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Evaluation of salinity tolerance of three olive (*Olea europaea* L.) cultivars

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ABSTRACT

Tarom region of Zanjan province is one of the olive production centers in Iran, which faced a crisis of salinity stress following the salinization of water resources. The selection of salinity tolerant cultivars using pivotal characteristics is one of the interesting challenges. This study was conducted to investigate the behavior of 'Zard', 'Abou-satl', and 'Arbequina' cultivars in the context of using marginal waters. Therefore, one-year-old self-rooted plants of these cultivars were potted and irrigated with 2, 5, 8, and 12 dS/m saline water for three months in the greenhouse, and certain physiological and morphological features were studied. The results show that 'Zard' and 'Abou-Satl' cultivars tolerated salinity of 8 and 12 dS/m, maintained their photosynthesis in medium salinity (5 dS/m), and grew well in these conditions. In contrast, the 'Arbequina' cultivar exhibited extreme susceptibility to salinity. In the high salinities, there was a lower slope in the increase of Na^+ concentration in the leaves of the 'Zard' cultivar. Also wet and dry biomass in this cultivar decreased much less than the others. A more severe reduction in the transpiration of the 'Zard' cultivar indicated better efficiency of water retention mechanisms and high water use efficiency. The photosynthesis rate of 'Zard' and 'Abou-Satl' cultivars were less affected under salinity stress. They reduced the accumulation of Na^+ and increased the K^+ concentration in leaves. These two cultivars had suitable responses to salinity and were recommended for planting in regions affected by salinity.

Keywords: gas exchange, growth parameters, nutrients, photosynthesis, 'Zard' cultivar

INTRODUCTION

Salinity and drought represent some of the most severe obstacles in agricultural production. About 20% of the world's arable land are saline (Zhu, 2001). Irrigation with unsuitable and saline water, increases soil salinity which in turn affects plants by salinity stress. As a result, the area of salinized soil is increasing every year. One of the best ways to minimize the destructive effects of water and soil salinity on plants is to use cultivars that can grow and have good performance in these conditions (Gholami and Rahemi, 2009; Noori et al., 2015). Like many other species, salinity tolerance in olives is cultivar dependent (Gucci et al., 1997; Chartzoulakis, 2005). In olive cultivars, gas exchange traits, such as transpiration, stomatal conductance, and photosynthesis rate, usually decrease with increasing salinity stress but remain constant in some olive cultivars such as 'Chetoui' and 'Chemlali' (Kchaou et al., 2013). Salinity causes disorders on physiological parameters, especially photosynthesis which can affect plant performance (Hasegawa et al., 2000; Morales et al., 2008; Chaves et al., 2009), so the photosynthetic parameters can be one of the indicators to determine cultivar tolerance to salinity (Tattini et al., 1997).

Salt tolerance in plants is associated with preventing the entry/or movement of saline ions (especially Na^+ and

Cl^-) from roots to aerial parts (Storey and Walker, 1999). In olive trees, the concentration of sodium and root chloride ions increases with increasing NaCl in the soil solution (Tattini et al., 1992; Chartzoulakis et al., 2002). Moreover, Na^+ and Cl^- ions from the root to the aerial parts of olive plants also have a linear slope. There are many genetic differences among olive cultivars regarding the accumulation of Na^+ in roots and ion transfer to aerial parts of the plant (Chartzoulakis et al., 2002).

'Zard' is one of Iran's native cultivars, compatible with olive orchards in northern Iran, i.e. Zanjan, Guilan, and Golestan (Azimi et al., 2016a, 2016b). 'Abou-Satl' as a promising early precocious cultivar, has an excellent adaptation to the Tarom region (Azimi et al., 2016a; Azimi et al., 2018). 'Arbequina', a highly productive cultivar, is one of the most promising and relatively dwarf cultivars and adapted in most olive orchards of Iran (Arji et al., 2012; Azimi et al., 2016b). In newly semi-dense and standard orchard establishment programs, the 'Arbequina' cultivar was used for several years. The country's orchards faced drought, declining surface and underground water quality, and increasing salinization of its water resources. Only limited studies on the effects of salinity and drought on the 'Abou-Satl' and 'Arbequina' cultivars have been done in Iran. This study was conducted to evaluate salinity stress effects on 'Zard', 'Abou-Satl', and 'Arbequina' cultivars and to investigate their tolerance to salinity stress and responses to irrigation with saline waters.

MATERIALS AND METHODS

Plant materials

This study was conducted in 2018 at the Tarom Olive Research Station in Zanjan Province. The station latitude and longitude were 49° 05′ East and 36° 47′ North, respectively, and the average elevation of the station was 350 m above sea level. As a first factor, the selected cultivars were the main Iranian olive cultivar, 'Zard' (control), and two foreign olive cultivars, 'Arbequina' and 'Abou-Satl', imported from Spain and Syria, respectively. 'Arbequina' cultivar has recently been extensively grown in Iran, and 'Abou-Satl' is a well-adapted and promising olive cultivar in many olive-growing regions of Iran. One-year-old plants of 'Zard', 'Arbequina' and 'Abou-Satl' cultivars that were uniform in growth and pruned to only one shoot were used in this research.

All plants of these cultivars were transferred to 10-liters pots containing sand, field soil, and coco peat in a 1:1:1 ratio (Table 1). They were maintained for six months in full irrigation and nutritional conditions. Salinity treatments, as a second factor, were applied after a six months adaptation period. Three levels of salinity and control were used in this experiment. Salt stress treatments prepared with NaCl salt, were 2 (Control), 5, 8, and 12 dS/m. The salinity of the water used to prepare solution treatments was 0.3 dS/m. The final electrical conductivity of the prepared solutions was measured using a portable EC meter at the time of irrigation. The study was carried out with three cultivars, four salinity levels, three replications, and two plants per each replication, a total of 72 plants, under greenhouse conditions.

Growth parameters

At the end of the experiment, shoot growth (cm) was measured. The roots, new leaves, and stem of each plant were separated. Fresh weight of biomass (BFW) and each part of the plant were weighted and then washed with tap and then distilled water consecutively. Each part of the plants was dried at 60 °C for 72h, and the dry biomass (BDW) was weighted. Leaf thickness was measured in five replicates.

Gas exchanges

The sub-stomatal carbon dioxide concentration (Ci), leaf transpiration (E), net photosynthetic assimilation rate (An), and stomatal conductance (gs) of the youngest fully expanded leaves of two plants from each treatment were measured using a portable photosynthesis-meter LCi (ADC Bio Scientific Ltd.). Chamber setup data was as follows: temperature 38.4 °C, set CO_2 level 385.8 µmol/mol, PAR 1730 µmol/m^2s, and humidity at 81.2%. Intrinsic and instantaneous water use efficiency were

Table 1. Physical and chemical properties of the soil mixture used in the experiment

OC (%)	Texture (-)	Clay	Silt	Sand	pH (-)	Lime (%)	EC (dS/m)	K	P
		(%)						(mg/kg)	
6.4	Sandy Loam	8	23	69	7.2	7.9	1.9	415	16.2

calculated with equations 1 and 2, respectively (Medrano et al., 2015):

Equation 1) $Int.WUE = A_n / E$

Equation 2) $Ins.WUE = A_n / g_s$

Mineral nutrient analysis

Leaves and roots were harvested and analyzed for Na^+, Ca^{2+}, and K^+. Roots were thoroughly washed with de-ionized water. Afterward, plant samples were dried in an oven at 65 °C for 48h. Mineral concentration in the prepared samples was measured by the atomic absorption method (GBC-Avanta, Australia). Concentrations of mineral nutrients were expressed as a percentage of dry weight (DW).

Statistical analysis

This experiment was carried out as a factorial based on a completely randomized design. Data analysis was performed by SAS 9.4 (Copyright (c) 2001 by SAS Institute Inc., Cary, NC, USA.) statistical software and means separated by LSD test.

RESULTS

Plant growth

After 90 days of salinity stress treatment, shoot growth, fresh and dry biomass (BFW, BDW), as well as fresh and dry matter of roots (RFW, RDW) decreased in all three cultivars with increasing salinity. The highest shoot growth was observed in the 'Abou-Satl' cultivar at the treatment of 2 dS/m. In contrast, the lowest shoot growth was in treatments of 8 and 12 dS/m of 'Zard' and 'Abou-Satl' cultivars (Table 2). The reduction percentage of shoot growth from 2 to 5 dS/m was 26.2%, 24.7%, and 15.9% in 'Zard', 'Abou-Satl' and 'Arbequina' cultivars, respectively.

The BFW in the 2 dS/m salinity treatment of the 'Abou-Satl' cultivar was higher than in the two others. The lowest BFW was found in 'Arbequina' at the salinity treatments of 8 and 12 dS/m. The amount of BDW in the salinity treatment with 2 dS/m in 'Abou-Satl' was higher than in the two others. In contrast, the lowest BDW was in the high salinity treatment of 'Arbequina', but there were no significant differences between the results of 5, 8, and 12 dS/m of this cultivar (Table 2). The amount of BFW and BDW reduction in the salinity treatments had a gentle slope in 'Zard'. BFW reduction from 2 to 5 dS/m were 9.7%, 21.3%, and 30.9%, for 'Zard', 'Abou-Satl' and 'Arbequina', respectively. The BDW reduction from 2 to 5 dS/m was 16.1%, 22.5%, and 48.4% in the 'Zard', 'Abou-Satl', and 'Arbequina' cultivars, respectively.

The RFW in 2 dS/m salinity treatment of 'Abou-Satl' was higher than in the two others, but there were no significant differences between 2, 5, and 8 dS/m of 'Abou-Satl' and the 2 dS/m salinity treatment of 'Arbequina'. The percentage of RFW reduction from the salinity of 2 to 5 dS/m was 24.2% in 'Zard' and 19.7% in the 'Abou-Satl' and 49.8% in the 'Arbequina'. The RDW of 'Abou-Satl' in salinity treatments was generally higher than in other cultivars. RDW reduction had a low slope in 'Zard' and 'Abou-Satl' cultivars. The reduction percentage of RDW from 2 to 5 dS/m salinity was 24.8% in the 'Zard', 27.3% in the 'Abou-Satl', and 54.6% in the 'Arbequina' cultivars.

The ratio of RDW to BDW increased with increasing the salinity. The highest ratio (RDW/BDW) was observed in 12 dS/m of 'Abou-Satl'. This ratio in the 'Zard' cultivar had a lower slope among salinity treatments. The increase of RDW to BDW in the salinity treatments of 12 dS/m in 'Abou-Satl' was higher than the others (Table 2).

Leaf thickness had a significant diversity between the cultivars and levels of salinity. The interactions of salinity

× cultivar were significant as well. Leaf thickness increased with increasing the level of salinity (Table 2). The thick leaves were observed in the 'Zard' cultivar, whereas the 'Arbequina' leaves had the lowest thickness.

Gas exchange

The highest concentration of sub-stomatal CO_2 was found in salinity treatment of 5 dS/m in the 'Zard' cultivar, while the lowest concentration of this feature was in the salinity treatment of 12 dS/m in the 'Arbequina'. The concentration of sub-stomatal CO_2 severely decreased in the 'Arbequina' cultivar. The reduction of sub-stomatal CO_2 percentage from the salinity 2 to 5 dS/m was 5.1% in the 'Zard', 0.4% in the 'Abou-Satl', and 29.3% in the 'Arbequina' cultivars (Table 3).

Transpiration (E) was reduced with the increase in salinity stress. The decreasing rate of transpiration was high for 2 to 5 dS/m in the 'Zard' and 'Arbequina' cultivars. The percentage of decrease in the transpiration in these two cultivars was 43.3% and 33.1%, respectively. In contrast, it was only 16.9% for the 'Abou-Satl' cultivar (Table 3).

Table 2. Effects of salinity stress and cultivar interaction on growth parameters (±SD)

Cultivar	Salinity (Nacl) (dS/m)	Shoot growth (cm)[y]	BFW (g)	BDW (g)	RFW (g)	RDW (g)	RDW/ BDW	Leaf thickness (mm)
Zard								
	2	68.17±0.01cd	51.36±1.61d	24±4.13cd	17.36±2.18b-e	4.96±2.41de	0.29±0.62c	0.55±0.01b
	5	50.33±0.01ef	46.37±1.53e	20.13±0.93de	13.16±3.21d-f	5.57±2.51de	0.23±1.04de	0.55±0.01b
	8	37.67±0.01h	45.22±3.21e	20.44±5.08de	14.22±1.71c-f	5.69±3.42de	0.28±0.44c	0.53±0.01cd
	12	28.67±0.01i	45.65±1.53e	22.2±0.26d	11.13±0.44ef	7.41±5.22c	0.33±1.11b	0.58±0.01a
Abou-Satl								
	2	95.67±0.01a	87.17±7.51a	41.48±3.48a	26.37±3.29a	11.6±3.86a	0.28±1.11c	0.51±0.02e
	5	72±0.02bc	68.64±4.58b	32.14±1.87b	21.17±1.95a-c	8.41±1.09bc	0.27±0.77c	0.51±0.08de
	8	56±0.01e	60.05±2.65c	27.78±1.41c	22.84±1.46ab	9.11±7.07b	0.32±0.57b	0.53±0.01c
	12	29.67±0.01i	36.89±5.86f	17.38±1.41ef	16.07±0.29b-f	7.46±0.57c	0.43±0.35a	0.53±0.01cd
Arbequina								
	2	75.67±0.01b	64.3±3.21bc	28.05±4.36c	19.02±5.57a-d	6.06±7.83d	0.21±0.97e	0.47±0.01f
	5	63.67±0.01d	44.46±1.53e	14.47±1.6fg	9.54±0.82f	2.75±1.25g	0.19±0.36f	0.51±0.01e
	8	46±0.01fg	27.64±4.36g	14.8±0.5fg	9.87±1.2f	3.3±1.89fg	0.23±1d	0.48±0.02f
	12	68.17±0.01cd	51.36±1.61d	24±4.13cd	17.36±2.18b-e	4.96±2.41de	0.29±0.62c	0.55±0.01b
Cultivar		***	***	***	***	***	***	***
Salt		***	***	***	NSX	**	***	***
C×S		***	***	***	*	***	***	***

BFW: Biomass Fresh Weight, BDW: Biomass Dry Weight, RWF: Root Fresh Weight, RDW: Root Dry Weight, [y]Means within the column followed by the same letter are not significantly different at p = 5% level, using LSD test

[x]NS, not significant; *, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

Table 3. Effect of salinity stress and cultivar on photosynthetic and nutrient parameters (±SD)

Cultivar	Salinity (Nacl) (dS/m)	Ci (vpn)	E (µmol H_2O/ m^2s)	g_s (µmol H_2O/ m^2s)	An (µmol CO_2/ m^2s)	An/E (µmol CO_2/ µmol H_2O)	K^+/Na^+ leaf (-)	K^+/Na^+ Root (-)
Zard								
	2	184.67±3.79b	1.57±0.09a	0.03±0.01a	3.84±0.18a	2.45±0.47ef	3.49±0.15b	0.83±0.09b
	5	194.67±5.13a	0.93±0.12d	0.02±0bc	2.96±0.09d	3.29±0.65b	1.29±0.1d	0.59±0.04de
	8	137.33±5.03f	0.89±0.09d	0.01±0.01cd	2.43±0.1e	2.62±0.81d	0.84±0.02e	0.41±0.02fg
	12	91±1h	0.36±0.08g	0.01±0.01d	1.44±0.03h	4.18±0.36a	0.52±0.04fg	0.36±0.04gh
Abou-Satl								
	2	159.67±5.51d	1.3±0.06b	0.03±0.01ab	3.18±0.04c	2.44±0.08f	6.66±0.17a	0.72±0.07c
	5	159.67±0.58d	1.08±0.02c	0.02±0bc	2.52±0.09e	2.37±0.06fg	2.79±0.32c	0.51±0.01e
	8	107±1g	0.75±0.03ef	0.01±0.01cd	2.11±0.01f	2.46±0.02ef	0.74±0.02ef	0.37±0.01gh
	12	101.67±3.21g	0.4±0.01g	0±0.01d	0.97±0.06i	2.82±0.08c	0.41±0.03gh	0.3±0.03h
Arbequina								
	2	176.67±2.89c	1.3±0.09b	0.02±0.01a-c	3.36±0.02b	2.58±0.75de	1.5±0.2d	0.99±0.13a
	5	125±2.65f	0.87±0.15de	0.01±0.01cd	1.94±0.15g	2.26±0.89g	0.71±0.05ef	0.67±0.05cd
	8	87.33±4.62h	0.62±0.11f	0.01±0.01d	1.56±0.03h	2.44±0.61f	0.36±0.01gh	0.51±0.03ef
	12	65.67±4.51i	0.4±0.01g	0±0.01d	1.02±0.11i	2.67±0.88d	0.23±0h	0.37±0.02gh
Cultivar		***	**	*	***	***	***	***
Salt		***	***	***	***	***	***	***
C×S		***	***	NS	***	***	***	***

An: Photosynthesis rate, g_s: Stomatal conductance, E: Transpiration, Ci: Sub-stomatal CO_2,
Means within the column followed by the same letter are not significantly different at p = 5% level, using LSD test.
NS, not significant; *, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

The percentage of photosynthesis reduction in salinity treatments, from 2 to 5 dS/m, was different among the studied cultivars. The photosynthesis reduction percentage in the 'Zard', 'Abou-Satl', and 'Arbequina' cultivars was 22.9%, 20.9%, and 42.2%, respectively. The higher rate of photosynthesis was in the 2 dS/m salinity treatment of all cultivars (Table 3).

Mineral nutrient concentration

The mineral composition of olive plants under salinity stress is presented in Figure 1. The Na^+ concentration in the olive plants slightly increased due to salt stress in both leaves and roots.

The concentration of Na^+ in the leaves of the 'Arbequina' cultivar was higher than that of the 'Zard' and 'Abou-Satl' cultivars and increased linearly with salinity (Figure 1). Leaf Na^+ was concentrated more severely beyond the 5 dS/m of salinity treatment in the 'Abou-Satl' cultivar. In the 8 and 12 dS/m of salinity treatments, the concentrations of Na^+ in leaves were 3.5% and 2.5% in 'Arbequina', 2.9% and 1.7% in 'Abou-Satl', and 1.9% and 1.5% in 'Zard' cultivars.

The increase of Na^+ concentration in the roots of 'Arbequina' was greater than that of the 'Zard' and 'Abou-Satl' cultivars and increased linearly with salinity (Figure 1). The changes of Na^+ concentration were 12.0% in 'Zard',

20.1% in 'Abou-Satl', and 38.6% in 'Arbequina' cultivars, as the salinity increased from 2 to 5 dS/m.

The K^+ concentration in the leaves of olive cultivars reduced with increasing salinity stress (Figure 2). The reduction in the K^+ concentration in the 'Arbequina' cultivar was higher than in the other two cultivars. Leaf K^+ concentration gradually reduced in 'Abou-Satl', and reduced more notably in 'Arbequina' in the salinity treatments. The reduction percentage of leaves K^+ concentration in salinity treatments increased from 2 to 5 dS/m, were 12.5, 9.9, and 13.7% in 'Zard', 'Abou-Satl', and 'Arbequina', respectively.

In contrast to the accumulation of K^+ in the leaves, the K^+ concentration in the roots of 'Arbequina' was higher than in the others (Figure 2). The reduction percentage of roots K^+ concentration with increasing the salinity stress from 2 to 5 dS/m was 18.9%, 10.5%, and 2.3%, in the 'Zard', 'Abou-Satl' and 'Arbequina' cultivars, respectively.

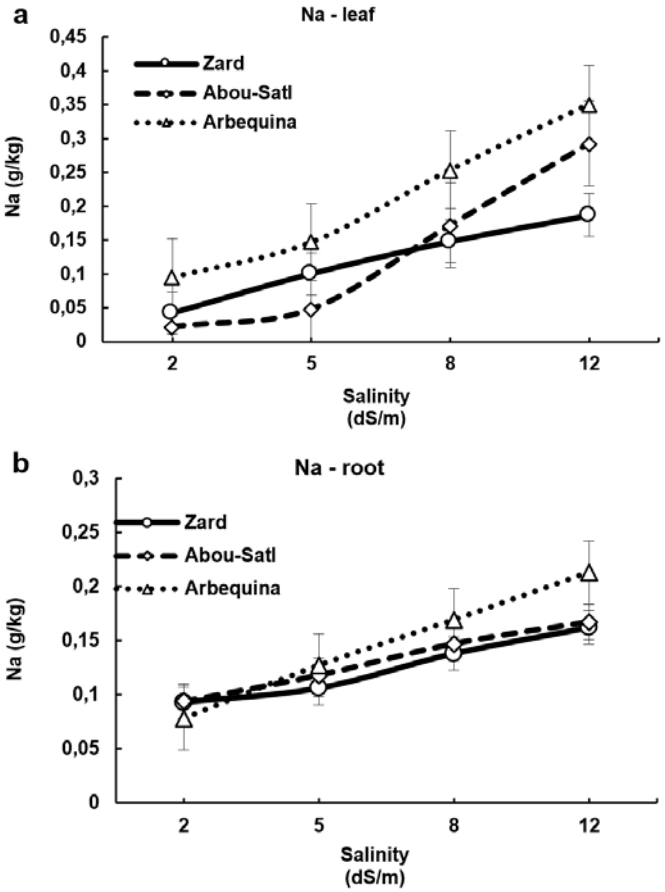

Figure 1. Na^+ concentration in leaves (a) and roots (b) in the olive cultivars at salinity treatments

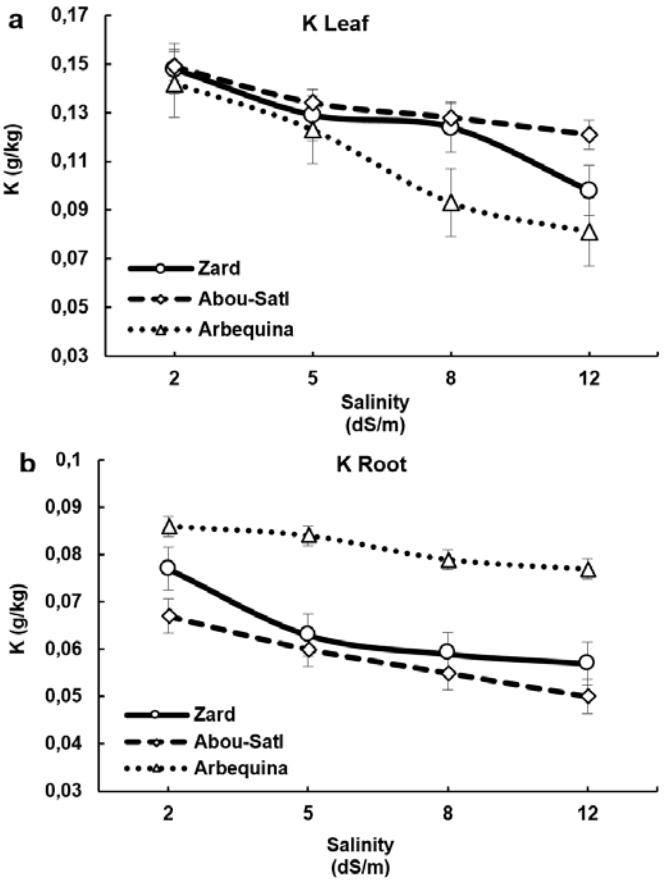

Figure 2. K^+ concentration in leaves (a) and roots (b) in the olive cultivar at salinity treatments

The Ca^{2+} concentration in the leaves of 'Abou-Satl' beyond the salinity of 5 dS/m was higher than the others. Changes of Ca^{2+} concentration in leaves were 6.3% for 'Zard'; however, there were 33.4% and 35.9% for 'Abou-Satl' and 'Arbequina', with increasing the salinity stress from 2 to 5 dS/m. The Ca^{2+} concentration slope versus the salinity stress in 'Zard' was lower than in the two other cultivars (Figure 3).

In all salinity treatments of the 'Arbequina' cultivar, the concentration of Na^+ in the leaves was higher than in the roots. In the 'Zard' cultivar, after 5, and in the 'Abou-Satl' cultivar, after 8 dS/m of salinity, the Na^+ concentration of the leaves was higher than in the roots. There was a significant difference among the studied cultivars for the translocation of Na^+ from roots to leaves and the Na^+ concentration in the leaves and roots (Figure 4).

In this study, the cultivars were divided into two groups. The first group was the 'Arbequina'. The concentration of Na^+ in the leaves of 'Arbequina' was higher than in the

roots. Roots of 'Arbequina' transferred the absorbed Na^+ to the aerial parts of plants. The roots of the plants of Zard' and 'Abou-Satl' (second group) cultivars accumulated Na^+ in their roots, but in higher salinity stresses (8 and 12 dS/m) the Na^+ translocation from roots to the aerial parts of plants increased (Figure 4).

In all salinity treatments, K^+ concentration decreased in the leaves and roots of all studied plants, meaning that the increase in the severity of salinity stress reduced K^+ uptake. In all plants, the K^+ concentration in the leaves was higher than the roots, and the slope of K^+ reduction with the increase in the salinity in all three cultivars was higher in the leaves than in the roots (Figure 4).

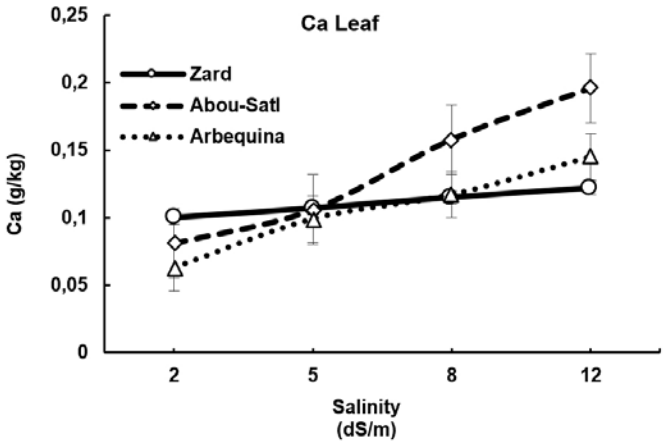

Figure 3. Leaves Ca^{2+} concentration in the olive cultivar at salinity treatments

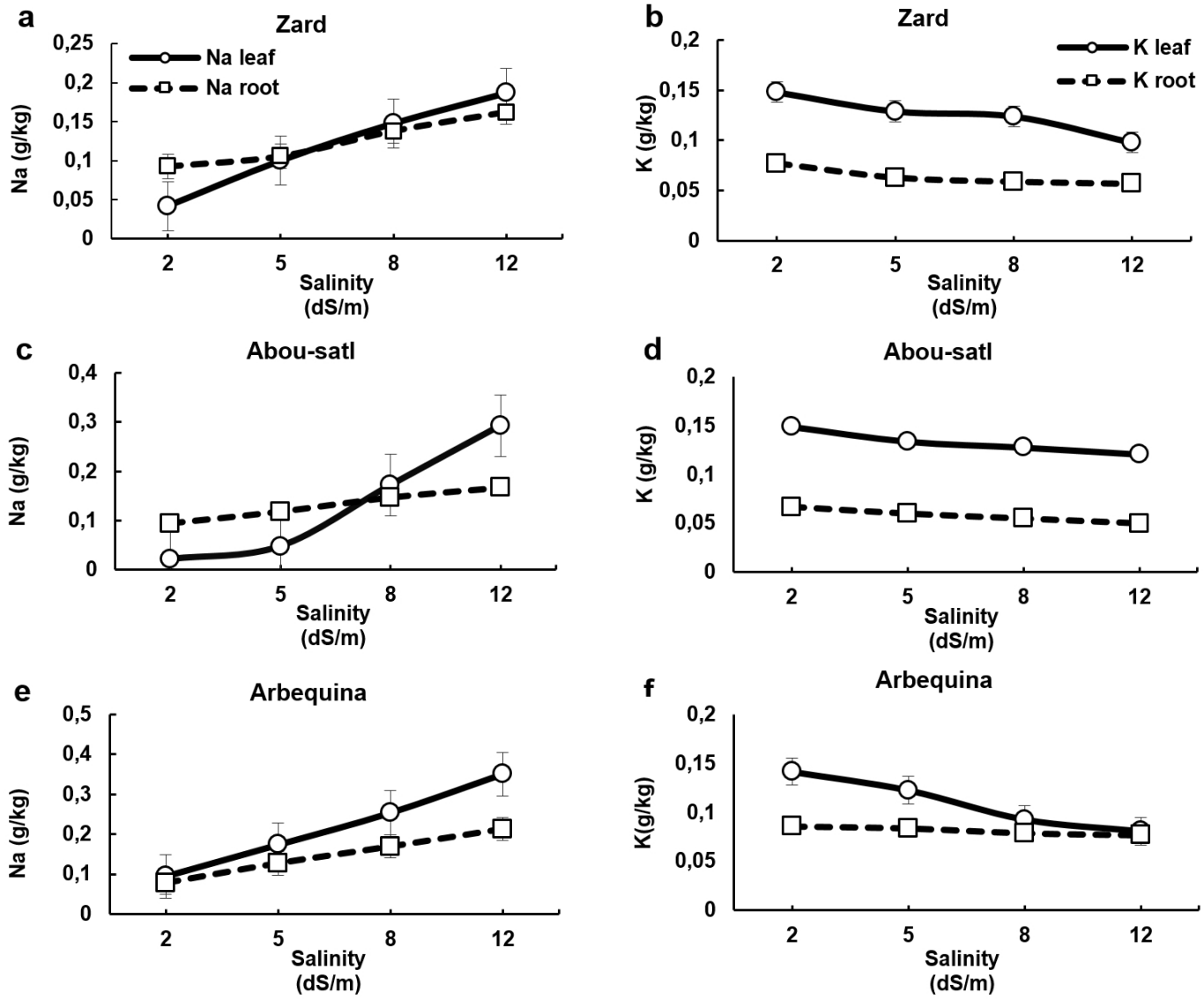

Figure 4. Na^+ and K^+ concentration in leaves and roots in the olive plants within 90 days at salinity stress treatments in the 'Zard' (a, b), 'Abou-satl' (c, d), and 'Arbequina' (e, f) cultivars

DISCUSSION

This study aimed to analyze the tolerance of three olive cultivars ('Zard', 'Abou-Satl' and 'Arbequina') to salinity stress, based on morphological, gas exchange traits, and changes in the accumulation of Na^+, K^+, and Ca^{2+} concentrations in roots and leaves (Ca^{2+} measured only in leaves). The results demonstrated that there were significant genetic differences among the cultivars in salinity stress tolerance based on these parameters. The results are in accordance with the results of other authors (Kchaou et al., 2010; Aparicio et al., 2014; Assimakopoulou et al., 2017).

Salinity stress negatively affected shoot growth, BFW, BDW, and RDW, and reduced their amounts. However, it increased the ratio of RDW to BDW and leaf thickness. Previous studies explained that salinity stress has adverse effects on many growth parameters in olive trees (Ahmed et al., 2008; Kchaou et al., 2010; Aparicio et al., 2014). Therios and Misopolinos (1988) indicated that salinity stress significantly reduced dry leaf and root weight. As salinity increased, the amount of leaf and dry root biomass decreased, depending on the genotype.

Salinity reduced shoot growth more severely than root growth; consequently the root-per-shoot biomass ratio increased (Therios and Misopolinos, 1988; Tattini et al., 1995; Chartzoulakis et al., 2002). High salinity treatments in the olives may alter the pattern of dry matter distribution, causing an increase in root biomass.

Some researchers believe that increasing leaf thickness is a general reaction to salinity, which is controlled by osmotic regulation (Sotiropoulos et al., 2002). Increasing leaf thickness raises the internal surface area per unit leaf area, where carbon dioxide and water vapor are released. As a result, the internal resistance decreases, and the absorption of carbon dioxide and water retention potential remain at higher levels. Vigo et al. (2005) stated that irrigated olive plants with high concentrations of brackish water, produce thicker leaves, which have thicker palisades, sponges chloroplasts, and denser hyphae in adaxial leaf surface (to reduce the transpiration from stomata) than the control plants adapted to salinity conditions.

When morphological traits were used as indicators of salinity stress, the 'Arbequina' cultivar was identified as the most sensitive to salinity in our experiment. BFW, BDW, RDW, RDW to BDW ratio, and leaf thickness of this cultivar, except shoot growth, reacted against salinity stress and were more affected than the 'Abou-Satl' and 'Zard' cultivars. In the 'Arbequina' cultivar, the rate of changes at the 8 dS/m of salinity stress treatment (in comparison with 2 dS/m) was about 50% or higher in most of its morphological traits. In the 'Abou-Satl' cultivar, the values of morphological traits were higher than in 'Zard' and 'Arbequina' cultivars. In general, the highest percentage of changes in the mentioned traits in the 'Abou-Satl' cultivar started from 8 dS/m of salinity and continued to 12 dS/m. In the 'Zard' cultivar, the value of morphological traits in most cases was less than in 'Abou-Satl', but the rate of changes in morphological traits (decrease or increase) among salinity treatments was less than in the other two cultivars.

Aragüés et al. (2010) suggested that the decrease in shoot growth of the 'Empeltre' cultivar at the 4.1 and 8.8 dS/m salinity stress treatments was 11% and 24%, respectively, while in the 'Arbequina' cultivar, it was 22% and 40%. This indicates the sensitivity of the 'Arbequina' cultivar to salinity stress compared to the 'Empeltre'. Aragüés et al. (2004) found that 55% of 'Arbequina' plants died, mainly in environments with salinities more than 10 dS/m, about 3.5 years after planting. Kchaou et al. (2010) represented that 'Arbequina' I18 was quite sensitive in moderate salinity (50 mM NaCl). On the other hand, Marin et al. (1995) found that the relative growth rate of the 'Arbequina' and some other olive cultivars, after 49 days of applying salinity stress, was more than 50%, which placed in the tolerant to salinity group.

Decreased growth in all cultivars was associated with a decrease in photosynthesis under severe salinity treatments (see Table 3). In all three cultivars, the photosynthesis reduction rate was significant at 5 dS/m salinity, but the severity of the photosynthesis rate decline was higher in 'Arbequina'. Many researchers

have explained that photosynthesis changed under the influence of different salinity levels and depended on the type of cultivar (Chartzoulakis et al., 2002; Ahmed et al., 2008; Kchaou et al., 2013; Aparicio et al., 2014). Tabatabaei (2006) also indicated that in the studied cultivars ('Zard', 'Mission', and 'Manzanilla'), photosynthesis and transpiration reduced with increasing salinity. In contrast, some researchers reported that photosynthesis is not dependent on salinity (Tattini et al., 1995; Loreto et al., 2003). The level of photosynthesis in olive trees was possibly limited by low water absorption in medium salinity.

Munns (1993) mentioned that the toxic effect of salt accumulation would be visible at higher Na^+ or Cl^- concentrations. Kchaou et al. (2010) identified that the 'Chemlali', which tolerated salinity (100 mM NaCl), along with the 'Chetoui' cultivar, are good choices for cultivation in saline conditions. Loreto et al. (2003) mentioned that the highest decrease of photosynthesis in saline conditions was observed in cultivars with higher intrinsic photosynthesis. Confirming previous studies, photosynthesis decreased in salinity treatments in the 'Zard', 'Abou-Satl', and 'Arbequina' cultivars. In the present study, the photosynthesis rate had a high negative correlation with the amount of Na^+ in leaves (0.781) and roots (0.797) ($P<0.01$). It means that the amount of photosynthesis was reduced with increasing Na^+ accumulation in the leaves and roots of olive cultivars. Kchaou et al. (2013) pointed out a significant correlation between photosynthesis reduction and Na^+ and Cl^- accumulation in the leaves.

In this study, the leaf Na^+ concentration increased with increasing salinity stress levels. Gucci and Tattini (1997) revealed that the Na^+ concentration in the stem and leaf tissues of 'Leccino', the sensitive cultivar, was higher than in 'Frantoio'. Decrease of Na^+ transfer from root to the aerial parts of the tree is the primary mechanism to regulate the accumulation of salt in the plant instead of excluding Na^+ from the root cells. The greatest limitation of Na^+ transfer compared to Na^+ adsorption is the evidence of differences in the ability of olive cultivars to regulate salt accumulation in the aerial parts of trees (Tattini, 1994). In contrast, Chartzoulakis et al. (2002) reported that the mechanism of Na^+ ion excretion in one-year-old olive plant is more effective in moderate salinity treatments. In most cultivars, Na^+ is transferred to the aerial part with increasing salinity stress and stored in the leaves and causes symptoms of toxicity. In this study, 45 to 50 days after salinity initiation, olive plants of the 'Konservolia' cultivar, which were irrigated with saline waters of 8 and 12 dS/m, showed necrosis signs in leaves, gradually fell down, and plants declined (results were not published). Grattan and Grieve (1999) confirmed that salinity could directly affect the absorption of nutrients; for example, Na^+ ions prevent the absorption of K^+ ions. Kchaou et al. (2010) declared that salinity treatments in olive trees had reduced the concentration of K^+ in leaves and roots.

The concentration of Ca^{2+} in the leaves of these cultivars increased significantly with increasing salinity stress (Figure 3). In olive leaves, the concentration of Ca^{2+} ions increased more than the other nutrients. Ca^{2+} may be used as an osmotic regulator in evergreen species inhabiting arid Mediterranean areas (White and Broadley, 2003; Therios, 2009; Cimato et al., 2010).

Extracellular salt was received by an unknown sensor and initiates a Ca^{2+} dependent pathway to regulate transport proteins to control the net Na^+ influx across the plasma membrane. Therefore, Na^+ transfers to vacuoles from intracellular spaces and protects cells from Na^+ hazards (Bressan et al., 1998).

The ratio of leaf K^+/Na^+ in the two cultivars of 'Abou-Satl' and 'Zard' was higher than in 'Arbequina'. Although in all three cultivars, the K^+/Na^+ ratio was higher in leaves than in roots (Table 3). Gucci and Tattini (1997) stated that the shoots K^+/Na^+ ratio in the 'Frantoio' tolerant cultivar was higher than the sensitive cultivar of 'Leccino'. They also reported that this ratio in the aerial parts of both cultivars was higher than in the roots. The ratio of K^+/Na^+ in the roots of the 'Arbequina' was more than the other two cultivars, except for the 12 dS/m salinity treatment. However, there were no significant differences between the cultivars at the 12 dS/m.

CONCLUSIONS

This research on three olive cultivars presented differences in olive cultivars' response to salinity stress, as assessed based on variability in morphological, mineral nutrients, and gas exchange traits.

Based on aerial biomass traits, cultivar 'Zard' tolerated salinity more than the other cultivars. Root biomass of cultivars 'Abou-Satl' and 'Zard' had the least reduction at all salinity levels. Generally, leaf thickness was higher in the salt tolerant cultivars. Gas exchange, carbon dioxide uptake, and water potential were high in the 'Zard' cultivar. Photosynthetic reduction rate was lower in cultivars 'Zard' and 'Abou-Satl' than in cultivar 'Arbequina' cultivar.

Na^+ and K^+ concentrations increased and decreased, respectively, in leaves and roots. K^+ concentration was generally higher in leaves than in roots of all cultivars. Na^+ translocation from roots to leaves was reduced efficiently in cultivars 'Zard' and 'Abou-satl'.

The results showed that cultivars 'Zard' and 'Abou-Satl' tolerated salinity levels of 8 and 12 dS/m, respectively, and maintained their performance at medium salinities. Therefore, 'Zard' and 'Abou-Satl' were suitable cultivars for the cultivation in olive orchards in northern Iran (Zanjan, Qazvin, Guilan, and Golestan provinces).

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