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ORIGINAL ARTICLE

Intra- and inter-cultivar impacts of salinity stress on leaf photosynthetic performance, carbohydrates and nutrient content of nine indigenous Greek olive cultivars

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Abstract Nine indigenous Greek olive cultivars ('Aetonicholia Kynourias', 'Arvanitolia Serron', 'Ntopia Atsicholou', 'Koroneiki', 'Lefkolia Serron', 'Ntopia Pierias', 'Petrolia Serron', 'Smertolia' and 'Chryssophylli') were evaluated for their tolerance to salinity stress (four levels of sodium chloride salt, i.e., 0, 50, 100 and 200 mM) under hydroponic conditions. Their photosynthetic performance, leaf carbohydrates (mannitol, glucose, fructose and sucrose) and nutrients (nitrogen, potassium, calcium, sodium and chloride) were assessed. Photosynthetic performance was reduced under salt stress and this was mostly evident in 'Koroneiki' and 'Ntopia Atsicholou' (approximately 20% of the corresponding controls), while 'Ntopia Pierias', 'Smertolia' and 'Petrolia Serron' did not exhibit significant changes with salinity level. Photosynthesis (A) was reduced mainly due to severe stomatal limitations. A weak correlation was detected between A and intercellular CO_2 (Ci) indicating a minor role of non-stomatal limitations. Carbohydrates in the leaves did not seem to undergo significant changes. Mannitol accumulated in 'Chryssophylli' leaves and glucose in 'Arvanitolia Serron' leaves under the highest salinity level. Potassium concentration per leaf water volume was significantly reduced (especially under the highest salinity level −45 to 60% of control). Calcium was not significantly affected although Ca/Na ratio was reduced, due to the great increase of sodium concentration. 'Lefkolia Serron' and 'Arvanitolia Serron' accumulated the least sodium in their leaves, exhibiting high K/Na ratio under the highest salinity level, indicating a better regulation of potassium influx under high sodium concentration. Based on the present data and on previous research 'Lefkolia Serron' and 'Arvanitolia Serron' are the two cultivars with the highest tolerance against salinity stress.

Keywords Carbon assimilation rate · Chlorophylls · Minerals · Salt stress · Water use efficiency

Introduction

In most Mediterranean countries olive (*Olea europaea* L.) is considered as the most important fruit species (Cimato et al. 2010). Its value, due to the health benefits of consumption of olives and olive oil, is recognized worldwide, and olive orchards are planted in new areas, characterized by Mediterranean type climate.

Although olive is relatively drought and salt tolerant (Chartzoulakis et al. 2002), irrigation increases yield and to some extent olive oil quality. As fresh water is mainly diverted from agricultural use to municipal and industrial use, in order to meet their demands, marginal quality water is often used for agriculture (Bourazanis et al. 2016), thus increasing salinity build up in soil (Bader et al. 2015; Chartzoulakis et al. 2002, 2006; Cimato et al. 2010; Tattini 1994).

Salinity affects many physiological and biochemical mechanisms of plants, from plant nutrition and carbon assimilation rate to hormone balance (Aparicio et al. 2014; Chartzoulakis et al. 2002; Goreta et al. 2007). Salinity

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effects are the results of ion toxicity and/or osmotic stress, which plant species try to cope with, employing different strategies. Olive responds to root-zone salinity mainly by excluding or limiting the flux of toxic ions (Na and/or Cl) to sensitive shoot organs and particularly to the leaves, via a reduced water mass flow and thereby lower growth rates (Assimakopoulou et al. 2017; Cimato et al. 2010; Loreto et al. 2003; Remorini et al. 2009). Tattini (1994) reported that the salt-tolerant olive cultivars are characterized by sodium exclusion by the roots and the ability to maintain an appropriate K/Na ratio in actively growing tissues. Nonetheless the effectiveness of exclusion mechanism depends on the salinity level, functioning quite effectively at low and moderate levels of salinity, while at higher levels sodium is usually transported and accumulated in the aerial parts (Chartzoulakis et al. 2002). The salt-induced osmotic imbalance could be partially overcome by an increase of osmolytes such as K, proline and other amino-compounds and carbohydrates, which in the case of olive is mainly the sugar-alcohol mannitol as well as glucose.

Although many experiments regarding olive salinity tolerance and response have been executed, there is still a controversy or a grey area on the criteria best suited to assign to a cultivar the tolerant or sensitive character. Many researchers prefer to use the so-called "physiological tolerance"—which is mostly based on the control of salt entry and allocation of salt at organ level (Cheeseman 1988; Flowers and Yeo 1995), while others the "horticultural tolerance"—which relates to the ability of the species to maintain growth and yield under saline conditions (Assimakopoulou et al. 2017; Cimato et al. 2010; Tattini et al. 2009). This is further complicated by the fact that the salt tolerance character of olive is mainly cultivar dependent, with many cultivars best suited to the "physiological tolerance" and others to the "horticultural tolerance".

There are many olive cultivars worldwide which have not been tested for their salt tolerance character and which are a great thesaurus of germplasm to be evaluated and exploited in future hybridization programs or even as salt-tolerant rootstocks for salt-sensitive cultivars.

The aim of the present trial was to evaluate nine indigenous olive cultivars for their salt tolerance, by assessing ion concentrations per leaf water volume (as a consequence of both nutrient and osmotic imbalances caused by salinity), photosynthetic performance and leaf carbohydrate concentration under four levels of sodium chloride salinity.

Materials and methods

Plant material: trial conditions

One-year old, own-rooted, uniform in appearance, olive trees of the nine Greek cultivars: 'Aetonicholia Kynourias' (thereafter Aetonicholia), 'Arvanitolia Serron' (Arvanitolia), 'Ntopia Atsicholou' (Atsicholou), 'Koroneiki', 'Lefkolia Serron' (Lefkolia), 'Ntopia Pierias' (Ntopia), 'Petrolia Serron' (Petrolia), 'Smertolia' and 'Chryssophylli' ("Kostelenos G.D. nurseries" selection), were transferred to 5.0 L pots filled with silica sand and perlite (1:1, v/v) and grown hydroponically in a greenhouse of the Technological Educational Institute of Peloponnese in Messinia (longitude: 22°1′43″E, latitude: 37°3′22″N), South Greece, from the beginning of April till the end of November. The uses of each cultivar as well as the mean fruit weight and oil percentage are presented in Table 1 (Kostelenos 2011). Before the onset of salt treatments (Tr), plants were irrigated for 1 month with half-strength Hoagland's No. 2 nutrient solution (BNS) (Hewitt 1966). After this period, four treatments were applied to the plants, Tr0: BNS + 0 mM sodium chloride (NaCl) (control), Tr50: BNS + 50 mM NaCl, Tr100: BNS + 100 mM NaCl and Tr200: BNS + 200 mM NaCl. During the first days of salt imposition, plants were irrigated with the NaCl solution in increments of 25 mM per day, in order to reach the desired salinity concentration gradually, avoiding salt shock. The duration of the salt imposition of plants was 6 months. More details are available in Assimakopoulou et al. (2017).

After the onset of the full salt stress per treatment, the electrical conductivity (EC) and pH of the nutrient solutions applied to the plants, as well as the relevant values of the drainage solutions from the pots were recorded every week. The mean pH of the nutrient solutions of all the treatments applied to the plants was 6.6, whereas the EC in the case of Tr0 was 2.0, Tr50 7.3, Tr100 11.9 and Tr200 21.2 dS m^{-1}. The mean pH of the drainage solutions from the pots from Tr0 treatment was 6.9, from Tr50 7.1, from Tr100 7.1 and from Tr200 7.2, whereas the relevant EC values were 2.8, 12.1, 20.8 and 29.2, respectively.

To control high temperatures inside the greenhouse during the experimental period and consequently avoid the appearance of sudden salt toxicity symptoms, shade curtains were used continuously. The average daily air temperature recorded was 18.9 °C in April, 21.2 °C in May, 26.1 °C in June, 29.2 °C in July, 28.8 °C in August, 28.1 °C in September, 23.7 °C in October and 19.1 °C in November, whereas the relative humidity ranged from 52.7 to 69%.

The effects of salinity stress on biometric characteristics (shoot growth, dry mass weight, etc.) of each cultivar, under the conditions described here, have been already reported by Assimakopoulou et al. (2017).

Carbon assimilation rate measurement, nutrient and carbohydrate analyses

At the end of the experiment carbon assimilation rate was measured on at least four fully expanded young leaves per plot, taking at least two measurements per leaf. Net

Table 1 Mean fruit weight and uses of the nine indigenous cultivars

Categories of cultivars based on their main uses (mean fruit weight/oil percentage per fresh weight)		
For oil production	Double use	For table olives
Atsicholou (2.1 g/20%)	Lefkolia (5.2 g/16–20%)	Aetonicholia (5.7 g/17–20%)
Koroneiki (1.1 g/20–25%)	Ntopia (4.0 g/20–25%)	Arvanitolia (5.7 g/15–18%)
Smertolia (2.0 g/20–25%)	Petrolia (4.9 g/20–25%)	
	For landscape use	
	Chryssophylli	

From Kostelenos (2011)

photosynthetic rate (A), stomatal conductance (gs), transpiration (E) and intercellular CO_2 concentration (Ci) were measured using a Li-Cor 6400 portable photosynthesis system (Li-Cor, Lincoln, NE). The water use efficiency (WUE) was estimated as the ratio of A/E while the ratio A/Ci was also calculated. Chlorophyll measurements were also performed using a Konica-Minolta chlorophyll meter (SPAD-502) while chlorophyll fluorescence was measured after 30 min dark adaptation of the leaves.

At the same time, the plants were destructively harvested and the youngest fully expanded leaves of comparable physiological age were collected in order to determine the concentrations of carbohydrates and the nutrients nitrogen (N), potassium (K), sodium (Na), chloride (Cl) and calcium (Ca). For nutrient analyses, the fresh samples were washed, dried to constant weight, ground to fine powder and dry-ashed in a furnace for 6 h at 500 °C. The concentration of Cl was determined by titration with 0.1 N silver nitrate, K and Na by flame emission spectroscopy, whereas Ca was determined by atomic absorption spectrometry (Varian SpectrAA, 240 FS) in the dry digest; N was determined by the indophenol-blue method in the wet digest (Allen et al. 1974; Kalra 1998). The water content of the leaves was determined after drying them till constant weight at 70 °C and nutrient concentrations were expressed as weight per liter of leaf water, in order to co-calculate both the nutrient and osmotic imbalances derived by salinity exposure. The ratios K/Na and Ca/Na of the leaves were also calculated.

The carbohydrate concentration of leaves was measured on freeze-dried samples. A microwave-assisted extraction was used, while carbohydrate HPLC analysis was performed according to Roussos et al. (2010). Four carbohydrates were detected in olive leaves, namely sucrose, glucose, fructose and mannitol.

Statistical analyses

The experiment was conducted as a factorial completely randomized design with four replications (of one tree). Therefore, 144 plants were grown in total (4 salt treatments × 9 cultivars × 4 replicates).

The effects of salt treatments within the same cultivar (intra-cultivar comparison), were evaluated by ANOVA and determined by Tukey HSD test at $p = 0.05$, whereas the correlation coefficients between photosynthetic parameters, leaf nutrient and carbohydrate concentrations were determined by Pearson correlation.

Parameters were also expressed as relative values to the corresponding control of each cultivar, in order to be able to determine significant differences among cultivars for every salt level (inter-cultivar comparison) and the results are shown in the figures. In the figures, SEs presented at the right side of each figure, were calculated from the residual variances of the MANOVA analysis.

Principal component analysis after varimax rotation was performed in order to summarize the effect of each salinity level on all the measured variables, in a reduced number of factors. Discriminant analysis by forward selection was also performed in order to classify the tested cultivars into groups characterized by similar responses to salinity, based on the measured variables.

Results

Intra- and inter-cultivar effects of salt treatment on the photosynthetic parameters of olive plants

Photosynthetic performance of olive trees was severely affected by the imposed salinity treatments (Table 2). Irrespective of the cultivar, carbon assimilation rate was significantly reduced under Tr100 and especially Tr200, while both gs and E have been negatively affected already by Tr50. The SPAD index increased under Tr200 compared to either control or Tr50.

Photosynthetic performance of ‘Ntopia’, ‘Petrolia’ and ‘Smertolia’ was not affected by salinity treatment. On the other hand though, ‘Aetonicholia’, ‘Arvanitolia’, ‘Atsicholou’, ‘Chryssophylli’ and ‘Koroneiki’ exhibited significant carbon assimilation rate reduction under Tr200 compared to control. ‘Lefkolia’ photosynthetic rate was negatively affected by Tr100 compared to control.

Table 2 Effect of salinity treatments on photosynthetic parameters and SPAD index of the nine olive cultivars (A, μmol CO_2 m^{-2} s^{-1}; gs, mol m^{-2} s^{-1}; Ci, ppm; E, mmol H_2O m^{-2} s^{-1}; WUE, μmol CO_2 $mmol^{-1}$ H_2O)

Cultivar	Salinity level	Photosynthetic parameters						
		A	gs	Ci	E	A/Ci	WUE	SPAD
All cultivars	0	5.82a[A]	0.073a	247.86a	1.64a	0.025a	3.55a	81.70b
	50	5.13ab	0.056b	231.79a	1.34b	0.024a	3.83a	82.24b
	100	4.41b	0.048b	225.83a	1.10b	0.021a	4.13a	83.70ab
	200	2.47c	0.030c	235.62a	0.65c	0.011b	3.78a	86.45a
Aetonicholia	0	5.76a	0.058a	215.83a	1.09a	0.027a	5.28a	83.17a
	50	5.31a	0.053a	223.33a	1.18a	0.025a	4.50a	80.32a
	100	4.82ab	0.047a	224.16a	1.09a	0.023a	4.40a	83.50a
	200	2.51b	0.032a	226.16a	0.63a	0.012a	3.98a	87.17a
Arvanitolia	0	8.25a	0.098a	242.00a	2.34a	0.035a	3.53a	80.37a
	50	5.96a	0.062ab	221.00a	1.67ab	0.028ab	3.57a	83.27a
	100	5.48ab	0.061ab	230.16a	1.53ab	0.025ab	3.58a	86.72a
	200	2.79b	0.026b	219.33a	0.73b	0.015b	3.82a	91.27a
Atsicholou	0	6.67a	0.084a	241.50a	2.17a	0.028a	3.46a	86.67a
	50	6.13a	0.071ab	236.83a	1.95a	0.026a	3.50a	84.62a
	100	4.65ab	0.058ab	208.16a	1.19ab	0.022ab	3.79a	84.25a
	200	1.53b	0.017b	227.16a	0.47b	0.008b	3.33a	85.75a
Chryssophylli	0	5.27a	0.065a	261.75a	1.20a	0.020a	4.39a	77.37a
	50	4.27ab	0.043b	226.33a	0.92ab	0.023a	4.57a	83.52a
	100	3.10ab	0.034b	225.50a	0.75b	0.015a	4.31a	79.35a
	200	2.50b	0.029b	238.17a	0.70b	0.012a	3.68a	82.05a
Koroneiki	0	4.96a	0.042a	209.00a	1.20a	0.027a	3.91a	74.65a
	50	4.70ab	0.037a	184.50a	1.15a	0.029a	4.01a	70.50a
	100	3.62ab	0.032a	191.00a	0.95a	0.021a	3.91a	77.05a
	200	1.03b	0.010b	248.00a	0.38b	0.005a	2.96a	81.35a
Lefkolia	0	5.59a	0.058a	219.83a	1.78a	0.026a	3.11a	85.05a
	50	5.33ab	0.049ab	202.83a	1.56a	0.027a	3.36a	86.90a
	100	3.07bc	0.033b	213.33a	0.94b	0.016ab	3.03a	87.72a
	200	2.39c	0.026b	190.83a	0.75b	0.014b	3.32a	92.92a
Ntopia	0	5.43a	0.093a	286.83a	1.68a	0.021a	3.40a	86.62a
	50	4.21a	0.054b	259.00a	0.99ab	0.016a	4.53a	87.35a
	100	4.00a	0.052b	258.33a	0.93ab	0.016a	4.09a	86.12a
	200	3.03a	0.051b	258.00a	0.62b	0.012a	4.57a	84.47a
Petrolia	0	4.28a	0.071a	284.00a	1.48a	0.016a	2.87a	86.52a
	50	4.26a	0.056a	255.17a	1.21ab	0.018a	3.46a	86.72a
	100	4.30a	0.050a	249.66a	1.12ab	0.018a	4.00a	87.07a
	200	2.62a	0.048a	260.00a	0.78b	0.010a	3.35a	87.05a
Smertolia	0	6.12a	0.091a	288.00a	1.69a	0.021a	3.62a	74.92a
	50	5.96a	0.085a	277.17a	1.55a	0.023a	3.89a	76.95a
	100	6.65a	0.066ab	232.16a	1.30ab	0.030a	5.31a	81.20a
	200	3.62a	0.040b	255.00a	0.82b	0.015a	4.72a	86.07a

[A] Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on Tukey HSD test at $a = 0.05$

Similarly, stomatal conductance presented a significant reduction already by Tr50 in 'Chryssophylli' and 'Ntopia', while 'Lefkolia' exhibited significant reductions by Tr100. Stomatal conductance seemed to be unaffected by salinity up to Tr100 in 'Arvanitolia', 'Atsicholou', 'Koroneiki' and 'Smertolia'. 'Aetonicholia' and 'Petrolia' did not exhibit any significant photosynthetic rate reduction under any salt treatment.

Intercellular CO_2 concentration was not affected by salinity in any of the cultivars tested, while transpiration

presented significant reduction by Tr200 in 'Arvanitolia', 'Atsicholou', 'Koroneiki', 'Ntopia', 'Petrolia' and 'Smertolia'. Instead, transpiration rate was significantly reduced by Tr100 in 'Chryssophylli' and 'Lefkolia', while it was not affected in 'Aetonicholia'. SPAD index did not exhibit any significant change in any of the cultivars tested.

The multifactor statistical analysis on the relative changes of each parameter in relation to each cultivar's control revealed that cultivar and salt treatment had a significant effect on the relative changes of A, E and SPAD compared to the corresponding control (Fig. 1a, d, g). The greatest reduction of A among cultivars was noticed in 'Arvanitolia' and the least one in 'Smertolia', while significant reduction was observed under Tr200. 'Smertolia' and 'Aetonicholia' exhibited the smallest reduction of E while 'Ntopia' the greatest one. Transpiration was severely reduced under Tr100 and Tr200 compared to Tr50. SPAD increased with increasing salinity level, with 'Smertolia' and 'Arvanitolia' presenting the highest values while 'Atsicholou' and 'Ntopia' the lowest.

The cultivar as well as salt treatment and their interaction significantly affected the relative change of stomatal conductance (Fig. 1b). The more the salt level increased the lower was stomatal conductance, with 'Petrolia' and 'Aetonicholia' presenting the greatest reduction, while 'Arvanitolia' and 'Chryssophylli' the lowest. 'Koroneiki' under Tr200 exhibited the highest gs reduction of all treatments.

Tr200 resulted in the lowest A/Ci values compared to Tr50 and Tr100, while there was no significant effect of either salt treatment or cultivar on the relative change of Ci (Fig. 1c, e).

Chlorophyll fluorescence (F_v/F_m) was not severely affected by Tr200 as can be seen in Fig. 2. A significant reduction was detected in 'Ntopia' while an increase in 'Smertolia' under Tr200 compared to Tr0.

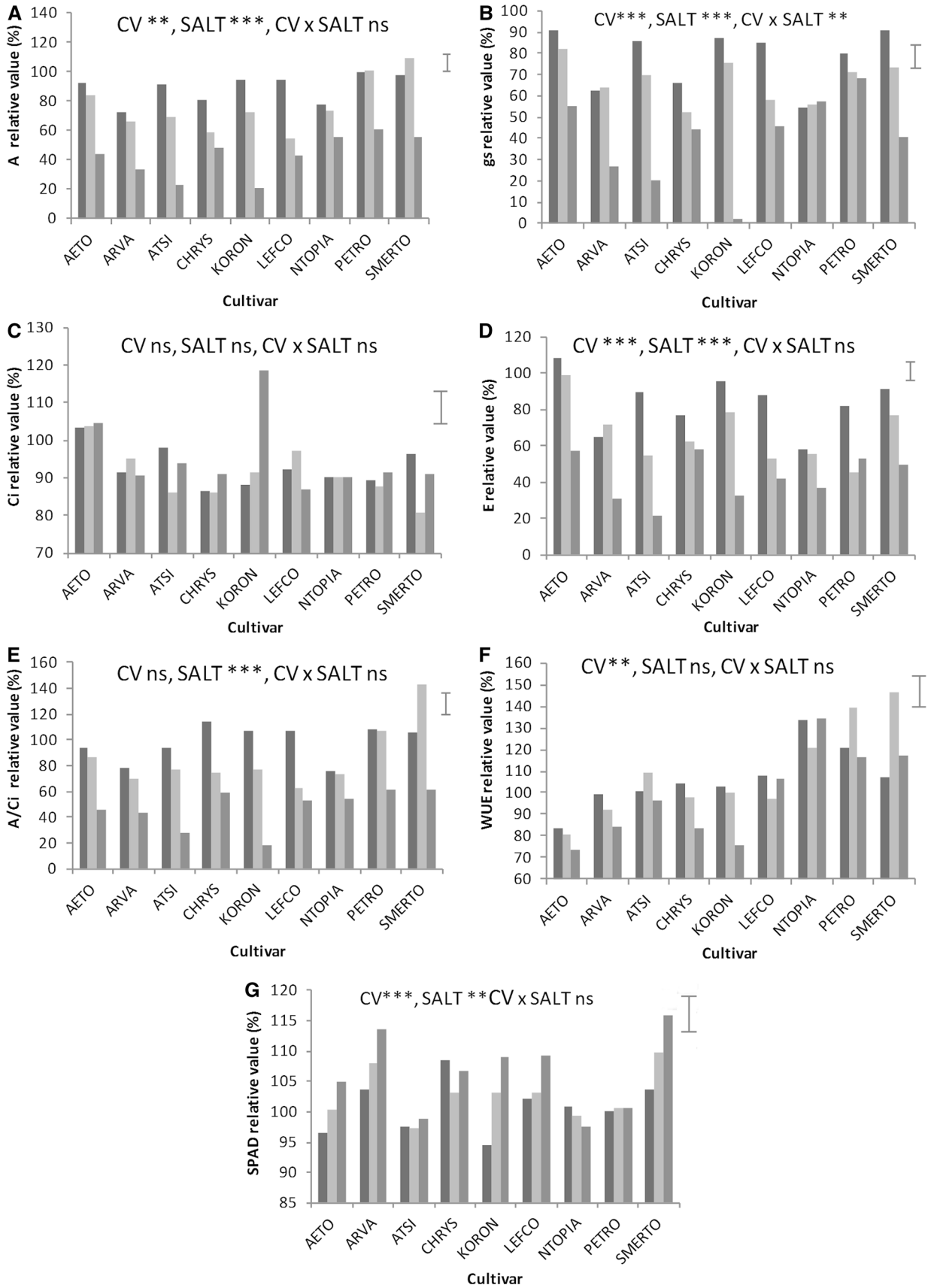
Intra- and inter-cultivar effects of salt treatments on carbohydrate concentration in olive leaves

Irrespective of the cultivar, salinity level had a significant effect on fructose concentration only, which exhibited a significant reduction under Tr200 (Table 3). Mannitol concentration increased significantly under Tr100 and Tr200 only in 'Chryssophylli', exhibiting a gradual increase from Tr0 through Tr200. In 'Koroeniki' though, a significant reduction occurred under Tr100 compared to control, while under Tr200 mannitol concentration increased again. Fructose concentration was lowest under Tr200 in 'Lefkolia' and 'Petrolia' (without any significant difference from control though) and in 'Smertolia'. Glucose concentration was found to be higher under salt imposition in 'Arvanitolia', especially under Tr50 and Tr200. Sucrose did not exhibit any significant change in any of the cultivars tested. Total carbohydrates were higher in 'Koroneiki' under Tr0 compared to Tr100, while sucrolysis index (the ratio sucrose/hexoses) was higher under Tr200 in 'Atsicholou' compared to Tr100 and under Tr100 in 'Smertolia' compared to other treatments.

The cultivar had a significant effect on the relative changes of all measured parameters under salt stress, as can be seen in Fig. 3. Salinity level affected significantly only the relative concentration of fructose (Fig. 3c). Irrespective of salinity level, mannitol relative concentration was found to be higher in 'Chryssophylli', 'Arvanitolia' and 'Atsicholou' and lowest in 'Koroneiki' and 'Smertolia' (Fig. 3a). Similarly 'Koroneiki' and 'Smertolia' presented the lowest relative concentration of glucose, fructose and total carbohydrates (Fig. 3b, c, e). The highest relative glucose concentration was determined in 'Arvanitolia', in 'Atsicholou' and 'Chryssophylli' while the lowest was determined in 'Koroneiki' and in 'Smertolia'. Fructose relative concentration was found to be highest in 'Petrolia', 'Atsicholou' and 'Ntopia'. Tr200 resulted in the lowest relative fructose concentration, compared to that under Tr50 and Tr100, irrespective of the cultivar. Sucrose relative concentration was high in 'Atsicholou' and 'Lefkolia' while low in 'Petrolia' and Aetonycholia (Fig. 3d). Total carbohydrates relative concentration was also high in 'Chryssophylli' and 'Lefkolia' while low in 'Koroneiki' and 'Smertolia' (Fig. 3e).

Intra- and inter-cultivar effects of salt treatments on nutrient concentration per leaf tissue water volume in olive leaves

Nitrogen concentration was not affected by salinity in most of the cultivars tested (Table 4), although there was a clear tendency to increase with increasing salinity. This was most evident in 'Chryssophylli' and 'Petrolia' where significant differences were observed. Potassium concentration was severely affected by salinity. Irrespective of the cultivar, K concentration decreased with increasing salinity level. The least decrease was detected in 'Arvanitolia' followed by 'Lefkolia', 'Petrolia' and 'Smertolia'. In 'Ntopia', 'Atsicholou' and 'Chryssophylli' the decrease was more severe, especially under Tr200. In 'Aetonicholia', K decreased significantly only under Tr200. Calcium concentration exhibited a decreasing trend by salinity in most cultivars up to Tr100, while under Tr200 its concentration increased to almost control levels. This was mostly noticed in 'Arvanitolia' and 'Chryssophylli'. Sodium concentration increased with salinity, irrespective of the cultivar, with significant differences between Tr200 and Tr0 in all cultivars. In 'Chryssophylli', 'Lefkolia', 'Smertolia' and 'Koroneiki' there was a difference


A
CV **, SALT ***, CV x SALT ns
A relative value (%)
B
CV***, SALT ***, CV x SALT **
gs relative value (%)
C
CV ns, SALT ns, CV x SALT ns
Ci relative value (%)
D
CV ***, SALT ***, CV x SALT ns
E relative value (%)
E
CV ns, SALT ***, CV x SALT ns
A/Ci relative value (%)
F
CV**, SALT ns, CV x SALT ns
WUE relative value (%)
G
CV***, SALT **CV x SALT ns
SPAD relative value (%)
AETO ARVA ATSI CHRYS KORON LEFCO NTOPIA PETRO SMERTO
Cultivar

◀**Fig. 1** Effect of salinity treatment on the relative values of photosynthetic parameters compared to their corresponding control. *Bars at the right side of each graph* represent the standard error of the analysis of multifactor Anova. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *CV* cultivar, *x* denotes interaction. *AETO* Aetonicholia, *ARVA* Arvanitolia, *ATSI* Atsicholou, *CHRYS* Chryssophylli, *KORON* Koroneiki, *LEFKO* Lefkolia, *PETRO* Petrolia, *SMERTO* Smertolia. *Dark grey* Tr50; *light grey* Tr100 and *grey* Tr200

concerning Na concentration even between Tr0 and Tr50. Chloride concentration changes followed similar pattern, with Tr0 exhibiting the lowest concentration and Tr200 the highest one. In many cultivars, Cl concentration did not increase significantly under Tr50 compared to control ('Aetonicholia', 'Arvanitolia', 'Chryssophylli', 'Ntopia', 'Petrolia' and 'Smertolia'). The higher the salinity level, the lower was K/Na ratio, while the same stood also for the ratio Ca/Na.

The cultivar had a significant effect on the relative concentrations of all nutrients, as can be seen in Fig. 4. Salinity on the other hand, did not affect the relative concentration of N (Fig. 4a), while it had a significant effect on the relative concentration of K, Na, Cl and Ca (Fig. 4b–e). The highest relative N concentration was determined in 'Ntopia' (Fig. 4a). Potassium relative concentration was highest in 'Arvanitolia', 'Lefkolia' and 'Aetonicholia' and lowest in 'Koroneiki', while the higher the salinity level was, the lower was potassium relative concentration, irrespective of the cultivar (Fig. 4b). Sodium and chloride relative concentration was highest in 'Petrolia', while lowest in 'Koroneiki' (Fig. 4c, d) irrespective of salt concentration. On the other hand, the higher the level of salinity was, the higher was sodium and chloride relative concentration irrespective of the cultivar. Calcium relative concentration was found to be higher in 'Aetonicholia' and lowest in 'Smertolia', which under all salt treatments presented the lowest calcium relative concentration value (Fig. 4e). Under Tr200 Ca relative concentration was higher than that under Tr100 and Tr50 except from 'Atsicholou' and 'Ntopia'.

Correlation between photosynthetic parameters, carbohydrates and nutrient concentration in olive leaves

In Tables 5 and 6 the correlation coefficients between measured parameters are shown, irrespective of the cultivar, in order to assess their relations. As can be seen, salinity treatment negatively affected photosynthetic parameters, such as A, E, gs and A/Ci as well as the concentration of K and the ratios Ca/Na and K/Na. On the other hand, it presented significant positive strong relation with the concentration of Na and Cl. The latter two minerals were negatively correlated with most of the photosynthetic parameters (A, E, gs, A/Ci) while the ratios Ca/Na and K/Na were positively related with the former parameters. Carbon assimilation rate was weakly but negatively related with Ci and positively with E, gs, A/Ci and WUE. SPAD index exhibited a weak relationship with N, Cl and Ca. Potassium was positively related with A, E and gs and negatively with Na and Cl, while Cl exhibited a weak negative relation with the ratios Ca/Na and K/Na. Carbohydrate concentration exhibited few and weak relations with most of the parameters measured. Salinity as well as Na and Cl, had a negative effect on fructose concentration only, while gs was negatively correlated with sucrose, mannitol and total soluble sugars. Nitrogen was positively but weakly related with fructose, glucose and total soluble sugars concentration.

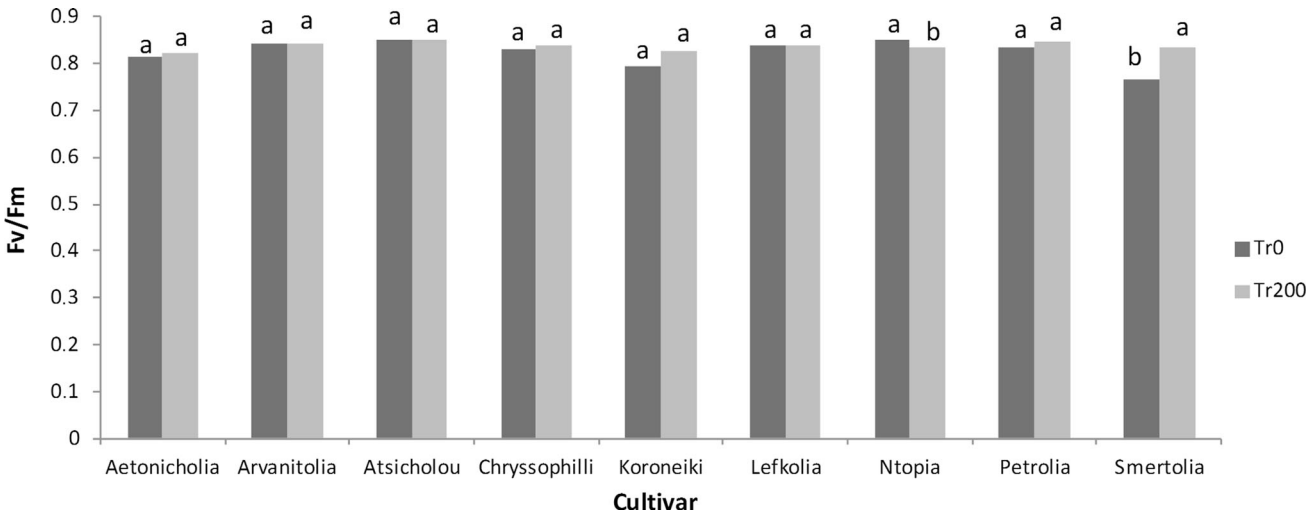


Fig. 2 Effect of salinity treatment on the chlorophyll fluorescence F_v/F_m ratio. *Different letters* above each column for the same cultivar indicate significant differences among treatments (Tr0 and Tr200) according to Student's *t* test at $a = 0.05$

Table 3 Effect of salinity treatments on the carbohydrate concentration in the leaves of the nine olive cultivars

Cultivar	Salinity level	Carbohydrates in the leaves (mg g^{-1} D.W.)					
		Mannitol	Fructose	Glucose	Sucrose	Total sugars	SI
All cultivars	0	48.43a[A]	14.03a	51.45a	6.39a	119.90a	0.103a
	50	50.44a	13.58a	55.16a	6.80a	125.37a	0.103a
	100	50.66a	13.13a	51.09a	6.69a	121.25a	0.111a
	200	53.67a	10.92b	54.22a	7.06a	125.85a	0.114a
Aetonicholia	0	47.66a	13.18a	43.69a	8.86a	113.41a	0.163a
	50	41.57a	12.07a	49.10a	7.73a	100.52a	0.154a
	100	57.82a	11.99a	49.01a	7.15a	125.98a	0.124a
	200	52.10a	9.23a	45.05a	8.99a	115.38a	0.170a
Arvanitolia	0	38.35a	11.43a	48.36b	5.30a	103.46a	0.089a
	50	44.34a	12.08a	64.40a	6.45a	127.29a	0.084a
	100	41.99a	9.39a	60.22ab	6.51a	118.11a	0.092a
	200	46.23a	8.08a	67.28a	5.41a	127.02a	0.071a
Atsicholou	0	52.12a	13.80a	51.85a	5.20a	122.98a	0.080ab
	50	56.06a	13.20a	62.63a	7.39a	139.30a	0.097a
	100	48.91a	14.67a	54.96a	5.21a	123.77a	0.077b
	200	61.45a	12.89a	58.98a	8.85a	142.18a	0.121a
Chryssophylli	0	40.15c	14.83a	62.64a	5.79a	120.54a	0.078a
	50	45.57bc	12.28a	64.93a	7.17a	129.97a	0.095a
	100	52.50b	15.98a	66.42a	5.00a	139.91a	0.061a
	200	64.52a	12.85a	76.03a	6.43a	159.85a	0.072a
Koroneiki	0	58.39a	16.89a	56.36a	6.66a	138.31a	0.092a
	50	50.88ab	14.77a	49.42a	8.60a	123.67ab	0.137a
	100	37.84b	12.76a	43.52a	6.75a	94.11b	0.121a
	200	55.77ab	11.77a	49.20a	5.66a	122.42ab	0.094a
Lefkolia	0	47.31a	12.46ab	51.96a	5.77a	117.52a	0.089a
	50	52.95a	13.56a	49.95a	5.97a	122.43a	0.095a
	100	56.31a	13.59a	51.67a	7.68a	129.26a	0.118a
	200	44.73a	8.98b	46.66a	8.25a	108.63a	0.152a
Ntopia	0	46.99a	13.12a	43.03a	5.28a	108.44a	0.096a
	50	50.20a	14.17a	48.89a	5.39a	118.66a	0.088a
	100	49.63a	12.94a	37.51a	7.95a	108.05a	0.169a
	200	48.07a	11.26a	39.55a	5.56a	104.46a	0.111a
Petrolia	0	50.40a	13.11ab	48.37a	8.52a	120.41a	0.158a
	50	58.10a	16.40a	54.44a	7.27a	136.23a	0.103a
	100	54.77a	13.19a	48.05a	6.94a	122.96a	0.124a
	200	49.22a	9.64b	44.99a	8.09a	11.95a	0.151a
Smertolia	0	56.64a	17.47a	56.79a	6.13a	138.72a	0.083b
	50	52.27a	13.65ab	52.65a	5.44a	124.03a	0.082b
	100	53.22a	13.63ab	60.28a	7.02a	141.19a	0.113a
	200	61.08a	13.57b	48.45a	6.25a	122.34a	0.085b

[A] Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on Tukey HSD test at $a = 0.05$

Possible discrimination of salinity levels and discriminant analysis among cultivars based on measured variables

Principal component analysis was carried out to minimize the number of variables that influence each factor and consequently facilitate the interpretation of the results. Seven components were extracted with eigenvalues higher than 1, explaining 78.2% of the variability in the original data. The first two factors were selected (explaining together 39.84% of the total variance) and the biplot produced is presented in Fig. 5.

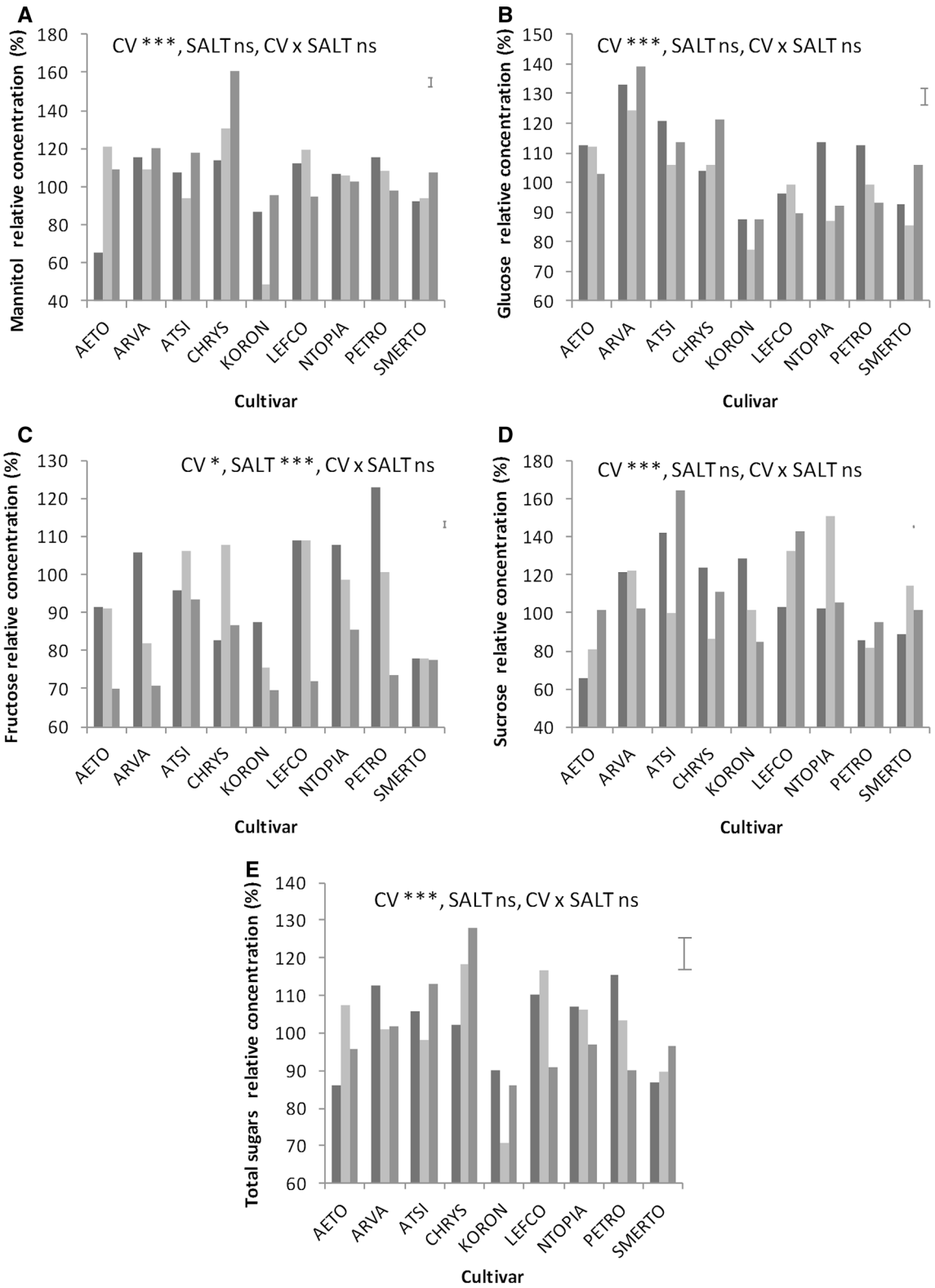


Fig. 3 Effect of salinity treatment on the relative concentration of carbohydrates compared to their corresponding control. *Bars at the right side of each graph* represent the standard error of the analysis of multifactor Anova. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; *CV* cultivar, *x* denotes interaction. *AETO* Aetonicholia, *ARVA* Arvanitolia, *ATSI* Atsicholou, *CHRYS* Chryssophylli, *KORON* Koroneiki, *LEFKO* Lefkolia, *PETRO* Petrolia, *SMERTO* Smertolia. *Dark grey* Tr50; *light grey* Tr100 and *grey* Tr200

Table 4 Effect of salinity treatment on mineral nutrient concentration (per leaf tissue water volume) in the nine olive cultivars

Cultivar	Salinity level (mM)	Nutrient concentration (g L^{-1} leaf tissue water)					K/Na	Ca/Na
		N	K	Ca	Na	Cl		
All cultivars	0	8.17a[A]	12.39a	6.12ab	0.076d	0.48b	163.03a	80.53a
	50	8.72a	9.48b	5.58b	2.46c	1.28b	4.49b	2.50b
	100	8.95a	8.03c	5.61b	3.78b	1.67b	2.48b	1.67b
	200	9.04a	6.85d	6.86a	6.33a	3.73a	1.26b	1.21b
Aetonicholia	0	9.37a	11.15a	6.23a	0.03c	0.17b	314.18a	175.58a
	50	9.97a	9.62a	5.83a	1.80bc	0.64b	5.63b	3.52b
	100	10.75a	9.01a	5.88a	3.03b	1.31b	3.14b	2.02b
	200	8.94a	5.24b	8.57a	5.77a	3.20a	1.04b	1.66b
Arvanitolia	0	7.68a	11.86a	5.71ab	0.009b	0.48b	1316.5a	634.40a
	50	7.91a	11.35a	4.75b	1.22b	0.66b	9.40b	3.91b
	100	8.12a	9.49ab	5.10ab	1.64ab	1.13b	4.81c	2.70bc
	200	8.14a	7.82b	6.21a	4.81a	3.68a	2.44c	1.71c
Atsicholou	0	7.94a	12.36a	5.39a	–	0.55c	–	–
	50	7.99a	8.98b	5.40a	2.31b	1.64b	3.90a	2.37a
	100	7.16a	6.52c	5.81a	4.49b	1.97b	1.64b	1.42b
	200	8.62a	5.87c	5.11a	7.11a	3.86a	0.83b	0.72b
Chryssophylli	0	9.91b	10.81a	6.79ab	0.15c	0.63c	89.3a	59.76a
	50	9.93b	7.55b	6.45b	2.64b	1.46bc	2.97b	2.54b
	100	10.75ab	7.01bc	6.41b	3.36b	1.65b	2.13b	1.95b
	200	11.63a	5.97c	7.93a	5.61a	3.78a	1.08b	1.44b
Koroneiki	0	9.23a	14.12a	8.56a	0.28d	0.83c	51.43a	30.30a
	50	8.87a	9.46b	8.91a	4.10c	1.84b	2.35b	2.21b
	100	9.26a	6.95b	7.02a	6.17b	2.62b	1.16b	1.46b
	200	9.58a	6.80b	11.14a	7.63a	4.47a	0.89b	1.16b
Lefkolia	0	7.75a	12.83a	5.25ab	0.07c	0.58c	171.45a	70.26a
	50	8.44a	10.38b	4.72b	2.23b	1.71b	4.87b	2.24b
	100	8.60a	9.39b	4.75ab	2.37b	1.44bc	4.31bc	2.23b
	200	8.24a	8.42b	5.66a	4.89a	3.06a	1.86c	1.24b
Ntopia	0	5.26a	12.24a	5.85a	–	0.53c	–	–
	50	8.48a	10.04ab	4.85a	2.24c	0.97c	4.52a	2.16a
	100	7.95a	8.39bc	6.11a	3.69b	1.77b	2.27b	1.64ab
	200	7.76a	6.49c	5.87a	4.94a	3.22a	1.32c	1.24b
Petrolia	0	8.17ab	14.03a	5.57ab	0.006c	0.07c	2280.0a	905.92a
	50	7.93b	9.33b	4.83b	2.66bc	0.93bc	3.63b	1.85b
	100	8.69ab	8.23b	5.70ab	4.56b	1.76b	1.89b	1.34b
	200	9.44a	8.17b	6.32a	9.30a	4.19a	0.92b	0.70b
Smertolia	0	8.78a	11.52a	5.78a	0.16d	0.49c	45.95a	23.74a
	50	9.01a	8.62b	4.49ab	2.92c	1.16bc	3.15b	1.67b
	100	9.29a	7.34b	3.70b	4.68b	1.90b	1.58b	0.80b
	200	9.02a	6.77b	4.95ab	6.92a	4.14a	1.00b	0.73b

[A] Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on Tukey HSD test at $a = 0.05$

The two medium levels of salinity presented common areas, characterized by the following variables, K, K/Na, Ca/Na, A/Ci, E, gs, A/E, sucrose, WUE, Ci, Ca, and N. Control treatment and Tr50 and Tr100 were characterized by common effect of salinity on K, K/Na, Ca/Na and fructose concentration. The higher salt level was clearly

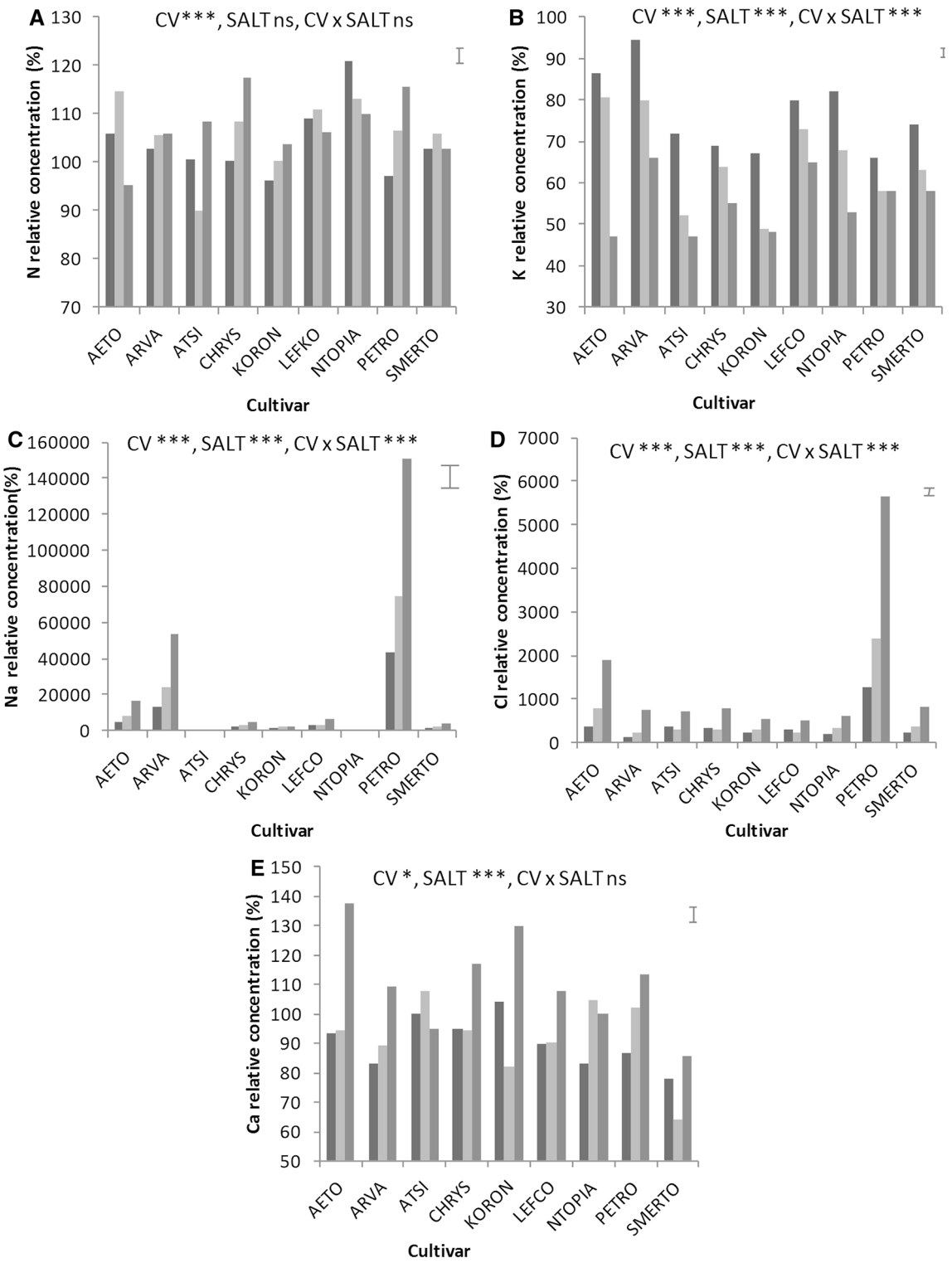


Fig. 4 Effect of salinity treatment on the relative concentration of nutrients compared to their corresponding control. *Bars at the right side of each graph* represent the standard error of the analysis of multifactor Anova. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; *CV* cultivar, *x* denotes interaction. *AETO* Aetonicholia, *ARVA* Arvanitolia, *ATSI* Atsicholou, *CHRYS* Chryssophylli, *KORON* Koroneiki, *LEFKO* Lefkolia, *PETRO* Petrolia, *SMERTO* Smertolia. *Dark grey* Tr50; *light grey* Tr100 and grey Tr200

Table 5 Correlation coefficient between salinity treatment, photosynthetic parameters and nutrients in the leaves of the nine olive cultivars

Parameters	Photosynthetic parameters								Nutrients in the leaves						
	Treatment	A	Ci	E	gs	A/Ci	WUE	SPAD	N	K	Ca	Na	Cl	Ca/Na	K/Na
Treatment	1														
A	−0.56***	1													
Ci	ns	−0.2*	1												
E	−0.55***	0.77***	ns	1											
gs	−0.58***	0.77***	0.29***	0.81***	1										
A/Ci	−0.44***	0.90***	−0.53***	0.57***	0.48***	1									
WUE	ns	0.33***	−0.36***	−0.28***	ns	0.43***	1								
SPAD	ns	ns	ns	ns	ns	−0.18*	−0.17*	1							
N	ns	−0.21*	ns	−0.31*	−0.23**	ns	0.28***	−0.32***	1						
K	−0.79***	0.33***	ns	0.36***	0.27***	0.29**	ns	ns	ns	1					
Ca	ns	−0.30***	ns	−0.25***	−0.35**	ns	ns	−0.24***	0.23*	ns	1				
Na	0.81***	−0.46***	ns	−0.47***	−0.48***	−0.38***	ns	ns	ns	−0.65***	0.20**	1			
Cl	0.84***	−0.53***	ns	−0.45***	−0.47***	−0.43***	ns	0.20**	ns	−0.61***	0.32*	0.89***	1		
Ca/Na	−0.53***	0.25***	ns	0.22***	0.27**	0.33***	ns	−0.28***	ns	0.36***	ns	−0.41***	−0.33***	1	
K/Na	−0.62***	0.29***	ns	0.24***	0.31***	0.36***	ns	−0.28***	−0.21*	0.37***	−0.31**	−0.46***	−0.34***	0.98***	1

ns not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 6 Correlation coefficient of carbohydrates with salinity treatment, photosynthetic parameters and nutrients in the leaves of the nine olive cultivars

Parameters	Photosynthetic parameters								Nutrients in the leaves						
	Treatment	A	Ci	E	gs	A/Ci	WUE	SPAD	N	K	Ca	Na	Cl	Ca/Na	K/Na
Fructose	−0.36***	ns	ns	ns	ns	ns	ns	−0.28***	0.36***	0.25***	ns	−0.35**	−0.36***	ns	ns
Glucose	ns	ns	ns	ns	ns	ns	ns	ns	0.22**	ns	ns	ns	ns	ns	ns
Sucrose	ns	ns	ns	ns	−0.21*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mannitol	ns	−0.17*	ns	ns	−0.20*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Total carbohydrates	ns	ns	ns	ns	−0.14*	ns	ns	ns	0.22**	ns	ns	ns	ns	ns	ns

ns not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

distinguished by Tr0, by being characterized by high concentration of Na and Cl, located on the positive side of Component 1 axis, while Tr0 was located on its negative side. Sodium and Cl were grouped together, while the same was evident for fructose, glucose, mannitol and total carbohydrates. Potassium, K/Na and Ca/Na were also grouped together as well as A, A/Ci, E and gs, indicating similar changes due to salinity imposition.

The discriminant analysis revealed a possible classification of the studied cultivars based on two discrimination functions composed by the following parameters: E, gs, WUE, N, K, Ca, Na, glucose and total carbohydrates (Table 7). "Arvanitolia" "Lefkolia" and "Petrolia" exhibited similar responses to salinity (grouped closely together, at the negative side of Function 1 and almost all at the negative side of Function 2) (Fig. 6). This is quite interesting since all three cultivars beside their relation based on their response to salinity stress, are indigenous of the same region of Greece, i.e., Serres County, North Greece. "Koroneiki" greatly differed from the rest eight cultivars, being at the positive side of Function 1, while "Aetonicholia" and "Chrysophylli" grouped closely together at the positive side of Function 2.

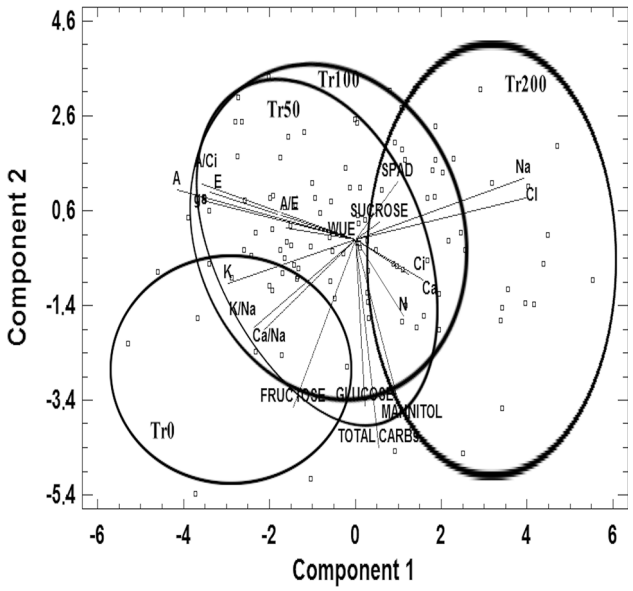


Fig. 5 Biplot presentation of the scores of the first two components of the principal component analysis of the parameters studied. *Ellipses* include points depicting the four salinity levels (Tr0, Tr50, Tr100 and Tr200), while *vectors* indicate the most important parameters characterizing each of the four salinity levels

Discussion

As salinity affects one-third of the world irrigated area and salinity tolerance is a discriminate characteristic of olive cultivars, the study and exploitation of the vast olive germplasm is highly important.

In the present experiment, salinity suppressed photosynthetic performance irrespective of the cultivar tested, according to the literature (Aparicio et al. 2014; Chartzoulakis et al. 2002; Kchaou et al. 2013; Tattini et al. 1997). Carbon assimilation rate was severely reduced by Tr100, reaching almost 50% of the control under Tr200. Photosynthetic performance under salt stress was cultivar dependent as has been previously reported (Bader et al. 2015; Chartzoulakis et al. 2002; Kchaou et al. 2013; Tattini et al. 1997). Three out of the nine cultivars tested did not show any significant reduction. Among the rest, only 'Lefkolia' exhibited significant reduction by Tr100, while all the others presented significant inhibition only under Tr200. Similarly, Kchaou et al. (2013) reported that some of the cultivars tested did not exhibit any changes in photosynthesis under Tr100, and one of these cultivars was the cultivar grown mostly in Tunisia under saline conditions.

Table 7 Coefficients of the first three discriminant functions with eigenvalues higher than one, for the studied cultivars

Parameters	Functions		
	1	2	3
E	0.0583	−0.539	0.633
gs	−0.0406	0.493	−0.208
SPAD	−0.307	−0.297	−0.277
WUE	−0.197	−0.255	0.138
N	0.755	0.852	−0.208
K	0.479	−1.77	0.392
Ca	0.959	−0.176	0.0952
Na	0.84	−1.9	0.804
Cl	−0.243	0.591	−0.192
Fructose	−0.0303	0.0252	−0.135
Glucose	−0.11	0.281	1.8
Total sugars	−0.0252	−0.196	−1.11

According to some reports, the greatest reductions in photosynthesis are observed in cultivars characterized by high photosynthetic rates (Kchaou et al. 2013; Loreto et al. 2003), while the lower ones in cultivars with inherent low A and gs (Chartzoulakis et al. 2002; Loreto et al. 2003). This was also the case in the present experiment, where 'Arvanitolia', the cultivar with the highest photosynthetic rate under control conditions, exhibited on average the most severe reduction compared to control conditions. 'Koroneiki' and 'Atsicholou' on the other hand under Tr200 exhibited the most severe photosynthetic rate reduction.

Stomatal conductance was also affected by salinity, where in two cultivars a significant reduction was observed already by Tr50 ('Chryssophylli' and 'Ntopia'), while in two others ('Aeonicholia' and 'Petrolia'), no reduction was observed, indicating genotyping differences (Bader et al. 2015; Tattini et al. 1997) and possible salinity sensitivity. As in photosynthetic rate, the greatest average decrease of gs compared to control conditions was observed in 'Arvanitolia', which was the cultivar with the highest gs under control conditions. According to Chartzoulakis et al. (2002) a high accumulation of Na and/or Cl in the leaves of cultivars with high A and gs, such as 'Arvanitolia', could explain the higher degree of gs reduction. Nonetheless, Na was found in 'Arvanitolia' leaves at lower concentration than in most of the other cultivars. Thus, Na accumulation does not explain the great A reduction in this cultivar, characterized by inherent high A under control conditions. Additionally, there was no strong negative relation between A or gs with Na and Cl (only moderately negative relation), justifying the minor role of these toxic ions to the photosynthetic limitations observed here, as has been reported earlier (Chartzoulakis et al. 2002; Loreto et al. 2003; Tattini et al. 1995).

Irrespective of the cultivar and salt treatment, a correlation coefficient of 0.77 was found between gs and A. According to the literature, stomatal closure limits photosynthesis in olive trees, suggesting that there are primarily stomatal limitations (Chartzoulakis et al. 2002; Loreto et al. 2003), while non-stomatal limitations also exist, especially in cultivars with low inherent photosynthetic rates (Loreto et al. 2003). There are though reports where no close relationship between A and gs was found, indicating non-stomatal limitations too (either biochemical or mesophyll limitations) (Loreto et al. 2003; Tattini et al. 1997). Since in the present experiment Ci was not affected by salinity level, it could suggest that under the conditions the experiment took place and for the specific cultivars, the non-stomatal limitations could be of minor importance compared to stomatal ones (Farquhar and Sharkey 1982; Melgar et al. 2008). Similar non-significant changes of Ci under salt stress have been reported up to Tr100 (Melgar et al. 2008), while sharp increases occurred at Tr200 (Tattini et al. 1997), as in 'Koroneiki' in the present experiment, suggesting the increasing role of non-stomatal limitations under high salt levels (Loreto et al. 2003). In a

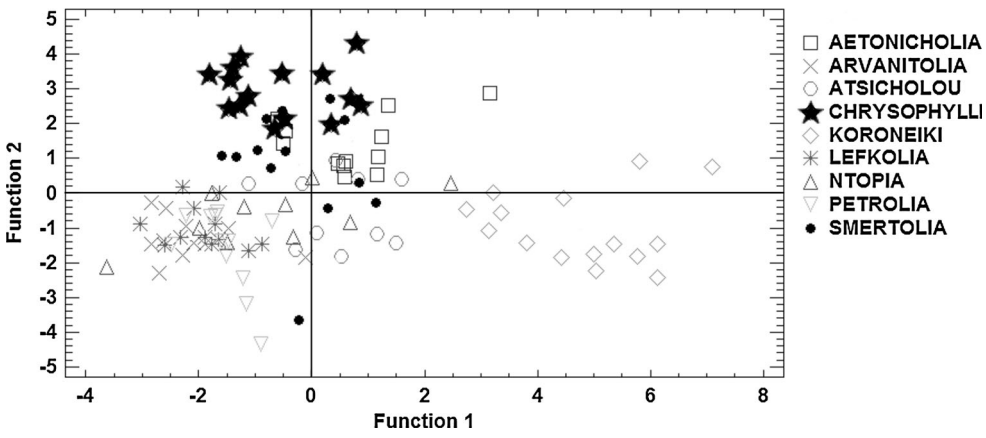


Fig. 6 Plot of discriminant factors presenting the grouping of the studied cultivars based on the measured parameters

few cultivars though the ratio A/Ci was severely reduced with salinity level, but this was mainly due to the greater A reduction rather than Ci increase. Water use efficiency was not affected by salinity, although E was severely affected, primarily under Tr200, due to stomatal closure (Tattini et al. 1997). This is justified in the present experiment by the close relationship found between A and E ($r = 0.77$) experiencing similar changes, resulting in non-significant WUE changes.

According to Kchaou et al. (2013), F_v/F_m ratio may decrease down to 0.5 in salt-stressed olive plants, while slight decreases could be considered as indications that salinity does not cause any severe damage in olive leaves chloroplasts. The F_v/F_m ratio did not exhibit significant changes in most of the cultivars studied, while SPAD index was found to be higher under Tr200 (irrespective of the cultivar), indicating that salinity does not have a deleterious effect on chlorophyll content and that olive tree has efficient antioxidant photoprotective mechanisms to cope with stress (Melgar et al. 2008). Similar results concerning the concentration of chlorophylls have been reported by Denaxa et al. (2012) in olive trees subjected to water stress. This increase was attributed to the lower water content of the leaves under drought, along with the possible antioxidant photoprotective mechanism of olive trees, resulting in concentrated chlorophyll levels. This could be the case in the present experiment, as salt stress induces besides ion imbalances, osmotic stress too, due to water shortage.

Carbohydrate levels in the leaves did not change dramatically with salt stress as has been reported in the literature (Gucci et al. 1998; Petridis and Therios 2012; Tattini and Gucci 1999; Tattini et al. 1996). Mannitol tended to increase up to a salt level, depending on the cultivar and only in 'Chryssophylli' this was clearly shown, as has been previously reported for other cultivars (Gucci et al. 1998; Rejšková et al. 2007; Tattini et al. 1996). Mannitol is an effective osmolyte, ameliorating by its accumulation the negative effects of salt on osmotic imbalances in the leaves (Rejšková et al. 2007). The other carbohydrates did not seem to be significantly affected by salinity, neither did the sucrolysis index, indicating that carbohydrate metabolism was not forced to the production of lower molecular weight sugars. Mannitol was the only carbohydrate accumulated in both cultivars that Chartzoulakis et al. (2006) used, with glucose remaining almost unchanged and fructose decreasing with salt level, as in the present experiment. Generally, carbohydrates apart from mannitol, do not seem to be the most significant factor in adaptation of olive under salt conditions (Gucci et al. 1998; Tattini and Gucci 1999) as has been clearly shown in the present experiment for these nine cultivars.

Salinity affected the nutrient concentration of olive leaves, having a significant impact mainly on K, Na and Cl concentration and less on Ca or N one. Potassium was found to decrease with increasing salinity level irrespective of the cultivar, as has been reported by other researchers too (Aparicio et al. 2014; Chartzoulakis et al. 2006; Goreta et al. 2007; Melgar et al. 2008; Soda et al. 2016), with 'Lefkolia' having the highest K concentration under Tr200. Among the nine olive cultivars, 'Lefkolia', 'Arvanitolia' and 'Ntopia' accumulated the least Na in the leaves under Tr200, less than the average concentration of the nine cultivars, indicating a possible tolerant character of these cultivars. Similarly, 'Arvanitolia' and 'Lefkolia' presented the highest K/Na ratio, while 'Lefkolia' exhibited also the lowest Cl concentration in the leaves. All these effects were most evident regarding the relative concentration of the minerals studied, where 'Arvanitolia' and 'Lefkolia' retained almost 65% of the K determined under control conditions. 'Lefkolia' exhibited also low Na and Cl accumulation compared to its control. According to the literature, the accumulation of Na in the upper parts of olive trees, and especially in the leaves could be used as a marker of the relative tolerance or sensitivity of the specific cultivar to salinity stress (Aparicio et al. 2014; Chartzoulakis et al. 2006; Rossi et al. 2015; Soda et al. 2016; Tattini 1994). This was also evident by the discriminant analysis where Na was one of the components of the two discriminant functions accounting for 69% prediction of cultivar grouping, while Cl was not, indicating the significance of Na for olive trees. Salt damage is prevented among others by the inhibition of salt transport to the shoot and leaves and the ability to maintain a higher K/Na ratio (Bader et al. 2015; Chartzoulakis et al. 2002; Perica et al. 2008; Tattini 1994). Based on that, it can be concluded that 'Lefkolia' and 'Arvanitolia', the two cultivars presenting the lowest Na accumulation under Tr200 and the highest K/Na ratio (above 1.8) in the leaves, are the ones exhibiting the highest salt tolerance among the nine cultivars (Kchaou et al. 2010; Perica et al. 2008; Tattini 1994). These two cultivars were the one grouped closely together after the discriminant analysis, indicating similar responses against salinity stress. On the contrary, 'Atsicholou', 'Koroneiki', 'Petrolia' and 'Smertolia' were the cultivars with the highest Na accumulation under Tr200 and the lowest K/Na ratio (below or equal to 1.0), indicating the sensitive character of these cultivars to salt stress. Concerning the effects of each individual salt level, the principal component analysis clearly showed that there were not many significant differences among Tr0 and Tr50 and/or Tr100 with Tr200 clearly distinguished by Tr0. These results support in general the salt-tolerant character of olive tree, at least up to a salt level of 100 mM.

It is quite interesting to discover that 'Lefkolia' and 'Arvanitolia' fulfill all the criteria to be considered salt tolerant, exhibiting both "physiological" and "horticultural" salt tolerance, as has been found by the present

experiment ("physiological" tolerance) and has been reported by Assimakopoulou et al. (2017) ("horticultural" tolerance). These two cultivars are thus promising genotypes for cultivation in salty soils and as genetic material in hybridization protocols and need to be further tested and studied.

Author contribution statement PAR participated in the trial design, carbohydrates analysis and wrote the manuscript. AA participated in the trial design, executed glasshouse trial and participated in nutrient analysis. AN did photosynthesis and chlorophyll fluorescence measurements. IS took part in trial execution in the glasshouse and participated in nutrient analysis. KN took part in trial execution in the glasshouse and participated in nutrient analysis. PK took part in trial execution in the glasshouse and participated in nutrient analysis. GK collected and described plant material, propagated it and took part in the trial execution in the glasshouse.

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