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Physiological responses of olive cultivars to salinity stress

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Key words: electrolyte conductivity, osmoregulation, proline, soluble carbohydrates.

Abstract: The aim of this study was to evaluate the tolerance of seven promising olive cultivars for southern parts of Iran ('Amigdalilolia', 'Dakal', 'Zard', 'Dezful', 'Tokhm-e-Kabki', 'Shiraz', and 'Conservalia') against salinity stress. Biochemical and physiological responses of the cultivars irrigated with saline water application (control, 4, 8, and 12 dS m^{-1}) were evaluated and the tolerant cultivars were identified. In contrast to the tolerant cultivars, the sensitive ones continue to grow with lower rate and died under salinity stress. In general, growth indices of olive cultivars were reduced with increasing salinity stress and the lowest growth indices were obtained under 12 dS m^{-1} treatment. Results indicated that the accumulation of higher levels of soluble carbohydrates and proline in the leaves of the tolerant cultivars helps them to deal with salinity stress. The results showed that saline waters up to 4 dS m^{-1} for irrigation can be used for olive cultivars, however, based on the result of this study, it is not recommended to use water sources with higher electric conductivities to irrigate sensitive olive cultivars. We concluded that the tolerant cultivars stopped growth and used their energy to defend against the salinity stress.

1. Introduction

Salinity stress is dependent on environmental condition (Kozlowski and Pallardy, 1997), farming, water management and genotype (Kozlowski and Pallardy, 1997). Olive (*Olea europea* L.) is one of the most valuable and widespread fruit trees in the Mediterranean area. Its cultivation is continuously being extended to irrigated land. Furthermore, in Mediterranean area salinity is becoming a major problem due to high rates of evaporation (Kozlowski and Pallardy, 1997). Olive is considered a moderately salt tolerant plant (Ayers and Westcot, 1976; Aragues *et al*., 2005; Weissbein *et al*., 2008). In comparison with other Mediterranean-grown tree crops, olive is more tolerant than citrus but less tolerant than date palm (Ayers and Westcot, 1976). The tolerance of olive cultivars are different to salinity stress (Therios and Misopolinos, 1988; Perica *et al*., 2004; Chartzoulakis, 2005).

The relationship between saline water and olive cultivation has been intensively studied for many years and significant progress has been made in the understanding of this topic (Ayers and Westcot, 1976; Wiesman *et al*., 2004). It is generally well established that saline conditions limit the vegetative and reproductive development of olives mainly as a result of interference with the osmotic balance in the root system zone and detrimental effects caused by specific toxic accumulation of chloride and sodium ions in the leaves (Weissbein *et al*., 2008). Salt stress reduces water availability in soil solution as a result of an increased osmotic potential, inducing the generation of reactive oxygen species (ROS) (Zhu, 2001; Melloni *et al*., 2003), the reduction of hormonal signals generated by the roots (Munns, 2002), altered carbohydrate metabolism (Gao *et al*., 1998), reduced the activity of certain enzymes (Munns, 1993; Chartzoulakis, 2005) and ultimately impaired photosynthesis (Chartzoulakis, 2005). Therefore, these physiological changes result reduced growth in either reduced cell division, expansion or promoting cell death (Hasegawa *et al*., 2000). Furthermore these criteria make plant reduce growth rate and yield, chlorophyll destruction which lead to leaf senescence. The plant response to salinity stress is depen-

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dent on environmental condition, farming, water management and plant genotype.

The aim of this study was to screen for the tolerance of seven olive cultivars from the Southern parts of Iran, against salinity stress. Tolerance was evaluated over several biochemical (proline content, stored carbohydrate, total chlorophyll and starch concentration), and physiological (Cell Membrane Injury) responses of these cultivars under salinity stress.

2. Materials and Methods

Experiment was carried out in the Department of Horticultural Sciences, Shiraz University during the growing season in 2012, using one year old cuttings of seven olive cultivars: 'Dakal', 'Zard', 'Shiraz', 'Tokhm-e-Kabki', 'Dezful', 'Amigdalilolia', and 'Konservalia' with four replicates for each cultivar. The cuttings were transplanted into 15 kg pots containing soil mixture (1:1:1) of soil (pure soil), sand and leaf mould. The physicochemical characteristics of the soil are shown in Table 1.

Table 1 - The physiochemical characteristics of the soil used

Characteristics	
Zn (ppm)	1.5
Fe (ppm)	7.6
Mn (ppm)	21.14
Cu (ppm)	1.76
K (ppm)	400
P (ppm)	23.8
Total Nitrogen (%)	0.094
OC (%)	1.54
Ph (%)	7.9
EC (%)	1.93
Clay (%)	34.4
Silt (%)	44.2
Sand (%)	21.4

During the establishment phase in greenhouse, olive cultivars were pruned uniformly in order to produce a single stem. The salinity stress treatments were applied by sub irrigation with different salinity levels [control (1.1), 4,8,12 ds/m]. In order to prevent salinity shock, the concentration of salts was gradually increased to reach a given level. The day and night temperature of the greenhouse was 35°C and 25°C, respectively. The saline water was prepared by dissolving sodium chloride (control, 4, 8, 12 ds/m) in the water. The pots were irrigated with saline water for 90 days. They were irrigated with saline water to the Field Capacity (FC) level, which was equivalent 20% of the dry weight of the soil of pot.

The total shoot length of olive cultivars was measured at the beginning and at the end of the experiment. Additionally, the number of fully expanded leaves and branches of each cultivar were recorded. At the end of the experiment, the average length of new shoot was measured. Using the data collected at the start and the end of salinity stress treatments, the rate of these changes was calculated.

Total chlorophyll measurement

Total chlorophyll content was determined by spectrophotometer (Saini *et al.*, 2001). Briefly, chlorophyll a and b contents were obtained by extraction in 85% acetone solution and measuring their absorbances using Camp spec M501 Single Beam UV/vis Spectrophotometer at λ= 663 nm and λ = 645 nm. The concentration of chlorophylls and carotenoids were calculated according to the following formula:

$$\text{Total chlorophylls (mg /g fw)} = [(20.2\times OD_{645\ nm} + 8.02\times OD_{663\ nm}) \times V] /(fw \times 1000)$$

where OD is optical density, V is the final solution volume in mL and fw is tissue fresh weight in mg. V is the final solution volume in mL and fw is tissue fresh weight in mg.

Proline measurement

Free proline was extracted from 0.5 g samples of fully expanded and young leaves with 3%, sulfuric acid and estimated by using ninhydrin reagent, according to the protocol described by Bates *et al*. (1973). The absorbance of the fraction with toluene was determined at 520 nm, using a spectrophotometer (Model UV-120-20, Japan).

Cell membrane injury (CMI)

Cell membrane injury was calculated according to the method of Blum and Ebercon (1981). For the CMI, 20 samples of stressed and unstressed young leaves were washed with distilled water to remove the dust and injured cells from samples. The samples were then immersed in 20 ml distilled water at room temperature. After 24 h the conductivity of the solutions was read. The samples were autoclaved for 15 min, cooled to room temperature and the conductivity of the solutions was read again. The electrolyte leakage was measured with a conduct meter (644 Conduct meter, Metrohm, Herisau, Switzerland). CMI was estimated from the formula:

$$\text{Id (Drought injury index)} = 1 - (1 - T1/T2)/(1 - C1/C2) \times 100$$

where T1 and T2 are the first and second measurement of the conductivity of the solutions in which the

treated samples were immersed and C1 and C2 are the respective values for the conductivity of the solutions.

Soluble carbohydrate extraction

To determine soluble carbohydrate concentration, 150 mg of dried leaf samples was extracted twice with 80% ethanol. The sample was centrifuged at 3500 rpm for 10 min and the volume of the supernatant was adjusted to 25 ml Soluble carbohydrate concentration was measured according to the method of Buysee and Merckx (1993). In summary, 1 ml of supernatant was transferred to a test tube and 1 ml phenol 18% and 5 ml sulfuric acid were added. The mixture was shaken immediately and its absorption was recorded at 490 nm using a spectrophotometer (Model UV-120-20, Japan).

Starch concentration

Starch concentration in the leaf samples was measured using anthron reagent (McCready, 1950). In this method, 5 ml of water (0°C) and 6.5 ml perchloric acid (52%) were added to the pellet used for sugar analysis and mixed for 15 min. About 20 ml water was then added and the sample was centrifuged. The supernatant was separated and the same procedure was repeated with the pellet for each leaf samples. The supernatants were combined and left for 30 min at 0°C. After filtration, the supernatant volume was adjusted to 100 ml. About 2.5 ml of cold 2% anthron solution was added, and the sample was heated at 100°C for 7.5 min. It was then transferred immediately to an ice bath and cooled to room temperature. Absorption at 630 nm was recorded using a spectrophotometer (Model UV-120-20, Japan).

Statistical analysis

The experiment was conducted as a complete randomized design with factorial arrangements. Analysis of variance was performed using the SPSS software package and significant differences among mean values were compared by Duncan Multiple Range Test (DMRT) ($P<0.05$).

3. Results

Effect of salinity on plant growth

After 90 days of salinity treatment plant growth (total shoot length, number of branches and leaf number) significantly reduced in all cultivars (Table 2, 3 and 4). The effect of salinity on these parameters showed a significant genotypic variation. As expected, the highest reduction of shoot length was found in 12 dSm^{-1} treatment; considering the mean over all treatments, Conservalia and Shiraz cultivars had the highest shoot length while 'Dakal' showed the lowest one under each salinity stress conditions (Table 2). The highest reduction in number of branches was found in 8 and 12 dSm^{-1} treatments on average, the lowest branch number was found in 'Conservalia' whereas the 'Amigdalilolia', 'Dakal' and 'Tokhm-e-

Table 2 - Effect of interaction between salinity treatment and cultivar on total shoot length (in comparison to the beginning of the experiment

Cultivar	Total shoot lenght (cm)				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Conservalia'	158.4 ab*	158.7 a	131.2 a-e	106.3 def	138.6 A
'Shiraz'	149.1 ab	137.7 a-d	137.7 a-d	124.1 a-f	138.8 A
'Tokhm-e-Kabki'	149.1 a-d	119.6 b-f	114.7 def	112.7 def	121.0 BC
'Dezful'	159.9 abc	136.0 a-d	119.2 b-f	121.4 b-f	134.1 AB
'Zard'	156.3 ab	124.7 a-f	127.3 a-f	118.0 c-f	131.6 AB
'Dakal'	154.4 ab	102.7 F	104.8 ef	103.9 ef	116.4 C
'Amigdalilolia'	142.4 a-d	121.8 b-f	106.4 ef	115.7 c-f	120.2 BC
Mean	151.4 A	128.5 B	120.2 BC	141.6 C	

* Mean separation within columns and rows by DMRT, at 5% level.

Table 3 - Effect of interaction between salinity treatment and cultivar on branch number of olive cultivars

Cultivar	Branch number				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Conservalia'	21.0 h-k *	24.3 f-j	8.3 l	8.0 l	15.4 D
'Shiraz'	28.3 c-h	18.6 Ijk	18.4 ijk	17.6 ijk	20.7 C
'Tokhm-e-Kabki'	40.0 a	35.6 a-d	25.2 e-i	30.2 b-g	32.7 A
'Dezful'	29.8 b-h	28.5 c-h	22.9 g-k	20.9 h-k	25.3 B
'Zard'	27.6 d-h	17.6 Ijk	16.0 k	17.0 h-k	19.3 C
'Dakal'	29.3 c-g	31.0 b-f	32.0 b-f	37.6 ab	32.7 A
'Amigdalilolia'	36.0 abc	34.6 a-d	33.0 a-e	22.6 g-k	31.4 A
Mean	30.3 A	28.0 B	21.8 C	22 C	

* Mean separation within columns and rows by DMRT, at 5% level.

Table 4 - Effect of interaction between salinity treatment and cultivar on leaf number of olive cultivar (Calculated as: leaf number at the beginning of the stress-the leaf number at ending the stress)

Cultivar	Leaf number				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Conservalia'	151.0 a-f *	138.6 c-g	21.0 Jk	-56.0 l	66.3 D
'Shiraz'	114.6 b-f	113.6 e-i	74.3 g-j	-11.3 kl	80.3 C
'Tokhm-e-Kabki'	190.6 a-d	207.1 ab	100.0 f-i	73.6 g-j	142.8 B
'Dezful'	224.6 a	217.6 a	135.0 c-g	113.3 e-i	172.6 AB
'Zard'	160.0 a-f	96.0 f-i	65.6 g-j	62.6 hij	96.8 C
'Dakal'	218.3 a	218.6 a	200.6 abc	130 d-h	191.9 A
'Amigdalilolia'	185.3 a-d	177.3 a-e	174.0 a-e	98 f-i	158.6 B
Mean	177.8 A	167.0 A	110.1 B	60.0 C	

* Mean separation within columns and rows by DMRT, at 5% level.

Kabki' obtained the most one (Table 3).

Leaf number was also significantly reduced by salinity treatments. At 12 dSm^{-1}, the reduction in leaf number ranged from -56.0 for 'Conservalia' to 130 for 'Dakal' with respect to control plants. The greatest number of leaves was found in controls and 4 dSm^{-1} treatments (Table 4).

Effect of salinity on cell membrane injury (CMI)

Cell membrane injury (CMI) was significantly increased in all studied cultivars from the treatment of 12 dSm^{-1} except in 'Amigdalilolia', 'Zard' and 'Dakal'. The highest percentage of CMI was obtained in 'Conservalia' with respect to the control treatment (Table 5). Furthermore, the maximum and minimum CMI belonged to 4dSm^{-1} and control, respectively. Therefore, CMI was influenced by both salinity treatment and type of cultivar (Table 5).

Table 5 - Effect of interaction between salinity treatment and cultivar on CMI of olive leaf (%)

Cultivar	Cell membrane injury (%)				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Corservalia'	74.7 abc*	72.8 abc	67.9 b-f	49.1 g	66.1 C
'Shiraz'	66.6 c-f	60.4 f	52.7 g	52.9 g	58.2 D
'Tokhm-e-Kabki'	72.4 abc	71.6 a-d	63.6 def	63.1 ef	67.7 BC
'Dezful'	69.7 a-e	66.2 c-f	68.7 a-e	53.3 g	64.5 C
'Zard'	69.6 a-e	73.0 abc	68.3 b-f	69.4 a-e	70.1 B
'Dakal'	70.3 a-e	77.1 a	68.9 a-e	68.6 a-e	71.2 B
'Amigdalilolia'	76.7 ab	75.2 ab	76.3 ab	76.0 ab	75.6 A
Mean	71.4 A	70.9 A	66.6 B	61.6 C	

* Mean separation within columns and rows by DMRT, at 5% level.

Proline content

Leaf proline content of olive cultivars was significantly influenced by salinity treatment and type of cultivar (Table 6). Maximum proline content was obtained in the leaf tissue of 'Dezful' while the minimum proline content was determined in the leaves of 'Conservalia'. In general, the highest proline content was observed in 8 dSm^{-1} treatments and the lowest one was observed in 4 dSm^{-1} treatments (Table 6).

Soluble carbohydrate content

The concentration of soluble carbohydrates of leaves under salinity stress was significantly changed in all studied cultivars and the highest content of soluble carbohydrates was found in Shiraz and Zard cultivars and the lowest was observed in the leaves of 'Amigdalilolia' (Table 7). The maximum soluble carbohydrate content was found in 4 dSm^{-1} treatment and the minimum content was observed in control and 12 dSm^{-1} treatments, respectively.

Starch concentration content

The stored carbohydrate content of leaves was significantly affected by salinity treatments and type of cultivar. 'Conservalia' had the highest stored carbohydrate content whereas 'Shiraz' showed the lowest (Table 8). Plants grown at 12 dSm^{-1} had the highest stored leaf carbohydrate content and the lowest one was observed in 8 dSm^{-1} treatments (Table 8).

Table 6 - Effect of interaction between salinity treatment and cultivar on proline content of olive cultivar leaf (µM g DW^{-1})

Cultivar	Proline content (µm G DW^{-1})				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Conservalia'	29.9 b-e*	21.1 g	24.0 efg	22.1 fg	24.3 C
'Shiraz'	36.7 ab	22.7 fg	26.8 d-g	23.0 efg	27.3 BC
'Tokhm-e-Kabki'	28.9 b-f	28.9 b-f	33.2 a-d	26.6 d-g	29.4 B
'Dezful'	35.4 abc	32.7 a-d	40.7 a	37.8 ab	36.7 A
'Zard'	26.6 d-g	26.5 d-g	35.7 abc	28.6 b-f	29.3 B
'Dakal'	24.1 e-g	29.9 b-e	33.8 a-d	29.9 c-g	28.7 BC
'Amigdalilolia'	22.4 fg	25.9 d-g	26.5 d-g	34.0 a-d	27.2 BC
Mean	29.1 B	26.8 C	31.5 A	29.5 AB	

* Mean separation within columns and rows by DMRT, at 5% level.

Table 7 - Effect of interaction between salinity treatment and cultivar on soluble carbohydrate content of olive leaf (%)

Cultivar	Soluble carbohydrate content (%)				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Conservalia'	30.6 abc*	28.3 c-g	26.9 d-h	27.2 d-h	28.3 AB
'Shiraz'	31.5 a	28.7 a-f	29.9 a-e	28.7 b-f	29.7 A
'Tokhm-e-Kabki'	28.0 c-h	31.3 ab	29.0 a-f	26.8 e-h	29.0 AB
'Dezful'	26.7 d-h	29.1 a-f	26.8 e-h	28.3 c-g	27.6 BC
'Zard'	30.1 a-d	30.0 a-e	28.9 a-f	29.3 a-f	29.6 A
'Dakal'	23.5 h	31.2 ab	30.6 abc	28.6 b-f	28.7 AB
'Amigdalilolia'	24.5 gh	25.7 fgh	28.4 c-g	27.3 d-h	26.5 C
Mean	27.9 B	29.4 A	28.6 AB	28.1 B	

* Mean separation within columns and rows by DMRT, at 5% level.

Table 8 - Effect of interaction between salinity treatment and cultivar on stored carbohydrate content of olive cultivar leaf (mg g DW^{-1})

Cultivar	Stored carbohydrate content (mg g DW^{-1})				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Corservalia'	279.6 a-e*	280.6 a-d	251.3 b-g	339.1 a	290.7 A
'Shiraz'	221.5 d-g	164.0 h	191.0 gh	296.9 abc	220.2 D
'Tokhm-e-Kabki'	238.7 c-g	242.8 c-g	239.0 c-g	284.2 a-d	249.7 BC
'Dezful'	221.6 d-g	277.0 a-d	244.9 c-g	261.8 b-e	251.3 B
'Zard'	251.9 b-f	233.4 c-g	244.2 c-g	237.3 c-g	241.7 BCD
'Dakal'	254.6 b-f	229.4 c-g	211.5 e-h	198.6 fgh	221.6 CD
'Amigdalilolia'	288.9 a-d	319.7 ab	233.4 c-g	229.6 c-g	269.1 AB
Mean	249.4 AB	249.1 AB	230.0 B	264.5 A	

* Mean separation within columns and rows by DMRT, at 5% level.

Chlorophyll content

The highest chlorophyll content was observed in the 'Dakal', 'Dezful' and 'Amygdalolelia' while the lowest chlorophyll content was determined in the leaves of 'Shiraz'. In general, the highest chlorophyll content was measured in plants grown with the 4dSm^{-1} treatment and the lowest was observed for 12dSm^{-1} treatment (Table 9).

Table 9 - Effect of interaction between salinity treatment and cultivar on chlorophyll of olive cultivar

Cultivar	Chlorophyll				
	EC (dSm^{-1}) of irrigating water				
	0	4	8	12	Mean
'Corservalia'	1.84 efg*	2.56 a	2.31 a-e	0.74 i	1.86 B
'Shiraz'	2.10 a-e	1.61 fg	1.55 g	0.20 I	1.36 C
'Tokhm-e-Kabki'	2.23 a-e	2.30 a-e	2.12 a-e	1.02 h	1.97 B
'Dezful'	2.52 a	2.47 abc	1.95 d-g	2.05 a-f	2.25 A
'Zard'	1.92 d-g	2.24 a-e	1.98 c-g	1.59 fg	1.93 B
'Dakal'	2.25 a-e	2.48 abc	2.42 a-d	2.01 b-g	2.29 A
'Amigdalilolia'	2.51 ab	2.25 a-e	2.27 a-e	1.80 efg	2.21 A
Mean	2.19 AB	2.27 A	2.09 B	1.36 C	

* Mean separation within columns and rows by DMRT, at 5% level.

4. Discussion and Conclusions

The purpose of this study was to evaluate the salinity tolerance of seven olive cultivars ('Conservalia', 'Amigdalilolia', 'Dakal', 'Tokhm-e-Kabki', 'Shiraz', 'Dezful' and 'Zard) based on the effect of salinity on growth characteristics, and on physiological and biochemical response of different cultivars, grown in south of Iran. Salt stress has been reported to have genotypic-linked response (Chartozoulakis, 2005). Different parameters were used to indicate the response of seven cultivars to NaCl stress.

The main result concerns the reduced number of leaves in salt treated olive plant respect to control ones. Such decrease was not only connected with the growth inhibition effects of salt but also to plants defoliation (Karimi *et al.,* 2009).

The impact of salinity stress on reducing growth indices was clear and confirmed the response shown in previous research on different species and cultivars of olives (Perica *et al*., 2004). It was clear that the decline in growth rate of olive trees under salinity stress was dependent on the duration of salt exposure, the concentration of salt and the potential of tolerance of cultivars. In the current study, olive cultivars showed different reactions to salinity stress. In general, vigorous cultivars were more susceptible to salinity stress than low growth cultivars. Partial growth reduction of olive tree growth can be related to the osmotic stress resulting of elevated level of ions in soil solution and irrigation water (Weissbein *et al*., 2008). Because no visual signs of salt toxicity in the plants e.g. tip burn, necrosis and/or shoot die back (Chartozoulakis, 2005) under low salinity level; In addition, monitoring leaf number changes indicated no sign leaf abscission due to salt toxicity or oxidative damages under low salt stress level. Low osmotic potential of soil solution under salinity stress limits growth of plant by reducing water uptake, transpiration and stomatal conductance, which is associated with the reduced photosynthesis (Ben-Asher *et al*., 2006). Hence it can be concluded that, osmotic stress is the primary reason of olive growth limitation under salinity stress.

The CMI of leaf is the quantitative index which shows either health of plasma membrane or the rate of membrane disruption in plant leaf tissue under salinity stress. The reduction in CMI of olive cultivar was parallel to intensity of sodium chloride concentration in the irrigation water. Under the severe salinity stress, the highest CMI rate was observed in the 'Amigdalilolia', 'Dakal' and 'Zard' (101.8%, 99.4% and 97.6%), respectively and the lowest one belonged to 'Conservalia' (65.6%). Furthermore, leaf tip burning and leaf abscission were observed that might be due to accumulation of specific ions in olive leaf under salinity stress.

Though the leaf proline content in 'Shiraz' and 'Conservalia' declined with the increase of salinity stress, it increased in the other cultivars. During the salinity treatment, proline content was induced to accumulate in plant leaf tissue by the accumulation of the sodium and chloride ions and water stress as a result of enhancing of salt in soil solution (Delauney and Verma, 1993). In this study, despite salinity stress injuries, the increase in proline content may have played an important role in protection of olive cultivars in stress situations. The data presented here agreed with previous studies which show the direct relationship between tolerance of salinity stress and proline accumulation. It has been demonstrated that proline can protect protein from denaturation and maintain cytoplasmic membrane in salinity stress (Khedr *et al*., 2003; Karimi *et al*., 2009).

Data were shown that soluble sugar content of leaves of tolerant olive cultivars ('Amigdalilolia', 'Dakal') was enhanced during salinity period.

However in semi-tolerant cultivars ('Dezful', 'Zard' and 'Tokhm-e-Kabki') the sugar content was not significantly changed and in sensitive cultivars ('Shiraz' and 'Conservalia'), this factor in comparison to control treatment was reduced. Soluble sugars play an important role in maintaining turgor pressure in osmotic stress. Furthermore, they can protect the plasma membrane in the stress situation (Sanchez *et al*., 1998). This study showed that among tolerant olive cultivars, the accumulation of sugars could be due to the reduced consumption of stored carbohydrate contents (Chartzoulakis, 2002). Among the sensitive olive cultivars in which the starch and soluble sugar content were limited, it can be concluded that this phenomenon was related to photosynthesis disruption and the subsequent consumption of stored carbohydrate. Under severe salinity stress condition, starch accumulation in the tissue of sensitive cultivars, may be due to disruption of enzyme activity and plant metabolism which led to stored carbohydrate in the tissue.

Salinity stress decreased chlorophyll concentration in the olive cultivar leaf. Tolerant cultivars were shown more chlorophyll concentration than susceptible ones. Therefore, it can be concluded that leaf chlorophyll concentration might be the best index for evaluating of olive cultivars to tolerate salinity stress as reported by Noble and Rogers (1994). The same authors suggested that chloroplast dysfunctions and decrease in the number and volume of chloroplast were influenced by salinity stress, and as a result, a reduction in chlorophyll content was a main reaction of olive trees to salinity stress. Winicov and Seemann (1991) observed that a salt - tolerant alfalfa cell line exhibited an 11-fold increase in chlorophyll content compared to the unadapted cell line. The increase in chlorophyll content in alfalfa was associated with large increase in the activity of ribulose-1, 5- bisphosphate carboxylase (Winicove and Seemnann, 1991).

In conclusion, the relative tolerance of olive cultivars to salinity stress was in the following order: tolerant cultivars were 'Amigdalilolia' and 'Dakal', semi-tolerant cultivars included 'Dezful', 'Zard' and 'Tokhm-e-Kabki' and finally sensitive cultivars were 'Shiraz' and 'Conservalia'.

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