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Comparative Study of Salinity Effects on Some Olive Cultivars

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Abstract: This study was conducted during 2016 and 2017 seasons on eleven own-rooted olive (*Olea europaea* L.) cultivars (Picual, Koroneiki, Manzanillo, Coratina, Aggezi Shami, Aggezi Akse, Kalamata, Dolce, Maraki, Frantoio and Sewia). The selected cultivars were thirteen years old and were drip irrigated with ground saline water (EC 7.06 dS/m – 5648 ppm) to select the most salinity tolerant ones for the expansion of cultivated area in new reclaimed lands in Egypt. Chemical analysis of leaves and roots indicated that tolerant cultivars tended to have decreased Na content and increased K and N content percentages as well as K/Na ratio. Leaf analysis showed that higher Ca content percentage, Ca/Na and K+Ca/Na ratios distinguished tolerant ones. In both seasons, Picual contained decreased Na content in leaves and roots, total phenols percentage, proline content and turgid weight/fresh weight ratio in leaves, whereas it accumulated higher values of K and N contents, K/Na ratio in leaves and roots, Ca content, Ca/Na as well as K+Ca/Na ratios in leaves. On the contrary, Dolce significantly recorded the highest leaf and root Na contents and leaf turgid weight/fresh weight ratio, whereas it contained lowest K and N percentages as well as K/Na ratio in leaves and roots. Also, Dolce maintained minimum Ca percentage, Ca/Na, K+Ca/Na ratios as well as relative water content percentage in leaves. Aggezi Akse recorded the highest leaf total phenols content. Coratina, Aggezi Akse and Dolce contained higher values of leaf proline content. Results indicated that sensitive cultivars were not included in this study. Picual and Kalamata cvs. were the most tolerant cultivars while Aggezi Akse and Dolce cvs. were the least tolerant, other cultivars were in between when irrigated with saline ground water (5648 ppm).

Key words: *Olea europaea* · Cultivars · Salinity · Sodium · Potassium · Calcium

INTRODUCTION

Salinity is a worldwide major problem facing crop production nowadays. It is estimated that approximately one-third of the world's irrigated lands and half the lands in semi-arid and coastal regions are affected by salinization; 10 million hectare (ha) of irrigated lands are abandoned annually because of excessive salinity. Hence, an effective way must be found to use soil of high salinity by the cultivation of tolerant cultivars [1]. The harmful effects of salinity on plants include negative effects on water relations, nutritional and osmotic imbalance, specific ion effects that lower photosynthetic rate and impair growth, oxidative stress or a combination of the previously mentioned effects [2-5].

Mediterranean and Middle East regions include a high olive (*Olea europaea* L.) plantation areas which suffer from water shortage in semi-arid and arid zones. To overcome water scarcity, the use of saline water is adapted in olive production. the use of saline ground water as the only source for irrigation are currently, the main causes of salinization in Egypt [6]. Olive is moderately tolerant to salinity. Cultivated olive, a typical crop species of the semi-arid regions, could successfully face the new scenarios of climate change through tolerant varieties selection to salt and drought stresses [7]. In Egypt, olive harvested area was 214147.62 Feddans, producing 1080091 Tons in 2019 [8]. Approximately 0.9 million ha (2.1 million Feddans) suffer from salinization problems in the cultivated irrigated areas in Egypt. Furthermore, 60% of the cultivated lands in the northern Delta, 20% of the southern Delta and Middle Egypt and 25% of the Upper Egypt regions are all salt-affected [6].

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The response to salinity is a genotypic or cultivar dependent characteristic [9-11]. Demiral [12] found that salinity negatively affected the K^+ and Ca^{++} contents of plant tissues. Ranking of cultivars on the basis of Na^+ accumulation and the K^+:Na^+ ratio were more consistent than ranking based on shoot growth parameters [13]. Kchaou *et al*. [14]; Bader *et al*. [15] and Hassan *et al*. [16] indicated that the degree of tolerance to salinity varied between different olive cultivars. Much research has been devoted to the interaction between salinity and olive cultivation, however, these were made on seedling stages and more information is needed to assure sustainable long-term olive production based on advanced agro-techniques for saline conditions in the field [1]. Young trees are generally less tolerant to saline water than mature trees. Tolerant cultivars use is recommended in problematic saline sites [17, 14]. However, the number of evaluated cultivars is very limited if compared to the thousands of genotypes under cultivation worldwide [7]. Due to the expansion of cultivated area in new reclaimed lands in Egypt, salinity tolerant cultivars are of great importance but there is no sufficient literature about salt tolerance degree of local and imported olive tree cultivars, also there is shortage in studies comparing between cultivars when irrigated with saline ground water under Egyptian conditions. The response of eleven selected cultivars to salinity is comparatively studied.

MATERIALS AND METHODS

Plant Materials and Experimental Conditions: This work was carried out during 2016 and 2017 seasons on eleven own-rooted olive (*Olea europaea* L.) cultivars (Picual, Koroneiki, Manzanillo, Coratina, Aggezi Shami, Aggezi Akse, Kalamata, Dolce, Maraki, Frantoio and Sewia). The selected cultivars were thirteen years old growing in a private farm. The selected cultivars were planted in sandy loam soil spaced at 6 × 3 m and were irrigated with saline ground water (EC 7.06 dS/m – 5648 ppm) using drip irrigation system (4400 m^3 /fed.). The trees were subjected to the regularly recommended cultural practices and were free from pathogens and physiological disorders. Each cultivar was represented by three trees, each tree was a replicate.

Soil and water samples were collected. Soil samples were air-dried, grounded to pass through a 2.0 mm sieve and then mixed. Soil texture was sandy loam. Chemical properties of the soil fractions at depth 0-30 cm and 30-60 cm and water sample were determined in Soils, Water & Environment Res. Ins. Labs and are presented in Tables 1, 2 and 3, respectively.

Fully expanded mature leaf samples from the middle portion of non-bearing shoots and fine root samples for each cultivar were collected in July for each season, then washed with tap water followed by distilled water. Leaf and root samples were oven dried at 70°C until completely dry. Leaf dry samples were digested in a mixture of sulfuric and perchloric acids according to Piper [18] to estimate Na, K, N and Ca percentages, the same was done for fine root samples to determine Na, K and N after digestion [19]. Also, K^+/Na^+ leaf and roots ratios, Ca^{++}/Na^+ and $K^+ + Ca^{++}/Na^+$ leaf ratios were calculated. Leaf Cl^- percentage was also extracted from dry samples with distilled hot water and titrated with standard silver nitrate solution and then determined according to A.O.A.C. [20].

The total phenols percentage was determined in fresh leaf samples at 760 nm (JENWAY 6405UV/Vis Spectrophotometer), using the Folin–Ciocalteu reagent according to Singleton *et al*. [21].

Proline estimation (mg/100g) was performed using dried leaf samples and absorbance at 520 nm was measured spectrophotometrically [22].

Twenty punched leaf discs from the middle of the leaves of each cultivar were obtained using hole puncher, fresh weighted (FW) then placed in distilled water (20 ml) in petri dishes with lids to dehydrate for 24 h in a dark place, reweighted for turgid weight (TW), then placed in an oven at 70°C for 72 h and dry weighted (DW) for the calculation of relative water content percentage according to Ben-Ahmed *et al.* [23] using the equation:

$$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100.$$

Also, the TW/FW ratio of leaf discs were estimated for each cultivar.

Experimental Layout: The experimental treatments (eleven olive cultivars) were arranged in randomized complete block design. Data recorded in 2016 and 2017 seasons were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [24] and the means were differentiated using Duncan multiple tests at the level of probability 5% [25].

Table 1: Chemical characteristics, available macro and micronutrients of the tested soil sample (0-30 cm) collected from the experimental area

			mEq/L.							
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	So_4^{--}	pH (1:2.5)	EC dSm^{-1}(1:5)	Texture class
18.42	16.91	29.31	0.64	-	4.72	27.12	33.44	8.00	7.08	Sandy loam
			mg/Kg							
N	P	K	Cu	Fe	Mn	Zn				
189.00	6.88	113.00	0.04	0.51	0.31	0.152				

Table 2: Chemical characteristics, available macro and micronutrients of the tested soil sample (30-60 cm) collected from the experimental area

			mEq/L.						
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	So_4^{--}	pH (1:2.5)	EC dSm^{-1} (1:5)
7.89	2.97	12.90	0.26	-	2.83	14.41	6.78	7.20	2.68
			mg/Kg						
N	P	K	Cu	Fe	Mn	Zn			
72.00	6.22	59.00	0.048	0.678	0.248	0.124			

Table 3: Chemical properties and available macro and micronutrients of the tested ground water sample collected from the experimental area

			mEq/L						EC	
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	So_4^{--}	pH	dSm^{-1}	ppm
16.06	11.74	38.26	0.17	0.00	1.75	29.15	35.33	8.30	7.06	5648.0
			mg/L							
N (NH_4^{+})	N (NO_3^{-})	P	B	Zn	Fe	Mn	Cu (µg/L)	SAR		
2.80	4.90	0.03	0.02	0.013	0.135	0.096	< 0.20	10.26		

RESULTS AND DISCUSSIONS

Dealing with soil EC, it was 7.08 and 2.68 dSm^{-1} in 0-30 and 30-60 cm soil fractions, respectively (Table 1 and 2). Concerning irrigation water EC (Table, 3), the estimated EC value was 7.06 dSm^{-1} (5648 ppm). It was recommended for olive cultivation that soil EC in the root zone growth area at a level lower than 6 dSm^{-1}. Also, it was suggested that in the long–term, 7.50 dSm^{-1} water is not suitable for sustainable olive cultivation [26, 27]. In this study, no salt toxicity visual signs were observed such as tip burn, necrosis and/or shoot die back in all examined cultivars under farm conditions [28]. About 3500 m^3/F. year was used for irrigation plus 900 m^3/F. year (leaching methodology) using drip irrigation. It was reported that using drip irrigation allowed water applying with a high frequency and maintaining a high humidity in the soil, which avoided the harmful salt concentration for the roots [29].

Leaf and Root Sodium Percentages: Data presented in Table (4) show leaf and root sodium percentages of studied olive cultivars in 2016 and 2017 seasons. In both seasons, Dolce cv. showed the highest significant leaf and root sodium values (1.40 and 1.42 % as well as 1.50 and 1.51 %, respectively), whereas Picual cv. leaves and roots statistically accumulated the lowest significant sodium values (0.56 and 0.63 % as well as 0.72 and 0.74 %, respectively).

It was concluded that the ability to regulate salt entry to the shoot can be used to screen genotypes for salt tolerance [17]. It's worthy to mention that in all studied cultivars, leaves sodium values were lesser than that of roots, which confirms Nabila *et al*. [30]; Olyaei *et al*. [5]; Kasırğa and Demiral [31] and Larbi *et al*. [32] findings, who noticed that sodium concentration level in the aerial parts of olive plants was lower than that of roots. In salt sensitive cultivars, sodium concentration increased more in the leaves than the roots [33, 34, 12, 35, 36, 15]. Mousavi *et al*. [7] mentioned that sodium ions concentration increase in leaves was higher than in shoots and roots of olive plants under severe conditions of salt stress. Moreover, Aparicio *et al*. [36] concluded that the most salt-tolerant genotypes (Ocal and Picudo cvs.) were characterized by an important accumulation of Na^{+} in root and an important inhibition of translocation of Na^{+} to leaf in nutrient solution in a growth chamber pot experiment. They also mentioned that Na in leaves of six olive cultivars ranged 0.53-2.62%, while root Na ranged

Table 4: Leaf and root sodium and potassium percentages of olive cultivars in 2016 and 2017 seasons

	Na %				K %			
	Leaves		Roots		Leaves		Roots	
Cultivars	2016	2017	2016	2017	2016	2017	2016	2017
Picual	0.56 H	0.63 F	0.72 F	0.74 E	1.15 A	1.10 A	1.13 A	1.12 A
Kalamata	0.82 G	0.89 E	0.89 E	0.94 D	1.11 AB	1.08 A	1.08 AB	1.04 AB
Koroneiki	0.89 EF	0.95 DE	1.15 D	1.16 C	1.04 B	1.07 A	1.02 B	0.93 C
Aggezi Shami	0.86 FG	0.91 DE	0.95 E	0.99 D	1.07 AB	1.05 A	1.02 B	0.98 BC
Frantoio	0.84 G	0.90 DE	0.91 E	0.97 D	1.09 AB	1.06 A	1.07 AB	1.03 AB
Sewia	1.09 D	1.16 C	1.22 B-D	1.20 C	0.88 C	0.82 B	0.78 C	0.62 D
Maraki	0.92 E	0.96 D	1.20 CD	1.22 C	0.76 D	0.84 B	0.72 C	0.63 D
Coratina	1.17 BC	1.23 B	1.28 BC	1.27 C	0.55 E	0.53 C	0.51 D	0.47 E
Aggezi Akse	1.22 B	1.23 B	1.31 B	1.38 B	0.54 E	0.43 D	0.35 EF	0.31 F
Manzanillo	1.14 CD	1.20 BC	1.25 BC	1.26 C	0.62 E	0.56 C	0.42 E	0.40 E
Dolce	1.40 A	1.42 A	1.50 A	1.51 A	0.33 F	0.23 E	0.30 F	0.12 G

Means designated with the same letter within column in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.

2.73-5.03% at 200 mM salinity. The ion exclusion and compartmentation at the root level regulates ion concentration in the xylem so as to prevent the accumulation of potentially toxic ions in the aerial parts [37, 34, 28]. This mechanism works effectively in all studied olive tree cultivars, since these cultivars tended to accumulate higher Na ions at the root level than leaves, without detection of any toxicity symptoms after a long period of salt stress (since 2007). Similarly, Olyaei *et al*. [5] found that leaf and root Na content of Iranian olive cultivars ranged 0.45-1.21 and 1.56-2.32% respectively. It was reported that olive trees can tolerate irrigation water salinity of up to 5 dS/m with a SAR of 18 and can produce new growth when leaf Na levels ranged 0.4-0.5% dry weight [35]. In this study, Trees under study irrigated with 7.06 dS/m water salinity, with a SAR of 10.26 and still growing and yielding when leaf Na level reached up to 1.42%.

The above results are in line with Tabatabaei [38] and Chartzoulakis [39] who concluded that there is a genotypic difference among olive cultivars in their Na^+ accumulation. Na^+ uptake reduction and accumulation by plants is one of the most important mechanisms of plant resistance to salt stress [40]. Similar result was obtained by Benlloch *et al*. [41]; El-Sayed *et al*. [42]; Marin *et al*. [43]; Hassan *et al*. [16] and Hassan [44] who reported that Picual cv. is tolerant to salinity. Moreover, Abd Elhameed [45] found that Picual cv. was lesser than Kalamata in Na^+ leaf content. Chartzoulakis *et al*. [9] found that Kalamata cv. is resistant to salinity, while Koroneiki is less tolerant. Chartzoulakis [39] declared that salt-tolerant cultivar Kalamata has a more efficient mechanism regulating salt translocation to the shoot, keeping leaf Na^+ concentration low, even exposed to 200 mM NaCl. The above results are in agreement with Melgar *et al*. [46] who found that root Na^+ content was higher in Koroneiki cv. than in Picual cv., reflecting differences in salinity tolerance. Similarly, Aly [47] mentioned that Aggezi transplants recorded the highest leaf Na content followed by Manzanillo then Koroneiki with significant differences. With respect to sodium allocation, Heimler *et al*. [49]; Rossi *et al*. [50] and Pandolfi *et al*. [11] reported that Leccino cv. (salt-sensitive) accumulated more sodium than Frantoio cv. (salt-tolerant) in all organs, especially in leaves. Rossi *et al*. [50] also mentioned that sodium is retained in root and translocated to aboveground organs in Frantoio lesser than in Leccino. Tabatabaei [1] reported that Manzanillo was sensitive to salinity. At 100 mM NaCl, Frantoio was able to accumulate less Na^+ than Manzanillo [13]. Hassan [44] found that Manzanillo cv. significantly achieved higher leaf Na percentages than Picual cv. in both seasons when irrigated with saline ground water.

Leaf and Root Potassium Percentages: Results of studied olive cultivars in 2016 and 2017 seasons are presented in Table (4). Dealing with leaf potassium percentage, higher percentages of Picual, Kalamata, Aggezi Shami and Frantoio cvs. were recorded with insignificant differences (1.15, 1.11, 1.07 and 1.09 % in the 1^{st} season as well as 1.10, 1.08, 1.05 and 1.06 % in the 2^{nd} season, respectively). The same trend of higher root potassium percentages was observed by Picual, Kalamata and Frantoio cvs. in the two studied seasons. However, Dolce cv. took the other way around in both seasons regarding leaf and root potassium content. Other cultivars were in between.

The decline of K concentration under salinity conditions was the case for olive trees, particularly in salt-sensitive cultivars [1]. Probably, the presence of K^+ enhances Na^+ exclusion by controlling channel selectivity. With high K concentration in leaves, the cultivar prevented osmotically Na transport from the roots to the aerial parts [51]. In contrast to Na^+, the cultivars took and translocated a large quantity of K^+ to the canopy [12] and leaves [31]. Tabatabaei [38] concluded that there is a genotypic difference among olive cultivars in their ability to accumulate K^+. Also, Kasırğa and Demiral [31] mentioned that the concentration of K was regarded as an indication of adaptation to salinity in olive. The above results are in agreement with Koubouris *et al*. [52] who revealed that the antagonistic role of Na and K was confirmed by plant tissue analysis. Similar results were obtained by Aparicio *et al*. [36] and Olyaei *et al*. [5] who mentioned that leaf and root K content ranged 0.75-1.43 and 0.42-0.90 % of six olive cultivars as well as 0.97-1.12 and 0.77-1.04% of Iranian olive cultivars, respectively.

Tattini *et al*. [33] found that K^+ content was always decreased to a greater extent in Leccino (salt-sensitive) than in Frontoio (salt-tolerant). El-Sayed *et al*. [42] noted that different accumulation of K content in 14 olive cultivar transplants, showing that Picual cv. significantly surpassed Aggezi, Koroneiki and Manzanillo cvs. in both seasons. Aly [47] found that Koroneiki surpassed in leaf K content followed by Manzanillo then Aggezi with significant differences. Perica *et al*. [13] demonstrated that Manzanillo accumulated less K^+ than Frantoio (0-100 mM salinity). Higher leaf K^+ content accumulated in Picual and Koroneiki cvs. than Manzanillo, Coratina and Eggazi Shami cvs. transplants [48]. Nabila *et al*. [30] showed that Picual accumulated higher root K content than Manzanillo. Similarly, Abd Elhameed [45] and Hassan [44] demonstrated that leaf K^+ content of Picual cv. was higher than that of Kalamata and Manzanillo cvs., respectively in both seasons. Mousavi *et al*. [7] found that higher leaf K^+ content of Royal de Cazorla cv. (tolerant) than Koroneiki cv. (susceptible) with insignificant difference in a hydroponic system.

Leaf and Root K^+/Na^+ Ratios: Table (5) represents K^+/Na^+ leaf and root ratios of different olive cultivars in 2016 and 2017 seasons. Both ratios were decreased drastically (2.04-0.23, 1.75-0.16, 1.58-0.20 and 1.50-0.08, in 2016 and 2017, respectively). The same trend was clear between olive cultivars in both seasons, as Picual cv. significantly maintained the highest leaf and root ratios (2.04 and 1.75 as well as 1.58 and 1.50 in the 1st season and the 2nd season, respectively) followed by Kalamata and Frantoio cultivars and they were at par, whilst Dolce cv. significantly exhibited the lowest ratios in both seasons. Studied cultivars tended to have higher leaf values than root.

Tattini [53] concluded that the resistance mechanism of salt-tolerant olive cultivars is probably related to the ability to maintain an appropriate K/Na ratio in actively growing tissues. Cultivars ranking based on Na^+ accumulation and K^+/Na^+ ratio was more consistent than ranking based on shoot growth parameters [17, 13]. Olive owes its tolerance of salinity to its ability to restrict transport to shoots, isolate Na in vacuoles and maintain a high K/Na ratio to support tissue metabolism [54]. Demiral [12] suggested that the translocation rate of the ions might be evaluated as reliable criteria giving clues to salt-tolerance levels of *O. europaea* cultivars. Similar result was found by Kasırğa and Demiral [31] who reported that the K/Na ratio was found higher in leaves than in roots. They also added that higher K/Na ratio of the plant leaves can be accepted as key indicator reflecting the level of adaptation of the cultivar to salt stress. A well balanced K^+:Na^+ ratio is crucial for the proper adjustment of stomatal function, activation of enzymes, protein synthesis, cell osmoregulation, oxidants metabolism, photosynthesis and turgor maintenance [55].

The salt-sensitive Leccino cv. showed a lower K^+/Na^+ selectivity ratio than the salt-tolerant Frantoio cv. [33, 53, 49, 10]. Tabatabaei [1] found that difference in K selectivity among cultivars was also observed in the K/Na ratio. Reduction in leaf K/Na ratio was became more pronounced in Manzanillo and Zard cultivars. Perica *et al*. [13] gave evidence that in 0, 33, 66 and 100 mM NaCl, Frantoio maintained the highest K^+/Na^+ ratio. At 166 mM NaCl, lower values were obtained by Leccino and Manzanillo. Aparicio *et al*. [36] found that the lowest decrease in K^+/Na^+ ratio was shown by the most tolerant genotypes (Ocal and Picudo cvs.) grown in nutrient solution in a growth chamber pot experiment. They also mentioned that leaf and root K^+/Na^+ ratio ranged 0.29-2.30 and 0.12-0.30% respectively. Abd Elhameed [45] showed that Picual had significant higher leaf K^+/Na^+ ratios than Kalamata in both studied seasons. Mousavi *et al*. [7] showed that the K^+/Na^+ ratio in Royal de Cazorla leaves was higher than in Koroneiki after the addition of 200 mM NaCl, confirming that salt tolerance as a consequence of both the ability to prevent Na^+ accumulation and to maintain high levels of K^+ in leaves.

Table 5: Leaf and root K^+/Na^+ ratios and nitrogen percentages of olive cultivars in 2016 and 2017 seasons

	K/Na ratio				N %			
	Leaves		Roots		Leaves		Roots	
Cultivars	2016	2017	2016	2017	2016	2017	2016	2017
Picual	2.04 A	1.75 A	1.58 A	1.50 A	1.88 A	1.90 A	1.75 A	1.70 A
Kalamata	1.35 B	1.23 B	1.24 B	1.12 B	1.91 A	1.82 B	1.67 B	1.63 B
Koroneiki	1.16 C	1.12 B	0.89 D	0.80 D	1.49 DE	1.45 DE	1.45 GH	1.41 G
Aggezi Shami	1.24 BC	1.16 B	1.08 C	0.98 C	1.53 CD	1.49 CD	1.47 EF	1.42 FG
Frantoio	1.31 B	1.17 B	1.17 BC	1.08 BC	1.65 B	1.56 C	1.59 C	1.54 C
Sewia	0.81 D	0.71 D	0.65 E	0.51 E	1.56 C	1.53 C	1.51 D	1.48 E
Maraki	0.83 D	0.88 C	0.59 E	0.52 E	1.55 CD	1.53 C	1.50 DE	1.52 D
Coratina	0.47 E	0.43 EF	0.41 F	0.37 F	1.49 DE	1.45 DE	1.46 FG	1.42 FG
Aggezi Akse	0.44 E	0.35 F	0.27 GH	0.22 G	1.46 E	1.43 DE	1.44 GH	1.41 G
Manzanillo	0.54 E	0.47 E	0.34 FG	0.32 FG	1.52 C-E	1.45 DE	1.48 EF	1.43 F
Dolce	0.23 F	0.16 G	0.20 H	0.08 H	1.48 DE	1.41 E	1.43 H	1.40 G

Means designated with the same letter within column in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.

Leaf and Root Nitrogen Percentages: Data in Table (5) present leaf and root nitrogen percentages of different olive cultivars in 2016 and 2017 seasons. Data indicated that Kalamata cv. topped the others in leaf nitrogen percentage with insignificant variation with Picual cv. in 2016 season, but in 2017 season they switched their positions with statistical difference. Aggezi Akse cv. recorded the least value with insignificant differences with Koroneiki, Coratina, Manzanillo and Dolce cvs. in the 1st season, whilst in the 2nd season, it was Dolce cv. without statistical variation with the previously mentioned cultivars. Notably, Picual cv. gave the significantly highest nitrogen root percentages in both seasons. The same trend of lower values was observed by Koroneiki, Aggezi Akse and Dolce cultivars in both seasons.

Nabila *et al*. [30] and Kasırğa and Demiral [31] reported higher leaves nitrogen content than roots in Manzanillo and Picual cvs. as well as Gemlik cv., respectively. El-Sayed *et al*. [42] found that a higher leaf nitrogen content was recorded by Picual with significant differences with Aggezi, Koroneiki and Manzanillo cvs. in the 1st season, whilst in the 2nd season, significant difference was just with Aggezi. Aly [47] noted that Koroneiki statistically surpassed both Aggezi and Manzanillo in both seasons. Abd Elhameed [45] reported that higher N content in leaves of Picual cv. than Kalamata cv. with significant differences in both seasons. Hassan [44] confirmed that Picual leaf N content significantly exceeded that of Manzanillo in both seasons when irrigated with saline ground water.

Leaf Calcium Percentage: Data of 2016 and 2017 seasons showed that Picual cv. achieved significantly the highest values (0.92 % in both seasons) and on the contrary, Dolce cv. acquired the lowest ones (Table, 6) regarding leaf Ca percentage.

Zidan *et al*. [56] stated that presence of K^+ and in particular Ca^{+2} ions has been shown to reduce Na^+ influx to plant cells. Calcium maintains the integrity and function of cellular membranes. In that way root selectivity for K instead of Na is maintained [57]. Ca^{2+} is assumed to play an important role in retention mechanisms and sodium exclusion, which may be considered responsible for survival under salt stress conditions [58]. Jacoby [51] and Fernández-Escobar [59] noted that the presence of Ca^{+2} enhances Na^+ exclusion. This presence can increase tolerance to salinity. According to Tattini *et al*. [33], Ca^{+2} tissue content was always decreased to a greater extent in salt-sensitive Leccino than in salt-tolerant Frontoio. Shaheen *et al*. [48] noted that Picual transplants gave the highest Ca^{+2} leaf content with significant difference just with Manzanillo in the 1st season. Kasırğa and Demiral [31] mentioned that leaf Ca concentration was regarded as an indicator of adaptation to salinity in olive. Mousavi *et al*. [7] found that higher leaf Ca^{++} content of Royal de Cazorla (tolerant) than Koroneiki (susceptible) with significant difference in a hydroponic system. Similarly, Hassan [44] showed that Picual significantly surpassed Manzanillo in leaf Ca content in both seasons when irrigated with saline ground water.

Leaf Ca^{++}/Na^+ and K^++Ca^{++}/Na^+ Ratios: Data presented in Table (6) reveal that in both seasons, Picual cv. significantly surpassed the others recording 1.63 and 1.46 as well as 3.67 and 3.22, respectively regarding leaf Ca^{++}/Na^+ ratio and K^++Ca^{++}/Na^+ ratio, while Dolce cv. gave the minimum values.

Table 6: Leaf calcium percentage, calcium/sodium ratio, potassium+calcium/sodium ratio and chloride percentage of olive cultivars in 2016 and 2017 seasons

	Ca % Leaves		Ca/Na ratio Leaves		K+Ca/Na ratio Leaves		Cl % Leaves	
Cultivars	2016	2017	2016	2017	2016	2017	2016	2017
Picual	0.92 A	0.92 A	1.63 A	1.46 A	3.67 A	3.22 A	0.36 D	0.41 C
Kalamata	0.78 B	0.76 B	0.95 B	0.86 B	2.30 B	2.08 B	0.34 E	0.37 E
Koroneiki	0.69 BC	0.67 BC	0.77 CD	0.70 CD	1.94 E	1.83 D	0.32 F	0.36 E
Aggezi Shami	0.73 BC	0.74 B	0.85 BC	0.81 B	2.09 D	1.96 C	0.46 A	0.50 A
Frantoio	0.74 BC	0.71 BC	0.89 BC	0.78 BC	2.20 C	1.95 C	0.43 B	0.46 B
Sewia	0.68 BC	0.67 BC	0.62 EF	0.58 E	1.43 G	1.29 F	0.36 D	0.36 E
Maraki	0.66 CD	0.65 BC	0.72 DE	0.68 D	1.55 F	1.55 E	0.45 A	0.50 A
Coratina	0.55 DE	0.50 DE	0.47 GH	0.40 F	0.94 I	0.84 H	0.37 D	0.39 D
Aggezi Akse	0.46 EF	0.44 EF	0.37 HI	0.36 F	0.81 J	0.70 I	0.39 C	0.41 C
Manzanillo	0.63 CD	0.60 CD	0.55 FG	0.50 E	1.08 H	0.97 G	0.36 D	0.39 D
Dolce	0.39 F	0.36 F	0.28 I	0.25 G	0.51 K	0.41 J	0.43 B	0.46 B

Means designated with the same letter within column in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.

Table 7: Leaf total phenols percentage, proline content (mg/100 g dry weight), relative water content percentage and turgid weight/fresh weight ratio of olive cultivars in 2016 and 2017 seasons

	Leaf total phenols (%)		Leaf proline content (mg/100g)		Leaf relative water content (%)		Turgid weight/Fresh weight ratio	
Cultivars	2016	2017	2016	2017	2016	2017	2016	2017
Picual	1.70 E	1.86 D	0.016 C	0.019 D	65.22 B	69.36 A	1.323 C	1.223 F
Kalamata	1.83 D	2.01 C	0.020 BC	0.023 CD	67.43 A	67.21 B	1.333 C	1.283 EF
Koroneiki	2.06 C	2.18 B	0.023 B	0.025 B-D	60.05 E	58.62 F	1.360 BC	1.263 EF
Aggezi Shami	2.04 C	2.16 B	0.023 B	0.025 B-D	61.27 D	63.49 D	1.380 BC	1.323 DE
Frantoio	1.86 D	2.03 C	0.023 B	0.023 CD	62.07 C	65.74 C	1.363 BC	1.380 CD
Sewia	1.90 D	2.06 C	0.020 BC	0.024 B-D	58.99 F	54.30 H	1.390 BC	1.440 A-C
Maraki	2.19 B	2.24 B	0.021 BC	0.021 CD	61.03 D	60.56 E	1.420 B	1.417 A-C
Coratina	2.23 B	2.21 B	0.030 A	0.035 A	61.13 D	55.16 G	1.363 BC	1.370 CD
Aggezi Akse	2.47 A	2.50 A	0.030 A	0.034 A	57.12 G	54.40 H	1.413 B	1.453 AB
Manzanillo	2.24 B	2.22 B	0.025 AB	0.026 BC	57.11 G	55.15 G	1.343 BC	1.393 B-D
Dolce	2.26 B	2.22 B	0.030 A	0.030 AB	52.90 H	52.97 I	1.490 A	1.473 A

Means designated with the same letter within column in each season are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 level of probability

Increasing the Ca: Na ratio in the external solution has been reported to alleviate the effects of salinity on depolarization and selectivity of the plasma membrane [60]. Kasırğa and Demiral [31] found that higher K+Ca+ Mg/Na ratio of the plant leaves can be accepted as key indicator reflecting the level of adaptation of the cultivar to salt stress.

Leaf Chloride Percentage: Referring to leaf chloride percentage, data presented in Table (6) show that both Aggezi Shami and Maraki cvs. significantly surpassed the others and on the contrary, Koroneiki cv. recorded the minimum values in both seasons.

The above results are in line with Tattini *et al.* [33] who concluded that Cl^- uptake and transport to the shoot of olive trees is lower than Na^+. Olive trees are less sensitive to leaf Cl^- than Na^+ especially at high salinities [27] and Cl^- ion does not cause toxicity in olive trees [61]. Similarly, Manzanillo cv. has been described as very resistant to high concentrations of chlorides in irrigation water [62]. In the same direction, Melgar *et al*. [29] found that after eight years of treatment, salinity did not affect leaf Cl^- concentration of Picual cv. trees and was always below the toxicity threshold (0.50%). Hassan *et al*. [16] reported that remarkable variations were noticed among one-year old own-rooted cultivars in relation to chloride content, Picual accumulated lowest leaf Cl^- content followed by Manzanillo then Aggezi Shami.

Leaf Total Phenols Content Percentage: Table (7) represents leaf total phenols content of the studied olive cultivars in 2016 and 2017 seasons. In both studied seasons, significant differences were observed, Aggezi Akse cv. recorded the highest content (2.47 and 2.50 %, respectively), Picual cv. took the other way around (1.70 and 1.86 %, respectively) and other cultivars were in between.

Saline water irrigation increases the contents of total phenols [63, 64]. It was concluded that high salinity induced the formation of total phenolic content in the leaves, which participate in the increase of antioxidant activity. Phenolic compounds play an important role in scavenging free radicals and protect plants against the damaging effects of reactive oxygen species due to salt stress [65]. In the same direction, Cavaca *et al*. [66] reported that phenols, as one of the major groups of secondary metabolites in olive, are important because of their involvement in the plant response against abiotic stressors. Olive cultivars may have a different response to abiotic factors, consequently leading to distinct olive leaf phenolic profiles and anti-oxidative activity. Similarly, Demir and Cetinkaya [67] concluded that cultivars may develop a defense mechanism against saline conditions through biosynthesizing polyphenols in different amounts.

Similarly, Farag *et al*. [68] found that Koroneiki had significantly higher leaf content than Picual. Also, Aparicio *et al*. [36] showed evidence that Picual cv. leaves contained lower total phenols than Frantoio cv. at 200 mM NaCl. Rossi *et al*. [10] mentioned that the concentration of total phenolic compounds was higher in old-leaves of Leccino (salt-sensitive) than in those of Frantoio (salt-tolerant). Higher leaf total phenols content of Dolce agogia than Frantoio was reported by Blasi *et al*. [69]. Moreover, Olmo-García *et al*. [70] found that Picual was the poorest variety of the eleventh studied cultivars, while Frantoio, Koroneiki and Manzanillo cvs. recorded higher total phenol values. Significant differences in leaf total phenolic content of 15 Italian olive cultivars were noted [71]. Pasković *et al*. [72] showed that temporal variation of olive leaf biophenolic composition may significantly depend on the cultivar.

Leaf Proline Content (mg/100g): Data presented in Table (7) shows leaf proline content (mg/100 g, dry weight) of olive cultivars in 2016 and 2017 seasons. Leaves of Coratina, Aggezi Akse and Dolce cvs. contained higher proline values than the others in both studied seasons (0.030 mg/100 g for all of them in 2016 season and 0.035, 0.034 and 0.030 mg/100 g in 2017 season, respectively). Picual cv. achieved the lowest values (0.016 and 0.019 mg/100 g, respectively) with insignificant differences with Kalamata, Maraki and Sewia cvs. in both seasons.

It was mentioned that there are differences in leaf proline content between different olive cultivars [42, 47, 45, 16]. The proline accumulation recorded in stressed plants has allowed them to improve water uptake to actively growing tissues, to preserve appropriate leaf water status and to increase photosynthetic activity as noted by Ben Ahmed *et al*. [73]. Similar results were reported by El-Sayed *et al*. [42] who found that Manzanillo cv. recorded lower proline value than Aggezi cv. in the 1st season and the results of the 2nd season were as follows Picual < Koroneiki < Aggezi. Similarly, Aly [47] found that Koroneiki cv. recorded lesser leaf proline content than Manzanillo cv. in both seasons. Shaheen *et al*. [48] showed that leaves of Coratina accumulated higher proline content than Koroneiki in the 2nd season. The above mentioned result is in agreement with Abd Elhameed [45] who found that Kalamata cv. significantly surpassed Picual cv. in estimated proline content at 6000 ppm salinity. Lowest proline content of Picual was reported by Hassan *et al*. [16] in comparison with Manzanillo and Aggezi Shami cvs. at 4000 mg/L salinity. However, the results of this study didn't agree with some findings of El-Sayed *et al*. [42], Aly [47], Shaheen *et al*. [48] and Hassan *et al*. [16]. This contradiction may be due to the difference between young and mature plants, as young plants are less tolerant to salinity [74]. In the same direction, Marin *et al*. [43] noted that results obtained with young plants might not be related to the tolerance of mature plants growing under field conditions.

Leaf Relative Water Content Percentage and Leaf Turgid Weight/Fresh Weight Ratio: With respect to leaf relative water content percentage (RWC %), data tabulated in Table (7) cleared that in 2016 season, Kalamata cv. significantly surpassed the others (67.43%), whereas Picual cv. was significantly superior in 2017 season (69.36%). In both seasons, Dolce cv. achieved statistically the lowest values. Aly [47] recorded that the highest relative water content was shown by Koroneiki followed by Manzanillo then Aggezi with significant variations in both seasons. Hassan *et al*. [16] mentioned that mean leaf water content showed high significant differences among the studied one-year-old cultivars. Picual recorded the highest relative water content (83.00%), while Aggizi Shami (76.25%) showed the lowest percentage and Manzanillo showed an intermediate percentage (78.25%). They also added that relative water content is important physiological parameters for measuring the water status of the plants.

Leaf turgid weight/fresh weight ratio (T/F ratio) is presented in Table (7), it's also notorious that Dolce cv. recorded the highest values in both studied seasons (1.490 and 1.473, respectively). On the contrary, Picual and Kalamata cvs. in 2016 season as well as Picual and Koroneiki cvs. in 2017 season acquired lower values (1.323 and 1.333 as well as 1.223 and 1.263, respectively). This may be due to lower accumulation of Na^+ in leaves of the previously mentioned cultivars (Table, 4) which resulted in lower turgid weight and led to lower T/F ratio.

It's worthy to mention that despite the numerous studies on the salt tolerance of olive cultivars, the results of these studies remained confusing due to different comparisons between cultivars. For instance, many researchers defined Picual as a salt tolerant cultivar [41-44, 16], but it was reported to be the least tolerant [36].

CONCLUSIONS

- In all studied cultivars, Na content of leaves was lower than that of roots, revealing that sensitive cultivars were not included in this study.
- An effective salt-exclusion mechanism operating in the root system was confirmed. However, the ability to use this mechanism differed between tested cultivars. Tolerant cultivars tended to have decreased Na content in leaves and roots, while the opposite was true for the least tolerant ones.
- Remarkable differences among the tested cultivars irrigated with saline ground water (EC 7.06 dSm^{-1} /5648 ppm) in their ability to accumulate K and Ca were clear, revealing selectivity for K and Ca which resulted in higher leaf and root K/Na ratio, Ca/Na and K+Ca/Na ratios in leaves of tolerant cultivars.
- Increased leaf total phenols and proline contents as well as leaf turgid weight/fresh weight ratio distinguished less tolerant cultivars.
- It seems that Na, K and Ca contents, besides K/Na, Ca/Na and K+Ca/Na ratios in leaves may be evaluated as reliable criteria for salt tolerance of olive cultivars.
- In spite of high irrigation water EC, no salt toxicity visual signs were observed among the studied cultivars. This may be due to following the recommended cultural practices including sufficient fertilization, drip irrigation and leaching that decreased the adverse effects of salinity.
- From the findings of this investigation, it seems that Picual and Kalamata cvs. were the most tolerant cultivars, while Aggezi Akse and Dolce cvs. were the least tolerant ones, other cultivars were in between when irrigated with saline ground water (5648 ppm).

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