



# Ecophysiological and biochemical aspects of olive tree (*Olea europaea* L.) in response to salt stress and gibberellic acid-induced alleviation

Ines Moula<sup>a,b,1</sup>, Olfa Boussadia<sup>a,1,\*</sup>, Georgios Koubouris<sup>d</sup>, Mortadha Ben Hassine<sup>a,b</sup>, Wafa Boussetta<sup>a</sup>, Marie Christine Van Labeke<sup>c</sup>, Mohamed Braham<sup>a</sup>

<sup>a</sup> Olive Tree Institute - Unit of Sousse, Sousse, Tunisia

<sup>b</sup> High Institute of Agronomic Sciences, Chott Mariem, Sousse, Tunisia

<sup>c</sup> Department of Plant Production, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

<sup>d</sup> Institute of Olive Tree, Subtropical Crops & Viticulture, Hellenic Agricultural Organization DEMETER, (NAGREF), Chania, Greece

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## ABSTRACT

Soil salinization is considered as one of the most important risks for agricultural soils. The objective of this experiment was to study the ecophysiological and the biochemical behaviour of two cultivars of *Olea europaea* L., 'Chemlali' and 'Koroneiki' under two salinity levels (100 and 200 mM NaCl) and the potential alleviation induced by gibberellic acid (GA3) foliar sprays. Salinity treatments significantly decreased photosynthetic assimilation rate and stomatal conductance compared to the control for both cultivars, but 'Chemlali' showed a higher resistance to increasing NaCl salinity compared to 'Koroneiki'. Leaf chlorophyll index also reduced gradually with increasing salinity concentration compared to the control. At the end of the experiment, a decrease in growth and dry matter accumulation was observed. Under high salinity stress, a significant decrease in root DW was recorded by 37% and 59% for 'Chemlali' and 'Koroneiki', respectively. High salinity stress decreased also shoot DW up to 51% for 'Chemlali'. However, mannitol concentration increased under increasing salinity levels compared to control for 'Chemlali' cultivars. Interestingly, foliar application of GA3 alleviated the negative effects of salinity on ecophysiological parameters especially for 'Koroneiki'. Indeed, GA3 improved photosynthetic assimilation up to 14% for 'Chemlali' and 36% for 'Koroneiki' compared to high salinity treatment. Both cultivars showed an increase in leaf chlorophyll index after applying GA3. Under high salinity combined with GA3, growth and dry weight were increased compared to salt stressed plants without GA3. The obtained results report that 'Chemlali' cultivar is more tolerant to salinity than 'Koroneiki' and suggest that GA3 plays an important role to reduce negative effects of NaCl salinity.

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## 1. Introduction

The olive (*Olea europaea* L.) is the dominant tree crop in the Mediterranean basin and has been expanding globally during the recent decades. It has been adapted to the climate variability of the Mediterranean region which is characterised by high temperature stress (G.C. Koubouris et al., 2015a; 2009), shortage of rainfall (Arampatzis et al., 2018; Kourgialas et al., 2019), expansion of salinity (Kourgialas et al., 2017) and recently extreme weather events (Koubouris, 2018). Evaluation of the effects of salinity on olive trees has been carried out through various studies. Changes in ecophysiological and biochemical parameters are some of these effects.

Under saline conditions, gas exchange properties are generally affected (Bonji and Loreto, 1989; Chartzoulakis et al., 2002; Sajid

et al., 2017; G.C. Koubouris et al., 2015b; Lui et al., 2017; Wang et al., 2018). The effects of salt stress might be direct, such as the diffusion limitations through the stomata and the mesophyll (Chaves et al., 2009; Acosta-Motos et al., 2017; Wang et al., 2018). When the leaves accumulate  $\text{Na}^+$  ions, mesophyll resistance tends to increase, and it is joined with an increase of stomatal resistance which gradually reduces the amount of  $\text{CO}_2$  reaching the chloroplasts (Loreto et al., 2003; Lui et al., 2017). This draw-down in the  $\text{CO}_2$  concentration leads, in turn, to a decrease in the photosynthesis (Delfine et al., 1999; Loreto et al., 2003; Sajid et al., 2017). It has been reported that the relationship between photosynthesis and stomatal conductance have a good linear correlation. This elaboration confirms that low stomatal conductance is the main limitation of photosynthesis in olive (Loreto et al., 2003). The drop in the photosynthetic rate can also be due to other non-stomatal limitations such as the alterations in photosynthetic metabolism and the inhibition of the Calvin Cycle enzymes like Rubisco (Yamane et al., 2012; Acosta-Motos et al., 2017).

Besides that, salt stress might also have secondary effects, such as the oxidative stress due to implantation of multiple stresses (Chaves

\* Corresponding author.

E-mail addresses: [io.olfa.boussadia@gmail.com](mailto:io.olfa.boussadia@gmail.com) (O. Boussadia), [koubouris@nagref-cha.gr](mailto:koubouris@nagref-cha.gr) (G. Koubouris).

<sup>1</sup> The first and the second authors contributed equally to this study

et al., 2009; Lui et al., 2017). Salt stress can contribute to the production of enormous reactive oxygen species (ROS) (Lui et al., 2017). ROS are known to generate damage to cell membranes and other cellular components (Chakraborty et al., 2016).

Studies on responses of olive tree to salinity include changes in growth. For example, shoot length, root length, leaf area and dry weight are restrained by salinity (Chartzoulakis et al., 2002; Chartzoulakis 2005; Perica et al., 2008; Kchaou et al., 2010; Sajid et al., 2017; Acosta-Motos et al., 2017; Lui et al., 2017).

Further response to salinity is osmotic stress due to ion concentration within the cell tissues (Lui et al., 2017).  $\text{Na}^+$  accumulates in the vacuole and leads to imbalance into the cell. The adaptive behaviour of olive tree to salinity has been reported to be variable depending on the intensity and duration of stress and it differs amongst cultivars (Chartzoulakis et al., 2002, 2006; Kchaou et al., 2010, 2013; Bader et al., 2015). Salt tolerance in olive cultivars is associated with effective mechanisms of ion exclusion and retention of  $\text{Na}^+$  and  $\text{Cl}^-$  in the roots (Tabatabaei 2007; Tattini et al., 1996; Chartzoulakis et al., 2002). Salt stressed plants need to maintain balance between osmotic potential in the vacuole and in the cytoplasm. The cytoplasm accumulates some organic solutes such as soluble sugars and proline, which contributes to the osmoregulation from the cell to the whole plant (Hu et al., 2000; Hasegawa and Bressan, 2000; Kafi et al., 2003; Tester and Davenport, 2003; Lui et al., 2017).

Management of olive tree cultivation under salt stress should enhance the understanding of the cultivar response and the execution of other agro-techniques. Application of sustainable agricultural practices is crucial for olive crop viability and adaptation to climate change (Koubouris et al., 2017). Phytohormones have been shown to modulate several physiological and biochemical mechanisms that lead to adaptation to unfavourable environments (Fatma et al., 2013; Ali et al., 2014; Lui et al., 2017).

Few studies pinpointed the role of GA3 to reduce salt stress impact. It has been concluded that GA3 is a safe plant growth regulator which alleviated the adverse effects of salinity on physiological aspects such as chlorophyll content, stomatal conductance and transpiration (Maggio et al., 2010; Misratia et al., 2013). Salt stress represses seed germination by negatively regulating GA biosynthesis in soybean (Shu et al., 2017). Also, foliar GA3 application improved growth in salt treated tomato plants by modifying the hormonal balance (Khalloufi et al., 2017). The exogenous application of GA3 ameliorated the negative effects of salt stress on growth, development and yield in many crops (Hamayun et al., 2010; Maggio et al., 2010; Javid et al., 2011; Shaddad et al., 2013; Shekafandeh et al., 2017).

To date, reports concerning the foliar application of GA3 under salt stress are limited. Therefore, the aim of this study was to investigate the effects of different levels of NaCl salinity on ecophysiological and biochemical response of two olive tree cultivars (*Olea europaea* L. cv 'Chemlali' and 'Koroneiki') and to assess the effects of foliar spray of gibberellic acid GA3 for salinity alleviation.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

One-year-old olive plants ('Chemlali' and 'Koroneiki') were employed. Roots were washed and plants were transplanted into a substrate mixture of sand and perlite (3/2 vol ratio) in 4 L plastic pots in a greenhouse at the Tunisian Olive Tree Institute (Tunisia, 35 49'N, 10 38'E) under normal daylight conditions. Trees were watered with Hoagland nutrient solution before imposing salt stress treatments. Plants were subjected to salt stress from April, 11th till June, 11th, 2016. Five treatments were studied: (T0) control, plants grown on standard nutrient solution; (T1) moderate salinity, plants grown on saline solution containing 100 mM NaCl with electric conductivity EC=8.51 mS/cm; (T2) high salinity, plants grown on saline solution

containing 200 mM NaCl with electric conductivity EC=17.24 mS/cm; (T1+GA) plants grown on moderate salinity plus 100 ppm gibberellic acid (GA3); (T2+GA) plants grown on high salinity plus 100 ppm gibberellic acid (GA3). During the experiment, the mean day and night temperature was 32° and 18 °C and the mean day and night air humidity was 65% and 85%, respectively. Control and salt stressed plants were arranged in a complete randomized design with ten replications for each cultivar.

### 2.2. Gas exchange measurements

Fully expanded leaves were used to measure simultaneously maximum net photosynthetic assimilation rate  $A$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance for water vapour  $g_s$  ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) using a portable gas exchange system (LI-6400, LI-COR, Lincoln, NE, USA). Measurements were performed twice weekly in 3 replicates for each treatment. Measurements of  $A$  and  $g_s$  were carried out at light saturation ( $1500 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ) between 9 am and 2 pm, at a fixed  $\text{CO}_2$  concentration ( $400 \mu\text{mol mol}^{-1}$ ), with a leaf temperature of 25 °C and relative humidity of 50%.

### 2.3. Non-destructive determination of leaf chlorophyll index

A non-destructive method was applied to determine the amount of chlorophyll present in the leaf sample using the SPAD-502 m (Spectrum Technologies, Inc, Aurora, USA). The SPAD-502 measurements were performed in the greenhouse weekly in three replicates for each treatment and for each cultivar between 9 am and 2 pm.

### 2.4. Soluble sugars quantification

Dried leaf samples were extracted with 80% ethanol and placed for 10 min at 75 °C and then for 3 h at 45 °C. Samples were centrifuged at 5000 rpm for 5 min and 200 mg of polyvinylpyrrolidone were added to 4 ml of the supernatant and then centrifuged at 5000 rpm for 5 min. The supernatant obtained was then transferred into eppendorf tubes and stored at -20 °C for analysis by HPLC (High Performance Liquid Chromatography) technique using CarboPac MA1 column. Analysis was performed in 3 replicates for each treatment and each cultivar.

### 2.5. Plant growth and dry mater accumulation

At the end of the experiment, ten plants were randomly harvested per treatment and cultivar. The plants were divided into root and shoot fractions. Growth was determined by measuring shoot length and principal root length. Dry mater was determined after drying the root and shoot fractions at 70 °C for 72 h.

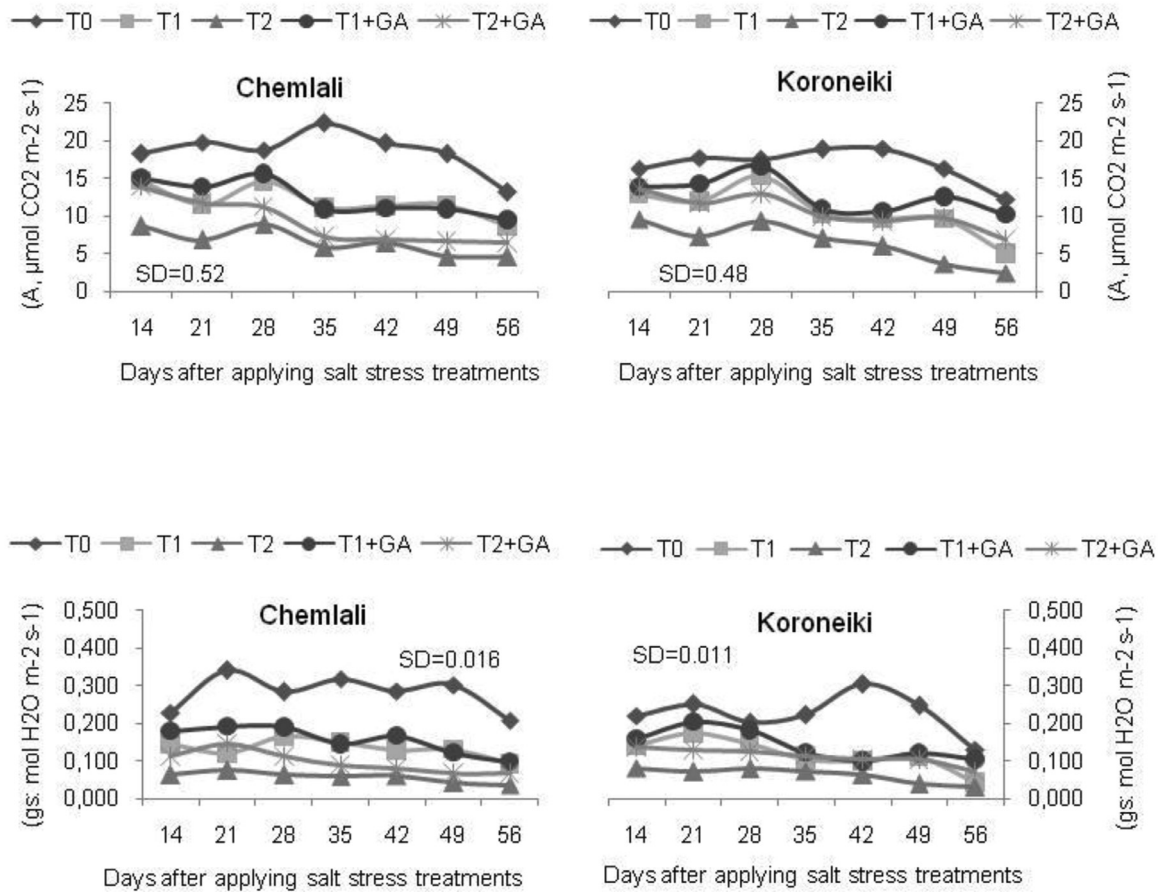
### 2.6. Statistical analysis

Data were statistically analysed using SPSS 21.0. Significant differences between treatments were determined by one-way analysis of variance. Duncan's multiple range test was used to compare the means ( $p = 0.05$ ).

## 3. Results

### 3.1. Leaf photosynthetic response to salinity stress

The effect of salt stress treatments combined with a foliar spray of gibberellic acid on the leaf photosynthesis ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of 'Chemlali' and 'Koroneiki' olive plants is shown in Fig. 1. After 56 days, leaf photosynthetic assimilation was significantly affected by ascendant salt stress levels for both cultivars. For 'Chemlali', a decrease of 34 and 65% for T1 and T2, respectively, was noted



**Fig. 1.** Leaf photosynthesis ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) of two olive tree cultivars (Chemlali and Koroneiki) under salt stress treatments and gibberellic acid foliar spray during 56 days after the start of the treatments. Each value represents the mean  $\pm$  standard deviation of three measurements ( $p = 0.05$ ).

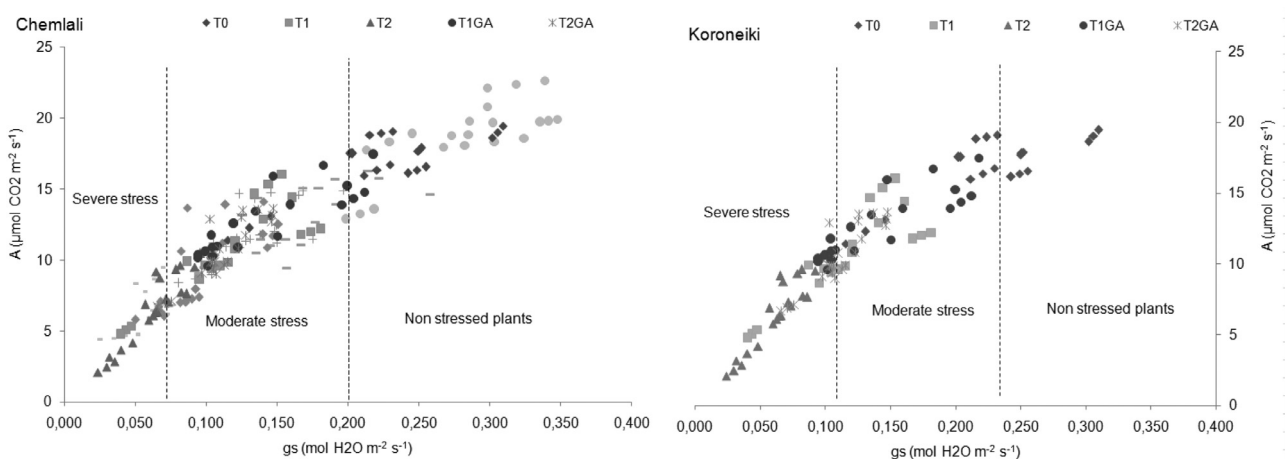
compared to control plants. For 'Koroneiki', the decrease reached 59 and 80% for T1 and T2, respectively. Foliar application of GA3 improved photosynthetic assimilation up to 14% for 'Chemlali' and 36% for 'Koroneiki' for T2+GA compared to high salinity treatment T2.

### 3.2. Leaf stomatal conductance response to salinity stress

Results related to the effect of salt stress treatments combined with foliar spray of GA3 on  $g_s$  showed that increasing salinity levels decreased stomatal conductance for both cultivars (Fig. 1). At the end

of the experiment, control plants showed the  $g_s$  values of 0.209 and 0.130  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  for 'Chemlali' and 'Koroneiki', respectively. However, high salinity treatment T2 showed the lowest value. A decrease of 82 and 77% for 'Chemlali' and 'Koroneiki', respectively, was noted. The foliar spray of GA3 improved  $g_s$  for 'Koroneiki' with a significant increase of 47% for T1+GA compared to moderate salinity treatment T1.

A clear correlation was found between photosynthetic assimilation and stomatal conductance under increasing levels of salt stress combined with GA3 application (Fig. 2). The distribution of the values



**Fig. 2.** Relationship between stomatal conductance ( $g_s$ ) and leaf photosynthesis ( $A$ ) of 'Chemlali' and 'Koroneiki' olive plants under salt stress treatments and gibberellic acid foliar spray during 56 days after the start of the treatments.

showed a linear relationship. Increasing levels of salt stress led to decreasing values of photosynthesis and stomatal conductance simultaneously, compared to control plants.

A clear correlation was found between the stomatal conductance and the photosynthetic assimilation under increasing levels of salt stress combined with GA3 application (Fig. 2). The distribution of the values showed a linear relationship ( $R^2_{\text{Chemlali}} = 0.88$ ;  $p = 0.01$  and  $R^2_{\text{Koroneiki}} = 0.86$ ;  $p = 0.01$ ). Maximum amounts of  $g_s$  and  $A$  were achieved in control plants T0. In our study, non-stressed plants presented the values of  $g_s > 0.200 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  and the values of  $A > 15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

However, these parameters were always lower in salt stressed plants. Based on the regression illustrated in Fig. 2, two salt stress ranges could be identified for both cultivars amongst increasing salinity levels. The first range is “moderate stress” which corresponded to values of  $g_s$  in the interval of  $0.075\text{--}0.200 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  and of values  $A$  in the interval of  $10\text{--}15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . The second range is “severe stress” which presented the values of  $g_s < 0.075 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  and the values of  $A < 10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Practically, plants grown under “moderate stress” were watered with a saline solution containing  $100 \text{ mM NaCl}$  ( $\text{EC}=8.51 \text{ mS/cm}$ ), and those grown under “severe stress” were watered with a saline solution containing  $200 \text{ mM NaCl}$  ( $\text{EC}=17.24 \text{ mS/cm}$ ).

Furthermore, the application of GA3 led to amelioration of the response intervals of salt stressed plants especially for the plants grown under “severe stress” where the values of  $g_s$  and  $A$  were ameliorated and were closer to values of plants grown under “moderate stress”. Thus, prediction of the degree of stress according to  $g_s$  and  $A$  is a useful way that can be exploited in practice.

### 3.3. Leaf chlorophyll index

Leaf chlorophyll index evolution of ‘Chemlali’ and ‘Koroneiki’ olive cultivars, as measured by non-destructive SPAD method, is shown in Fig. 3. For both cultivars, control plants T0 showed the highest chlorophyll index during the experimental period, whereas high salinity plants T2 showed the lowest one. Significant differences appeared from the 28th day after applying salt stress treatments. This significant difference was maintained until the end of the experimental period. After 56 days, moderate salinity treatment T1 decreased by 18 and 26% and high salinity treatment T2 decreased by 27 and 31% for ‘Chemlali’ and ‘Koroneiki’, respectively, compared to control plants T0. Both cultivars showed an increase in Leaf chlorophyll index with the foliar application of GA3 compared to moderate and high salinity treatments.

### 3.4. Soluble sugars under salinity and GA

After 56 days of treatments, the effects of salt stress combined with GA3 foliar spray were investigated and the results are shown in Table 1. Mannitol was the most abundant amongst the sugars. For ‘Chemlali’, mannitol concentration showed higher values under salt stress compared to the control. Glucose and fructose didn’t show any differences under salinity treatments.

### 3.5. Plant growth and dry matter accumulation

At the end of the experimental period, plant growth was significantly affected by salt stress treatments (Table 2). A pronounced decrease in root length was noted for both cultivars compared to control plants. Shoot length decreased by increasing levels of salinity, namely treatments T1 and T2.

However, foliar application of GA3 increased shoot length for ‘Chemlali’ under high salinity combined with GA3, T2+GA, by 8% compared to control (T0). GA3 also increased shoot length for ‘Koroneiki’ under moderate salinity combined with GA3, T1+GA, by 2% compared to the control (T0). Root and shoot dry weights were significantly affected by salt stress treatments for both cultivars (Table 3). Control plants showed the highest root and shoot dry weights. However, under high salinity stress T2, a significant decrease in root DW was recorded by 37 and 59% for ‘Chemlali’ and ‘Koroneiki’, respectively. High salinity stress decreased also shoot DW up to 51% for ‘Chemlali’ compared to control.

## 4. Discussion

The aim of the present study was to investigate the salinity tolerance of two olive cultivars (‘Chemlali’ and ‘Koroneiki’) and the alleviation potential of applying GA3 as a foliar spray. The results showed that both rates of leaf photosynthesis and stomatal conductance were negatively affected by NaCl treatments. The photosynthetic assimilation rate decreased with the increase of salt stress level. These results agree with earlier findings of Tattini et al., (1997); Chartzoulakis et al., 2002; Centritto et al., (2003); Chartzoulakis (2005); Tabatabaei (2006) and Iqbal and Ashraf (2013).

Indeed, Tabatabaei (2007) reported that the reduction of photosynthetic rate was due to several factors: (1) dehydration of cell membranes which reduce their permeability to  $\text{CO}_2$ , (2) salt toxicity, (3) reduction of  $\text{CO}_2$  supply because of hydroactive closure of stomata, (4) enhanced senescence induced by salinity, (5) changes of enzyme activity induced by changes in cytoplasmic structure, and (6) negative feedback by reduced sink activity.

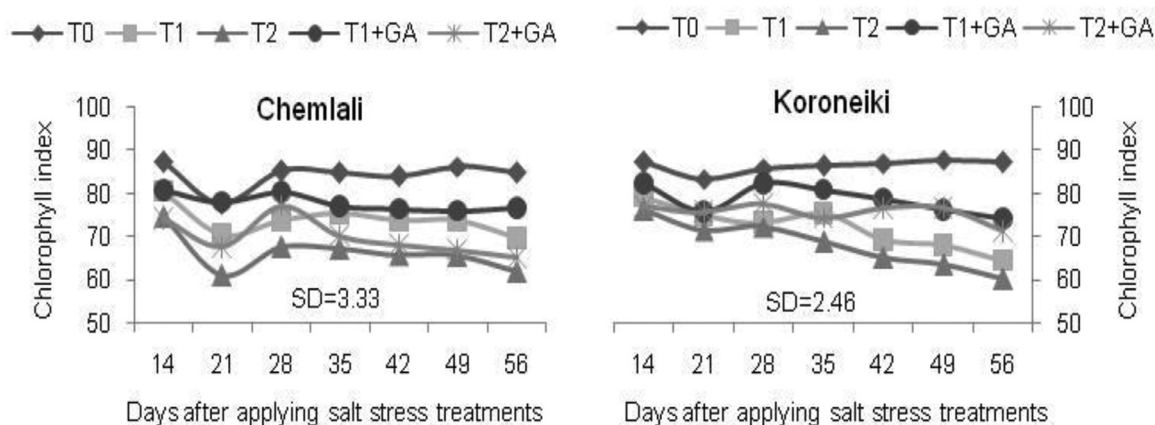


Fig. 3. Chlorophyll index evolution of ‘Chemlali’ and ‘Koroneiki’ olive leaves under salt stress treatments and gibberellic acid foliar spray during 56 days after the start of the treatments. Each value represents the mean  $\pm$  standard deviation of three measurements ( $p = 0.05$ ).



**Table 1**

Effects of salt stress treatments and gibberellic acid foliar spray on soluble sugars of 'Chemlali' and 'Koroneiki' olive plants 56 days after the start of the treatments. Each value represents the mean  $\pm$  standard deviation of three measurements. Means followed by the same letter do not differ statistically (Duncan test,  $p = 0.05$ ). The first letter is for the statistical analysis within the salt treatments for each cultivar separately and the second is for the statistical analysis for both cultivars.

		Mannitol	Glucose	Fructose	Sucrose	Myo-inositol
T0	'Chemlali'	2.09 $\pm$ 0.19 <sup>b,b</sup>	0.53 $\pm$ 0.02 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a,a</sup>	0.13 $\pm$ 0.06 <sup>b,a</sup>	0.12 $\pm$ 0.01 <sup>a,a</sup>
	'Koroneiki'	2.82 $\pm$ 0.07 <sup>a,a</sup>	0.45 $\pm$ 0.07 <sup>a,a</sup>	0.07 $\pm$ 0.04 <sup>a,a</sup>	0.21 $\pm$ 0.05 <sup>a,a</sup>	0.10 $\pm$ 0.02 <sup>ab,a</sup>
T1	'Chemlali'	2.83 $\pm$ 0.55 <sup>a,a</sup>	0.42 $\pm$ 0.15 <sup>a,a</sup>	0.06 $\pm$ 0.03 <sup>a,a</sup>	0.41 $\pm$ 0.23 <sup>a,a</sup>	0.13 $\pm$ 0.04 <sup>a,a</sup>
	'Koroneiki'	3.11 $\pm$ 0.51 <sup>a,a</sup>	0.58 $\pm$ 0.10 <sup>a,a</sup>	0.06 $\pm$ 0.02 <sup>a,a</sup>	0.24 $\pm$ 0.01 <sup>a,a</sup>	0.13 $\pm$ 0.03 <sup>a,a</sup>
T2	'Chemlali'	2.95 $\pm$ 0.32 <sup>a,a</sup>	0.39 $\pm$ 0.25 <sup>a,a</sup>	0.06 $\pm$ 0.02 <sup>a,a</sup>	0.37 $\pm$ 0.11 <sup>a,a</sup>	0.09 $\pm$ 0.02 <sup>a,a</sup>
	'Koroneiki'	2.94 $\pm$ 0.23 <sup>a,a</sup>	0.51 $\pm$ 0.11 <sup>a,a</sup>	0.04 $\pm$ 0.01 <sup>a,a</sup>	0.33 $\pm$ 0.13 <sup>a,a</sup>	0.10 $\pm$ 0.01 <sup>b,a</sup>
T1+GA	'Chemlali'	2.78 $\pm$ 0.31 <sup>a,a</sup>	0.33 $\pm$ 0.10 <sup>a,b</sup>	0.03 $\pm$ 0.00 <sup>a,b</sup>	0.23 $\pm$ 0.03 <sup>ab,a</sup>	0.11 $\pm$ 0.01 <sup>a,a</sup>
	'Koroneiki'	2.75 $\pm$ 0.08 <sup>a,a</sup>	0.58 $\pm$ 0.11 <sup>a,a</sup>	0.07 $\pm$ 0.02 <sup>a,a</sup>	0.26 $\pm$ 0.06 <sup>a,a</sup>	0.09 $\pm$ 0.01 <sup>b,a</sup>
T2+GA	'Chemlali'	3.38 $\pm$ 0.32 <sup>a,a</sup>	0.50 $\pm$ 0.13 <sup>a,a</sup>	0.04 $\pm$ 0.01 <sup>a,b</sup>	0.26 $\pm$ 0.05 <sup>ab,a</sup>	0.10 $\pm$ 0.02 <sup>a,a</sup>
	'Koroneiki'	2.94 $\pm$ 0.29 <sup>a,a</sup>	0.50 $\pm$ 0.11 <sup>a,a</sup>	0.09 $\pm$ 0.02 <sup>a,a</sup>	0.32 $\pm$ 0.08 <sup>a,a</sup>	0.09 $\pm$ 0.01 <sup>b,a</sup>

**Table 2**

Effects of salt stress treatments and gibberellic acid foliar spray on shoot and root length of 'Chemlali' and 'Koroneiki' olive plants 56 days after the start of the treatments. Each value represents the mean  $\pm$  standard deviation of three measurements. Means followed by the same letter do not differ statistically (Duncan test,  $p = 0.05$ ). The first letter is for the statistical analysis within the salt treatments and the second is for the statistical analysis within the cultivar.

Cultivars	Root length (cm)		Shoot length (cm)	
	'Chemlali'	'Koroneiki'	'Chemlali'	'Koroneiki'
T0	41,38 $\pm$ 1,11 <sup>a,a</sup>	42,33 $\pm$ 1,15 <sup>a,a</sup>	80,33 $\pm$ 7,37 <sup>ab,a</sup>	87,00 $\pm$ 9,90 <sup>a,a</sup>
T1	34,40 $\pm$ 7,21 <sup>ab,a</sup>	37,5 $\pm$ 1,32 <sup>ab,a</sup>	72,18 $\pm$ 2,64 <sup>bc,b</sup>	84,67 $\pm$ 5,86 <sup>ab,a</sup>
T2	31,10 $\pm$ 3,57 <sup>bc,a</sup>	31,3 $\pm$ 2,72 <sup>c,a</sup>	64,88 $\pm$ 3,84 <sup>c,b</sup>	73,83 $\pm$ 3,75 <sup>c,a</sup>
T1+GA	25,03 $\pm$ 4,97 <sup>c,b</sup>	39,00 $\pm$ 3,27 <sup>ab,a</sup>	70,25 $\pm$ 3,77 <sup>c,b</sup>	89,00 $\pm$ 8,19 <sup>a,a</sup>
T2+GA	31,83 $\pm$ 2,02 <sup>bc,a</sup>	34,05 $\pm$ 5,57 <sup>bc,a</sup>	86,50 $\pm$ 6,26 <sup>a,a</sup>	77,58 $\pm$ 3,18 <sup>ab,a</sup>

Furthermore, stomatal conductance decreased significantly under salt stress treatments. The degree of reduction in  $g_s$  might be due to the closure of stomata caused by excessive accumulation of Na<sup>+</sup> ion in the guard cells, which reduces the availability of internal CO<sub>2</sub> (Thiel and Blatt, 1991). Stomatal closure reduces the loss of water by transpiration which leads to altered chloroplast activity as a result of altered chloroplast light harvesting and energy conversion systems (Tabatabaei 2007).

Increasing salt stress levels decreased leaf chlorophyll index measured using SPAD-502 m. This result strongly agrees with the finding of Shaheen et al., (2011). Decreased chlorophyll concentration in NaCl treated plants might be due to accumulation of Na<sup>+</sup> ions in chloroplast which affects the plant growth, photosynthesis and PSII function (Kao et al., 2003). Ashraf and Harris (2013) reported that salt stress can break down photosynthetic pigments and reduce chlorophyll (Chl a and b). The salt-induced alterations in leaf chlorophyll content could be due to impaired biosynthesis or accelerated pigment degradation.

A significant increment of mannitol content in salt stressed plants of 'Chemlali' was reported. The increase in mannitol in response to

increasing NaCl concentration supports the idea that this carbohydrate plays an active role in the process of osmotic adaptation to salinity in olive (Tattini et al., 1996). As Na<sup>+</sup> accumulates in the vacuole, the synthesis of organic solutes (compatible solutes or osmoprotectants) is stimulated. This is done to balance the osmotic potential in the cytoplasm with that in the vacuole (Tester and Davempport, 2003).

Glucose content in salt treated plants showed no significant difference compared to control plants. Tattini et al., 1996 concluded that the increase in mannitol is a mechanism of response rather than a consequence of salinity in olive. Whereas glucose is most likely the main sugar involved in metabolic functions and storage in olive leaf.

Furthermore, imposing either moderate or high salinity caused a significant depression in plant growth and dry matter accumulation. Growth suppression was observed in both cultivars. During the experimental period, chlorosis, tip burn and leaf drop occurred gradually for the salt stressed plants. This pattern agrees with previous works of Chartzoulakis et al., 2002 and Tabatabaei (2006).

In the present work, foliar application with GA3 had beneficial effects on ecophysiological parameters as well as growth attributes. The adverse effects of salt stress on photosynthetic assimilation and stomatal conductance were mitigated by the GA3 treatment, especially for 'Koroneiki'. In a previous work, Misratia et al., (2013) reported that the reduction in photosynthetic rate and stomatal conductance at different salinity levels occurred without GA3 application, while with the foliar application of GA3 these ecophysiological parameters were enhanced. An earlier study of Badger and Price (1994) assessed the role of carbonic anhydrase (CA) in the photosynthetic process. The enzyme CA contributes to the conversion of HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub> for fixation by Rubisco, the conversion of CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup> for fixation by PEP carboxylase, and the provision of a rapid equilibration between CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> which enhances the diffusion of CO<sub>2</sub>. In another work of Soussi et al., (1998) it was shown that NaCl caused reduction in CA activity and suggested that it may be due to the inactivation of Rubisco. It has been generally reported that the alteration

**Table 3**

Effects of salt stress treatments and gibberellic acid foliar spray on root and shoot dry weight (DW) and root/shoot ratio of 'Chemlali' and 'Koroneiki' olive plants 56 days after the start of the treatments. Each value represents the mean  $\pm$  standard deviation of three measures. Means followed by the same letter do not differ statistically (Duncan test,  $p = 0.05$ ). The first letter is for the statistical analysis within the salt treatments and the second is for the statistical analysis within the cultivar.

	Root DW (g plant <sup>-1</sup> )		Shoot DW (g plant <sup>-1</sup> )		Root/shoot ratio	
	'Chemlali'	'Koroneiki'	'Chemlali'	'Koroneiki'	'Chemlali'	'Koroneiki'
T0	12.18 $\pm$ 1.59 <sup>a,b</sup>	18.39 $\pm$ 0.02 <sup>a,a</sup>	28.35 $\pm$ 1.42 <sup>a,b</sup>	33.55 $\pm$ 0.33 <sup>a,a</sup>	0.43 $\pm$ 0.03 <sup>b,b</sup>	0.55 $\pm$ 0.00 <sup>a,a</sup>
T1	8.27 $\pm$ 1.97 <sup>b,a</sup>	9.81 $\pm$ 0.57 <sup>b,a</sup>	18.34 $\pm$ 1.21 <sup>b,b</sup>	21.79 $\pm$ 0.56 <sup>c,a</sup>	0.45 $\pm$ 0.08 <sup>b,a</sup>	0.45 $\pm$ 0.01 <sup>b,a</sup>
T2	7.62 $\pm$ 0.96 <sup>bc,a</sup>	7.53 $\pm$ 0.26 <sup>d,a</sup>	13.90 $\pm$ 0.98 <sup>c,b</sup>	19.69 $\pm$ 0.31 <sup>d,a</sup>	0.55 $\pm$ 0.03 <sup>a,a</sup>	0.38 $\pm$ 0.01 <sup>d,b</sup>
T1+GA	5.51 $\pm$ 0.17 <sup>c,b</sup>	9.562 $\pm$ 0.36 <sup>b,a</sup>	13.49 $\pm$ 0.84 <sup>c,b</sup>	24.30 $\pm$ 0.91 <sup>b,a</sup>	0.41 $\pm$ 0.01 <sup>b,a</sup>	0.39 $\pm$ 0.00 <sup>cd,a</sup>
T2+GA	6.20 $\pm$ 0.98 <sup>bc,b</sup>	8.39 $\pm$ 0.39 <sup>c,a</sup>	16.39 $\pm$ 1.04 <sup>b,b</sup>	20.73 $\pm$ 1.3 <sup>cd,a</sup>	0.38 $\pm$ 0.04 <sup>b,a</sup>	0.40 $\pm$ 0.01 <sup>c,a</sup>

of Rubisco activity reduces diverse physiological processes such as net photosynthetic rate (Jiang et al., 1993). Conversely, application of GA3 appeared to mitigate photosynthetic assimilation through the amelioration of the carboxylase activity of Rubisco (Shah 2007), as well as the enhancement of the rates of cyclic and non-cyclic phosphorylation (Naidu and Swamy, 1995). Gibberellin increased the accumulation of proline in two Iranian olive cultivars (Shekafandeh et al., 2017).

In the present study, GA3 treatment alleviated the inhibitory effect of salt stress and restored plant growth. This result is in agreement with earlier findings of Hamayun et al., (2010) on soybean (*Glycine max* L.) and Iqbal and Ashraf (2013) on wheat (*Triticum aestivum* L.).

Moreover, shoot length and dry biomass significantly recovered when GA3 was added to salt-stressed plants. The plant growth promotion as a response to GA3 could be explained through the role of GA3 in cell elongation and cell division. This caused in turn an increase in total dry matter accumulation (Khan et al., 2010). It has been shown that the increase of vegetative growth as a response to GA3 occurs as a result of the enhancement of stomatal conductance and leaf expansion of lettuce and rocket plants under GA3 treatment (Miceli et al., 2019).

## 5. Conclusion

Salinity treatments induced negative effects on ecophysiological and vegetative growth of 'Chemlali' and 'Koroneiki' olive tree cultivars. Indeed, photosynthesis, stomatal conductance and leaf chlorophyll index were negatively affected for both cultivars. Nevertheless, 'Chemlali' showed a higher resistance to increasing NaCl salinity. Mannitol accumulation increased under salinity stress for 'Chemlali' and seems to play a major role in stress tolerance. Plant growth and dry matter accumulation were affected by the imposed salinity stress. On the other hand, GA3 application mitigated the drastic effects of salt stress on the ecophysiological parameters, especially for 'Koroneiki'. Amelioration of the carboxylase activity of Rubisco, as well as the enhancement of the rates of cyclic and non-cyclic phosphorylation may be the responsible processes of the recovered ecophysiological performance and vegetative growth.

## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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