450ppm (Bilecik). It is stated that plants which contain low amounts of Na^+ in their roots and other regions under salt conditions may be accepted to have salt tolerance (Greenway & Munns, 1980; Akram *et al.,* 2007). Accordingly, it can be stated that the Kaman 5 cv. is more tolerant to salinity stress since it accumulates less Na^+ in comparison with the other 2 cultivars.

Another important factor for the salinity stress tolerance is chlorine accumulation. In our study high Cl^- ion accumulation was observed in plant leaves depending on increasing salt concentrations. According to the control application the Cl^- content increased by 81, 82%-209, 12% for the 1, 5 dS/m and 3 dS/m salt applications respectively. The average Cl^- accumulation in the cultivars was determined to be between 10385 ppm (Kaman 1) – 15493 ppm (Bilecik) (Table 3). Less Cl^- accumulation was determined in Kaman 1 and Kaman 5 cultivars in comparison to the Bilecik cv. It can be stated that in general there is a greater tendency for salt tolerance in genotypes which accumulate less Cl^- ions under salinity stress or those that can keep Cl^- ions away. In terms of Cl^- accumulation it was determined that Kaman 1 and Kaman 5 cultivars were more tolerant to salt than the Bilecik cv. Irregular changes were observed in the Mg^{++} and P content depending on the increasing salt concentrations, however regular decreases were observed in the N content. General increases were observed in K^+ and Ca^{++} content depending on increasing salinity stress. The potassium content increased by 38, 88% in the 5 dS/m salt application in comparison to the control group. Even though the highest Na^+ content was determined in the Bilecik cv. with the highest K^+ content, a potassium content of 1, 22 % was observed in the Kaman 5 cv. which has the lowest Na^+ (Table 3). The findings of our study were similar to those of Lotfi *et al.,* (2009) regarding the increase of K^+ content in walnuts under salinity stress. Levitt (1980b) stated that in environments with high amounts of NaCl the Na^+ ion intake is more and the K^+ ion intake is decreased due to competition.

The increase of Na^+ and Cl^- content in strawberries under salt conditions may prevent K^+ intake. It has been determined that the Rapella strawberry cv. K^+ content is not affected by salt application (Awang & Atherton, 1994).

It is stated that K/Na and Ca/Na ratios play an important role in the measurement of salt tolerance (Yu *et al*., 1978; Muhammed *et al*., 1987; Heimler *et al*., 1995; Lopez & Satti 1996; Maathuis & Amtmann, 1999). It is emphasized that for optimum yield efficiency the K/Na ratio of the plant cytoplasm should be lower than 1 (Greenway & Munns, 1980). In our study significant decreases were observed in the K/Na and Ca/Na ratios depending on increasing salt concentration. As a matter of fact the K/Na ratio decreased by % 48, 76 - % 95, 38 in other applications in comparison with that of the control group. It was also determined that the Ca/Na ratio decreased by % 42, 42- % 67, 21 in other applications in comparison with the control group. The K/Na ratio was determined to be greater than 1 for Bilecik and Kaman 5 cultivars, whereas it was determined to be smaller than 1 for the Kaman 1 cv. The decrease in the K/Na and Ca/Na ratios with the decreasing K^+ and Ca^{++} content depending on the increasing Na^+ content under salinity stress conditions is a common result of many studies. As a matter of fact Al-Karaki (2000) has stated that the exclusion ability from high Na^+ accumulation and high amounts of K/Na and Ca/Na in leaves increase salt tolerance in tomato.

Decrease in total chlorophyll content may be observed due to ion accumulation and functional disorders observed during stoma opening and closing under salinity stress. (Seemann & Critchley (1985); Aranda & Syvertsen (1996); Molazem *et al*., (2010); Nawaz *et al*., (2010). Another reason for the decrease of chlorophyll content under salt conditions is stated to be the rapid maturing of leaves (Yeo *et al*., 1991). Decrease in chlorophyll content under salinity stress is observed more in salt sensitive genotypes in comparison to cultivars with low tolerance (Khan *et al*., 2009). In our study, a statistically significant decrease in the chlorophyll a and chlorophyll b content was observed depending on increasing salt concentrations in comparison to the control application. In the 5 dS/m salt application decreases in the chlorophyll a and chlorophyll b of % 38, 27 and % 32, 32 were observed respectively in comparison to the control group.

The tolerance to Na^+ of plants under salt conditions (NaCl), the prevention of the replacement of Mg^{++} with Na^+ and the continuous increase of chlorophyll amount is accepted as an important indicator of salt tolerance (Katsuhara *et al*., 1990; Demiroğlu *et al*., 2001). It is stated that plants with high chlorophyll content under salinity stress are more tolerant to salt. In our study, no statistically significant difference regarding chlorophyll content was found between cultivars. However, the highest chlorophyll a content was determined in Kaman 1 cv. whereas the highest chlorophyll b content was determined in Kaman 5 cv. Plants make changes in the cell and tissue levels in order to decrease the effect of stress factor.

Plants synthesize proline under arid and salinity stress conditions in order to protect themselves and to regulate their physiological status (Edreva, 1998). Hence, it can be stated that plants and their cultivars which synthesize large amounts of proline are more tolerant to stress conditions (de Lacerda, *et al*., 2003; de Lacerda, *et al*., 2005; Mehdi *et al*., 2010; Demiral & Türkan, 2005)**.** According to the findings of our study, proline content increased with increasing salt concentration. The proline increase ratios of salt applications in relation with the control application were determined to be between 24, 59 % - 65, 96 %. A statistically significant difference was not found between the proline content of cultivars; however the highest proline content of 7, 74 g/kg was determined in the Kaman 5 cv. In a study they carried out on wheat, Kong *et al*., (2001) have determined higher proline synthesis ratios in salt tolerant cultivars in comparison to those of salt sensitive cultivars. It has been determined in a study carried out on tomatoes that the proline synthesis amount of salt tolerant genotypes is greater than that of the salt sensitive plants (Perez- Alfocea *et al*., 1993).

The shoot and leaf water concentrations of plants under optimum conditions is significantly greater than those of plants under high salinity conditions (Hester & O'leary, 2003). The water intake of plants is limited based on salinity. Under these conditions plants try to overcome water stress by increasing the concentrations of their intracellular osmotic compounds.

Leaf relative water content is a criterion that is frequently used to define the water content of plants (Schonfeld *et al*., 1988). It is thought that plants with high leaf relative water content have a more stable osmotic balance (Morgan, 1984). The relative water content decreases under salinity stress conditions (Srivasta *et al*., (1998); Katerji *et al*., (2003); Kaya *et al*., (2003).

The leaf relative water content depending on salt applications was determined to be between 44.43 (control) – 66. 92 (3 dS/m). A statistically significant difference was not determined between the leaf relative water content of cultivars; however the highest the leaf relative water content was determined in the Bilecik cv. In our study, the leaf relative water content decreased with increasing salinity in accordance with literature.

Conclusion

In our study, when the proline accumulation, chlorophyll content, K/Na ratio and Na^{++} and Cl^{-} accumulation amounts are taken into account, it was determined that the Kaman 5 cv. is more tolerant to salinity stress in comparison with the other two cultivars. The proline accumulation and total chlorophyll content, K/Na ratio and Na accumulation may be used as tolerance parameters while determining the salt tolerance of walnut genotypes.

Acknowledgfment

This work was financially supported by Gazisomanapaşa University.

References

Akram, M., M.A. Malik, M.Y. Ashraf, M.F. Saleem and M. Hussain. 2007. Competitive seedling growth and K^+/Na^+ ratio in different maize (*Zea mays* L.) hybrids under salinity stress. *Pak. J. Bot.*, 39: 2553-2563.

Al-Karaki, G.N. 2000. Growth, water use efficiency and sodium and potassium acquisition by tomato cultivars grown under salt stres. *J. of Plant Nutrition*, 23: 1-8

Aranda, R.R. and J.P. Syvertsen. 1996. The influence of foliar applied urea nitrogen and salina solutions on net gas excehenc of citrus leaves, *J. Amer. Soc. Hort. Sci.*, 121: 501-506.

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

Awang, Y.B. and J.G. Atherton. 1994. Salinity And Shading Effects On Leaf Water Relations And Ionic Composition Of Strawberry Plants Grow On Rockwool. *J. Hort. Sci*., 69: 377-383.

Barr, H.D. and P.E. Weatherley. 1962. A reexamination of the relative turgidity technique for estimating water deficit in leaves. *Aust. J. Biol. Sci*., 15: 413-428.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205-207.

Caprile, J.and Grattan S. 2011. Evaluating the Salt and Boron Tolerance of English Walnut Rootstock, http://cekings.ucdavis.edu/files/66721.pdf

Chookhampaeng, S. 2011. The effect of salt stress on growth, chlorophyll content proline content and antioxidative enzymes of pepper (*Capsicum Annuum* L.) seedling. *European Journal of Scientific Research,* 49: 103-109.

Cramer, C.R., E. Epstein, A. Lauchli, A. 1988. Kinetics of root elongation of maize in response to short-term exposure to NaCl and elevated calcium concentration *J. Expt. Bot.,* 39: 1573-1582.

de Lacerda, C.F., J. Cambraia, M.A. Oliva, H.A. Ruiz and J.T. Prisco. 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ. Exp. Bot*., 49: 107-120.

de Lacerda, C.F., J. Cambraia, M.A. Oliva and H.A. Ruiz. 2005. Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environ. Exp. Bot*., 54: 69-76.

Demiral, T. and İ. Türkan. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot*., 53: 247-257.

Demiroğlu, G., M.A. Khalvati and R. Avcıoğlu. 2001. Effect of different salt concentrations on the resistance of maize cultivars (2) some physiological characteristics and ion accumulation in early growth ; *Turkish Journal of Field Crops,* 6: 55-59.

Edreva, A., 1998. Molecular basis of stress in plants. Seminars on molecular basis of stres physiology in plants. *The Ege Science and Technology Centre*, İzmir, pp. 22-26.

Fulton, A.E. Oster J. and Nanson B. 1988. Salinity Management of Walnut. In: *Walnut Production Manual* (Eds.): D.E. Ramos and David E. Ramos, University of Califonia. Division Of Agriculture and Naturel Resources, pp. 54-65.

Gasim, A.A. 1998. Effect of salinity on growth, proline accumulation and chlorophyll content during vegetative growth, flowering and seed formation of *Brassica juncea* L. *Agric. Sci.*, (1): 145-152.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology*, 30: 149-190.

Hasegawa, P.M., R.A. Bressan, and A.V. Handa. 1986. Cellular mechanisms of salinity tolerance. *Hort Sci*., 21: 1317-1324.

Heimler, D., M. Tatini, S. Tici, M.A. Coradeshi and M.L. Traversi. 1995. Growth, ion accumulation and lipid composition of two olive genotypes under salinity. *J. Plant Nutrition*, 18: 1723-1734.

Hester, L.B. and J.W. O'leary. 2003. Effects of salinity on growth and cation accumulation of *sporobolus vırgınıcus* (poaceae). *Am. J. Bot*., 90(10): 1416-1424.

Katerjı, N., J.W. Van Hoorn, A. Hamdy and M. Mastrorıllı. 2003. Salinity effect on crop development and yield analysis of salt tolerance according to several classification methods. *Agric. Water Management,* 62: 37-66.

Katsuhara, M., T, Mimura and M. Tazawa. 1990. ATP-Regulated ion channels in the plasma membrane of a characeae alga, nitellopsis obtuse. *Plant Physiol*., 93: 343-346.

Kaya, C., D. Hıggs, H. Kırnak and I. Taş. 2003. Amelioratrive effect of calcium nitrate on cucumber and melon plants drip irrigated with saline water. *Journal of Plant Nutrition*, 26: 1665-1681.

Khan, M.A., M.U. Shırazı, Muhammad Alı Khan, S.M. Mujtaba, E. Islam, S. Mumtaz, A. Shereen, R.U. Ansarı And M. Yasın Ashraf. 2009. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Trıtıcum Aestıvum* L.). *Pak. J. Bot*., **41(2):** 633-638.

Kong, Y., G. Zhou, Y. Wang. 2001. Phsiological characteristics and alternative respiratory pathway under salt stress in two wheat cultivars differing in salt tolerance. *Russian J. Plant Physiol.,* 48: 565-600.

Lauchli, A., 1986. Responses and adaptation of crops to salinity. *Acta Hort.,* 190: 243-246.

Lewitt, J. 1980a. *Responses of plants to environmental stress*. *Acedemic Press.* New York, pp. 489-530.

Lewitt, J. 1980b. *Responses of plants to environmental stresses*. Vol. II, 2nd Ed. *Academic Press*, New York, pp. 607.

Lopez, M.V. and S.M. Satti. 1996. Calcium and potasium – enhanced growth and yield of tomato under sodium chloride stres. *Plant Sci.,* 114: 19-27.

Lotfi, N., M. Vahdati, B. Kholdebarin, D. Hassani and R. Amiri. 2009. Mineral composition of some walnut cultivars (*Juglans regıa* L.) for evaluation of ionone and ionomics under salt stress condition. *Acta Hort.* (ISHS) 839: 293-300.

Maathuis, F.J.M. and A. Altmann. 1999. K^+ Nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. *Ann. Bot.,* 10: 123-133.

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

Marschner, H. 1995. *Mineral nutrition of higher plants*. *Academic Pres*, 657-680.

Mer, R.K., P.K. Prajıth, D.H. Pandya, A.N. Pndey. 2000. Effect of salt on germination of seeds and growth young plants of *Hordeum Vulgare, Tiritikum Aestivum, Cicer Arietinum and Brassica Juncea*. *J. Gron. Crop. Sci*., 185: 209-217.

Mehdi, H., A.C. Chaabouni, D. Boujnah and M. Boukhris. 2010. The response of young pistachio trees grown under saline conditions depends on the rootstock, *A no. XX, 2010—XIV GREMPA, Meeting on pistachios and almonds.*

Molazem, D., E.M. Qurbanov and S.A. Dunyamaliyev. 2010. Role of proline, Na and chlorophyll content in salt tolerance of corn (*Zea mays*) L. *American-Eurasian J. Agric. & Environ. Sci.,* 9: 319-324.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants: Ann. Rew. *Plant Physiol.* 35: 299-319.

Muhammed, S., M. Akbar and H.U. Neue. 1987. Effect on Na/Ca And Na/K ratios in saline culture solution on the growth and mineral nutrition of rice (*Oryza Sativa*). *Plant and Soil,* 104: 57-62.

Munns, R. and A. Termaat. 1986. Whole-plant responses to salinity. *Aust. J. Plant Physiol*., 13: 143-160.

Nawaz, K., A.I. Talat, K. Hussain and A. Majeed. 2010. Induction of salt tolerance in two cultivars of sorghum (*Sorghum bicolor* L.) by exogenous application of proline at seedling stage, *World Applied Sciences Journal* 10: 93-99.

Okubo, M. and T. Sakuratani. 2000. Effects of sodium chloride on survival and stem elongation of two Asian pear rootstock seedlings. *Scientia Hort.,* 85: 85-90.

Perez-Alfocea, E., M.T. Estan, A.S. Cruz and M.C. Bolarin. 1993. Effects of salinity on nitrate, total nitrogen, soluble protein and free amino acid levels in tomato plants. *J. Hort. Sci.,* 68: 1021-1027.

Pessarakli, M. and T. Hayat. 2006. Growth Responses of Bermudagrass and Seashore Paspalum under Various Levels of Sodium Chloride Stress. *Journal of Food, Agriculture, and Environment* 4: 240-243.

Schonfeld, M.A., R.C. Johnson, B.F. Carver and W. Morhinweg. 1988. Water relations in winter wheat as drought resitance indicators: *Crop Sci.,* 28: 526-531.

Shannon, M.C. and C.M. Grieve. 1999. Tolerance of vegetable crops to salinity. *Scientia Horticulturae,* 78: 5-38.

Seemann, J.R. and C. Critchley. 1985. Effects of salt stress on growth. ion content, stomatal behaviour and photosynthetic capacity of a salt sensitive species, *Phaseolus vulgaris L. Planta,* 164: 15-16.

Sixto, H., J.M. Grau, N. Alba and R. Alia, 2005: Response to sodium chloride in different species and clones of genus *Populus* L. *Forestry,* 78: 93-104.

Srıvasta, T.P., S.C. Gupta, P. Lal, P.N. Muralia and A. Kumar. 1998. Effect of salt stress on physiological and biochemical parameters of wheat. *Ann. Arid Zone*., 27: 197-204.

Taleisnik, E. and K. Grunberg. 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.

Ünlükara, A., A. Kurunç, G.D. Kesmez and E.Yurtseven. 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.

Yu, B., H. Gong and Y. Lıu. 1978. Effects of calcium on lipid composition and function of plasma membrane and tonoplast vesicles isolated from roots of barley seedlings under salt stres. *J. Plant Nutr.* 21: 1589-1600.

Yeo, A.R., K.S. Lee, P. Izard, P.J. Boursier and T.J. Flowers. 1991. Short and long term effects of salinity on leaf growth in Rice (*Oryza sativa* L.), *J. Exp. Bot*., 42: 881-889.

Withan, F.H., D.F. Blaydes and R.M. Devlin. 1971. *Experiments in plant physiology*, Nostrand Reinhold Co., New York.

(Received for publication 20 September 2011)