Research Article

Giovanni Gugliuzza*, Carla Gentile, Dario Scuderi, Eristanna Palazzolo, Vittorio Farina

Effects of salt stress on growth of *Quercus ilex* L. seedlings

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Abstract: High salt concentration is one of the main factors that affects plants' growth, especially in urban areas. Many Mediterranean sclerophyllous species manifest high resistance to salt, although few information exists in the literature on *Quercus ilex*. The aim of this work was to evaluate the response of *Q. ilex* seedlings to salt stress conditions generated by an increasing concentration of sodium chloride on water supply irrigation. A 2-year experiment was conducted by using salt water at different concentrations (first year 50–100–200 and second year 75–150–300 mM NaCl). At increasing salt concentration, a plant growth reduction was registered in both years. Until 200 mM NaCl, the plants grew and did not show any visible damage on the leaves, while at 300 mM NaCl, all the plants died. Also, the photosynthetic rate decreased at increasing salt concentration. Sodium was accumulated in the plant parts and this accumulation occurred at the expense of potassium uptake.

Keywords: *Quercus ilex*, salt stress, carotenoids, photosynthesis

1 Introduction

In the arid zones of the world, crop production consumes large quantities of water, and groundwater is often used for irrigation. The increased anthropic pressure, climate changes, the use of inorganic fertilizers, and irrigation with saline water are considered the sources of increasing salt concentration in the groundwater [1–4]. The scarce water resources in dry climates and the increased demands for water by civilian, industrial, and agricultural practices force the use of low-quality water for irrigation in urban landscape [5]. In urban environments, trees are subjected to abiotic and biotic stresses such as heat, low air humidity, restricted irrigation volumes, soil compaction, water stress related to reduced rainfall infiltration, de-icing salt, air pollution, insect damages, and NaCl soil contamination [6,7]. Soil salinity in urban environments is caused primarily by the application of NaCl to "deice" the winter streets and sidewalks [8], whereas in the coastal areas, it is a consequence of the sea-salt aerosol formed by air bubbles and spume droplets mixed into the surface layer of the sea by wave action [9]. High salt concentration is a major environmental factor limiting plant growth [10,11]. Salt stress not only contributes to a reduction in the landscape value of the trees but also causes major changes in its physiological and biochemical processes as well as in its morphology [12,13], its susceptibility to pests or diseases, and often, is the cause of plant death.

Quercus ilex L. is an evergreen Mediterranean sclerophyllous species typical of the broadleaf woodlands that can grow up to 20 m tall with a fissured blackish bark. *Q. ilex* has been largely used as an ornamental plant in the landscaping of parks and gardens and is very common on roadsides all along the coast of the western Mediterranean [14–17]. It is found under a variety of conditions of environmental pollution due to its capacity to accumulate pollutants using leaves as bio-accumulator [18–20]. This characteristic is due to the dense cover of hairs that allows the retention of airborne particulate, whereas the abundance of wax components promotes the incorporation of lipophilic organic pollutants [21].

Many *Quercus* species (*Q. lyrata* Walt., *Q. michauxii* Nutt., *Q. nigra* L., *Q. virginiana* Mill., and *Q. nuttallii* Palmer) are susceptible to salinity, showing a decrease in photosynthesis, stomatal conductance, tree height, and trunk diameter under saline conditions [22]. A recent study on *Q. ilex* [23] observed the responses to mild root zone salinity stress and evidenced the strategies adopted by this plant to allocate potentially toxic ions. The effect of medium salt concentrations (150 mM NaCl, 15 days) on photosynthesis, hydric relations, and ion partitioning saplings was done

*** Corresponding author: Giovanni Gugliuzza,** CREA – Research Centre for Plant Protection and Certification, c/o Dipartimento SAAF, Università degli Studi di Palermo , Ed. 4 Viale Delle Scienze, 90128 Palermo, Italy, e-mail: giovanni.gugliuzza@crea.gov.it
Carla Gentile: Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Palermo, Italy
Dario Scuderi, Eristanna Palazzolo, Vittorio Farina: Dipartimento Scienze Agrarie Alimentari e Forestali (SAAF), Università degli Studi di Palermo, Palermo, Italy

by Guidi et al. in 2017 [24]. Another study on transcriptome analysis conducted by Natali et al. [25], always on *Q. ilex* at max 150 mM NaCl, showed how salinity strongly changed the profile of expressed transcripts; indicating a higher genetic expression with respect to a biochemical and physiological response.

However, few information exists on *Q. ilex* susceptibility to high salinity levels. For their use in urban greening, where salt stress conditions are frequently reported, investigating the salt tolerance of *Q. ilex* represents a fundamental piece of information. Hence, the aim of this work was to evaluate the response of *Q. ilex* seedlings to salt stress conditions generated by an increasing concentration of sodium chloride in irrigation water.

2 Methods

2.1 Plant material

Q. ilex seedlings from the 'Crucicchia' forest nursery in Castellammare del Golfo (TP), managed by the Azienda Foreste Demaniali of Regione Sicilia, were transplanted in 7 L plastic pots filled with a 7:3 (v/v) mixture of sand and sandy soil. Seedlings were grown in an east–west-oriented greenhouse (540 mq) with a steel structure and polymethacrylate methyl cover located in Bagheria (38°5′28″ N: 13°31′18″E; 23 m a.s.l.), Sicily, Italy. The yearly maximum, average and minimum temperatures were 45.3, 21.7 and 2.5°C for the first year and 46.4, 20.3 and 1.7°C for the second year.

A 2-year experiment was conducted: during the first year, 1-year-old plants were manually watered twice a week with 1 L per plant by using four solutions at different NaCl concentrations (0, 50, 100, and 200 mM NaCl) for 90 days during the growing season, occurring in spring; in the second year, the same experiment was conducted with a new plot of 2-year-old plants which were manually watered twice a week with 2 L per plant by using four solutions at different NaCl concentrations (0, 75, 150, and 300 mM NaCl) for 90 days during the growing season, occurring in spring.

A completely randomized experimental design was adopted with six plants per treatment per four treatments – corresponding to the irrigation water NaCl concentrations – for a total of 24 plants per year.

At the end of the experiment, samples of leaves, stems, and roots from each plant were taken for fresh weight determination. Dry weights (DWs) were determined after oven drying the samples at 60°C for 48 h. Before drying, the number and area of leaves were measured by leaf area meter (Win DIAS).

2.2 Gas exchange

Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA).

Data were recorded at 12:00 solar time at the end of the experiment, on two mature leaves per plant. Environmental photosynthetic active radiation values were measured by Li-Cor sensor and fixed by an LED light source during the measurements.

2.3 Ion analysis

A sample of fresh leaves, shoots, and roots was washed with distilled water three times and then dried at 60°C to constant weight (6–8 h). The plant material was ground with a ball mill. The cations were determined after a digestion of 0.1 g dry matter with HNO_3, and then, the extract was taken to determine the free Na^+ and K^+ concentrations. The contents of K^+ and Na^+ were determined using atomic absorption spectroscopy following wet mineralization [26,27].

2.4 Carbohydrate content

A sample of fresh leaves, shoots, and roots was washed with distilled water three times and, then, dried at 60°C to constant weight (6–8 h). The carbohydrate content, either free or present in polysaccharides, was obtained with the anthrone method reported in Loewus [28].

Carbohydrates were first hydrolyzed into simple sugars using diluted hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural which when mixing with anthrone forms a green-colored product with an absorption peak at 630 nm

2.5 Antioxidant activity

Analysis of leaves' antioxidant activity was performed during the second year.

2.5.1 Extracts preparation

Ten grams of samples of young leaves were manually picked at the end of the experiment. After washing, leaves were freeze dried for 12 h and then were finely ground in a food grinder and stored at −80°C until extraction.

The powdered samples were extracted with 70% ethanol at a ratio of 1:10 (g/mL). Extraction was repeated two times for each sample. After a cleanup step via centrifugation (10 min at 10,000*g*, 4°C) and filtration through a Millex HV 0.45 µm filter (Millipore, Billerica, MA), the supernatants were recovered, combined, and used for the determination of antioxidant activity and phenolic content.

2.5.2 Total phenolic content

The phenolic content of ethanol extracts was determined by the reduction of phosphotungstic-phosphomolybdic acid (Folin–Ciocalteu's reagent) to blue pigments, in an alkaline solution according to Folin and Denis [29,30]. Quantitation was made by reference to a curve constructed with gallic acid, and the results were expressed as mg gallic acid equivalents (GAE) per 100 g DW.

2.5.3 Total antioxidant activity (TAA)

The TAA of ethanol extracts was evaluated using the ABTS radical cation decolorization assay [31]. $ABTS^{\bullet+}$ was prepared by reacting ABTS with $K_2S_2O_8$. Samples were analyzed at five different dilutions, within the linearity range of the assay, as previously described [32]. TAA was expressed as µmol Trolox equivalent/100 g DW.

2.5.4 Determination of total chlorophyll and carotenoid contents

The chlorophyll and carotenoid contents of leaves were analyzed according to the protocol described by Lichtenthaler and Buschmann [33] with minor variations. Briefly, freeze-dried leaves' powder (5 mg) was mixed with 5 mL of dimethyl sulfoxide and incubated at 65°C for 6 h. After incubation, the mixture was centrifuged at 15,000*g* for 5 min. The supernatant was collected, and the absorbance was read at 663 nm to measure chlorophyll A content, 647 nm to measure chlorophyll B content, and 470 nm to measure carotenoids' content. The chlorophyll and carotenoid concentrations were calculated using the following equations: chlorophyll A = $12.25A_{663} - 2.79A_{647}$; chlorophyll B = $21.50A_{647} - 5.10A_{663}$; total chlorophyll = $20.29A_{647} + 8.02A_{663}$; carotenoid = $(1{,}000A_{470} - 1.82$ chlorophyll A − 95.15 chlorophyll B)/225.

2.5.5 Statistics

Statistical analyses were based on a one-way analysis of variance. The effects of salinity treatments were considered statistically significant when $P < 0.05$. In these cases, the Tukey test of significant differences was carried out and results are given in the tables and figures. All statistical analyses were performed by using the SYSTAT 10 software.

3 Results

3.1 Dry matter accumulation

In the first year, plant biomass, in terms of fresh (data not shown) and DW, decreased as NaCl concentration increased (Table 1). Significative differences were observed in terms of plant DW between 0 and 200 mM NaCl with a weight reduction until 40%. A slight increase in roots' DW was registered at 50 mM NaCl while significative DW reductions were observed also between 50 and 200 mM NaCl. On the aerial parts of the plant, significative DW reductions at increasing NaCl concentrations were observed starting from 100 mM NaCl, reaching a 57% DW reduction at 200 mM NaCl.

Table 1: First-year DWs of *Q. ilex* plant components irrigated with water at different NaCl concentrations

NaCl (mM)	Plant DW (g)	Roots DW (g)	Shoot DW (g)	Leaves DW (g)	Above ground DW (g)	Leaves, *n*	Leaf area (cm^2)
0	39.01 ± 4.08a	19.81 ± 3.21ab	9.40 ± 1.97a	9.80 ± 0.52a	19.20 ± 2.25a	116 ± 6.65a	481.18 ± 37.3a
50	35.61 ± 6.63ab	20.64 ± 0.89a	7.30 ± 1.67ab	7.67 ± 1.40ab	14.97 ± 3.02ab	115 ± 7.34a	330.98 ± 58.4b
100	32.10 ± 3.81ab	19.23 ± 1.25ab	6.40 ± 0.72ab	6.47 ± 1.47b	12.87 ± 1.90bc	82 ± 8.71b	323.55 ± 54.6b
200	24.15 ± 3.65b	15.82 ± 1.01b	5.20 ± 0.53b	3.13 ± 0.11c	8.33 ± 0.62c	56 ± 8.62c	162.80 ± 6.86c

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

Table 2: Second-year DWs of *Q. ilex* plant components irrigated with water at different NaCl concentrations

NaCl (mM)	Plant DW (g)	Roots' DW (g)	Shoot DW (g)	Leaves' DW (g)	Above ground DW (g)	Leaves, *n*	Leaf area (cm^2)
0	124.24 ± 17.39a	87.20 ± 9.34a	20.99 ± 2.50a	16.05 ± 1.53a	37.04 ± 0.97a	188 ± 15.13a	1092.4 ± 88.2a
75	69.84 ± 7.41b	41.33 ± 4.43b	17.07 ± 1.52ab	11.44 ± 1.89b	28.51 ± 3.41b	161 ± 18.01ab	806.9 ± 172.1ab
150	42.43 ± 7.81b	22.33 ± 4.50b	11.83 ± 2.49b	8.27 ± 1.09b	20.10 ± 3.90c	111 ± 8.82b	519.9 ± 128.7b

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

The highest DW reduction (−68%) was observed in the leaves at 200 mM NaCl. This reduction was determined partially by the leaf number reduction (−52%) and partially by the leaf area reduction (66%). Neither plants died nor leaf damage was observed in all the treatments.

During the second year, shoots of the plants subjected to the treatment of 300 mM NaCl did not grow, leaf damage appeared, and all the plants died within 3 weeks from the beginning of the experiment.

For the other treatments, as in the first year, plant biomass, in terms of fresh (data not shown) and DW, decreased as NaCl concentration increased (Table 2). A significative plant DW reduction (−44%) was observed between 0 and 75 mM NaCl, while no statistical differences were observed between 75 and 150 mM NaCl concentration. The same behavior was observed in terms of roots' and leaves' DW (−75 and −50%). Leaves' DW reduction was caused by both the leaf number reduction and the leaf area reduction.

3.2 Ion levels

In both years, an increase in Na ions and a decrease in K ions were registered in all parts of the plant due to NaCl concentration increasing (Tables 3 and 4).

In the first year, statistically significant increases in leaves' Na concentration were registered among 0, 50, and 100 mM NaCl while statistically significant decreases in K ions were registered at NaCl concentration increase from 50 to 100 mM (Table 3). In the shoots, every NaCl

Table 3: First-year sodium (Na) and potassium (K) levels expressed as ppm/DW of *Q. ilex* plant components irrigated with water at different NaCl concentrations

	Leaf		Shoot		Root	
NaCl (mM)	Na (ppm)	K (ppm)	Na (ppm)	K (ppm)	Na (ppm)	K (ppm)
0	107.0 ± 2.16a	317.7 ± 4.16a	76.7 ± 2.50a	322.7 ± 4.72a	115.7 ± 2.08a	214.3 ± 7.02a
50	153.0 ± 2.64b	285.3 ± 7.50a	119.3 ± 2.08b	133.3 ± 2.08b	116.3 ± 3.21a	141.7 ± 1.53b
100	191.3 ± 3.21c	194.0 ± 5.56b	155.7 ± 4.04c	120.0 ± 1.02c	130.5 ± 1.72b	107.3 ± 1.15c
200	196.7 ± 3.51c	184.5 ± 6.81b	176.7 ± 3.06d	76.5 ± 4.20d	251.0 ± 3.34c	95.0 ± 2.16d

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

Table 4: Second-year sodium (Na) and potassium (K) levels expressed as ppm/DW of *Q. ilex* plant components irrigated with water at different NaCl concentrations

	Leaf		Shoot		Root	
NaCl (mM)	Na (ppm)	K (ppm)	Na (ppm)	K (ppm)	Na (ppm)	K (ppm)
0	122.1 ± 3.53a	306.0 ± 2.42a	73.4 ± 2.12a	123.0 ± 7.02a	110.0 ± 0.70a	156.3 ± 2.25a
75	158.3 ± 8.50b	284.7 ± 3.21a	126.2 ± 13.07b	120.0 ± 1.05a	125.7 ± 3.51b	107.3 ± 1.15b
150	161.7 ± 3.78b	214.4 ± 4.94b	127.6 ± 6.42b	79.8 ± 1.41b	130.3 ± 2.08b	101.4 ± 1.41b

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

increase corresponded to a statistically significant increase in Na and a decrease in K. Similar behavior was registered in the roots.

During the second year, a significative increase in Na was registered starting from 75 mM NaCl in all the plant components while no statistical differences were observed between 75 and 150 (Table 4). In the roots, a significative K reduction was observed starting from 75 mM NaCl, while in leaves and shoots, significative K reduction was observed at 150 mM NaCl.

3.3 Carbohydrates

In both years, a carbohydrate reduction was observed at NaCl concentration increasing in all plant parts (Tables 5 and 6). In the first year, significant carbohydrate reductions were registered in roots and shoots with a reduction of more than 50% of their content in the roots between 0 and 200 mM NaCl (Table 5).

In the second year, significant carbohydrate reduction was registered in leaves and shoots between 0 and 75 mM NaCl with the reductions of more than 70% in all plant parts (Table 6).

3.4 Gas exchange

In both years of observation, plants' assimilation rate and stomatal conductance were influenced by NaCl concentration variations.

In the first year, a slight photosynthesis increase was observed at 50 mM NaCl and a significant decrease at 200 mM NaCl with a reduction of around 80% of photosynthetic activity (Figure 1a). A similar trend was observed for conductance where significant reductions were observed starting from 100 mM NaCl and a reduction of around 50% at 200 mM NaCl (Figure 1c).

In the second year, significant reductions of photosynthesis were observed at every NaCl concentration increase with a 57% reduction at 150 mM NaCl (Figure 1b). The same behavior was observed for conductance with significant variation among the treatments (−63% at 150 mM NaCl) (Figure 1d). In both years of observation, plants' gas exchanges were influenced by NaCl concentration variations.

Table 5: First-year carbohydrate (Carb.) content (g/100 g DW) of *Q. ilex* plant components irrigated with water at different NaCl concentrations

	Leaf	Shoot	Root
NaCl (mM)	Carb. g/100 g DW	Carb. g/100 g DW	Carb. g/100 g DW
0	7.45 ± 0.67a	3.52 ± 0.12a	7.20 ± 0.14a
50	6.86 ± 0.17ab	3.20 ± 0.04b	6.21 ± 0.13b
100	5.43 ± 0.11bc	2.60 ± 0.15c	3.66 ± 0.09c
200	4.43 ± 0.07c	2.52 ± 0.04c	3.36 ± 0.06c

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

Table 6: Second-year carbohydrate (Carb.) content (g/100 g DW) of *Q. ilex* plant components irrigated with water at different NaCl concentrations

	Leaf	Shoot	Root
NaCl (mM)	Carb. g/100 g DW	Carb. g/100 g DW	Carb. g/100 g DW
0	7.37 ± 0.38a	2.14 ± 0.09a	2.88 ± 0.07a
75	1.64 ± 0.11b	1.03 ± 0.04b	1.24 ± 0.10b
150	1.46 ± 0.04b	0.70 ± 0.06b	0.37 ± 0.02c

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

3.5 Antioxidant, chlorophyll, and carotenoids

A progressive increase in TAA and GAE was observed as consequence of NaCl increasing with significant differences between 0 and 150 mM NaCl.

A significant reduction in Chla and Chlb was observed at 75 mM NaCl while no statistical differences were observed between 75 and 150 mM NaCl (Table 7). A slight, but not significant, reduction of carotenoids was observed at increasing NaCl concentration.

4 Discussion

It is well known that salinity, for many species, may have detrimental effects on plant growth. Salinity-induced modulations in growth, photosynthetic pigments, photosynthesis, ion concentration, and changes in the activity of various antioxidative enzymes.

In non-halophytic species, increasing soil salinity induces different responses in growth plant parameters, with symptoms such as a decrease in root and/or stem development or in leaf area damages while high concentrations lead to the death of the plant. Deciduous species tend to be more tolerant to salt spray or to soil-borne salt than evergreen coniferous

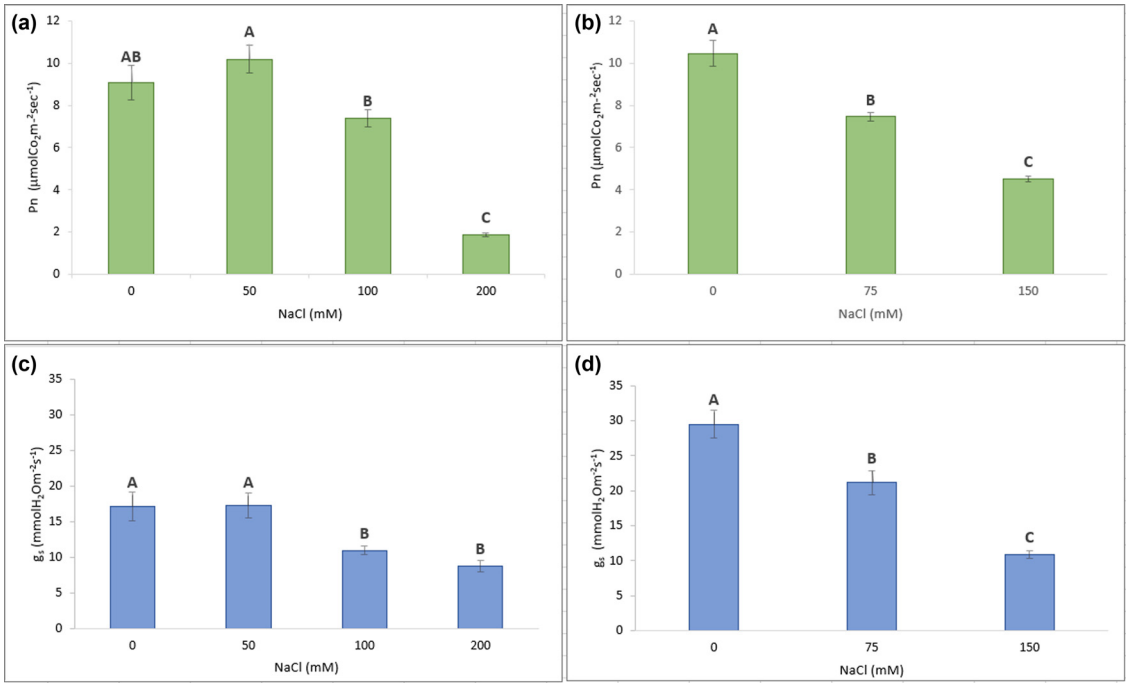

Figure 1: Net photosynthetic rate (P_n) (a and b) and leaf stomatal conductance (g_s) (c and d) measured during the first (a and c) and second (b and d) year of observation. Within each plot, different letters indicate significantly different means by Tukey's multiple range test. First year air temperature 27°C, PAR 1,300 µmol m^{-2} s^{-1} VpdL 3.5; second year air temperature 32°C, PAR 1,350 µmol m^{-2} s^{-1} VpdL 4.3.

species. In some evergreen species, the symptoms of salt damage manifested as chlorosis [34] while in deciduous trees, they manifested as foliage discoloration [35].

No previous reports are available in the literature regarding the effects of high salt concentration on *Q. ilex*. In our experiment, NaCl concentration of 200 mM was the highest concentration which allowed plant survival with no partial desiccation or leaf damage, while at 300 mM leaf damage appeared, plants did not grow and died within 3 weeks from the beginning of the experiment.

4.1 Dry matter accumulation

At 50 mM NaCl, a slight increase in roots' DW was registered in our experiment. Alaoui-Sosse et al. [36] evidenced that an addition of 40 mM NaCl to the nutrient solution in oak seedlings reduced the length and weight of roots and first-flush stems. The number and the area of the leaves of each flush were not significantly affected regardless of the applied treatment. In contrast, the second vegetative flush was properly expanded even with a concentration of 40 mM of NaCl in the culture medium. Similar responses were observed on species of other botanical families, such as Mirtacae – *Eucaliptus camaldulensis* [37], Poaceae – *Phragmites karka* [38], and Zygophyllaceae – *Zygophyllum xanthoxylum* [39], where a lower salt concentration stimulated growth and dry matter accumulation.

Plant growth in terms of biomass decreased at NaCl concentration increases, as observed in other oak species, even at lower salt concentrations than those we used [32], and for many species like *Phyllirea angustifolia* [11] and

Table 7: Second-year TAA, GAE, and chlorophyll (Chl) levels of *Q. ilex* plant components irrigated with water at different NaCl concentrations

NaCl (mM)	TAA g Trolox/100 g DW	GAE mg/100 g DW	Chl a g/100 gDW	Chl b g/Kg DW	Chl a/Chl b	Carotenoids g/100 g
0	1.58 ± 0.52a	230.4 ± 62.8a	3.56 ± 0.13a	4.51 ± 0.99a	0.81 ± 0.16a	1.37 ± 0.29a
75	2.80 ± 0.59ab	395.7 ± 74.1ab	2.50 ± 0.39b	2.84 ± 0.65b	0.86 ± 0.10a	1.04 ± 0.18a
150	3.87 ± 0.29b	484.6 ± 50.8b	2.54 ± 0.29b	2.77 ± 0.41b	0.92 ± 0.15a	0.89 ± 0.16a

Data are represented as mean ± standard deviation. Within each column, different letters indicate significantly different means by Tukey's multiple range test.

halophyte *Salvadora persica* [40]. This result is in contrast with Gudi et al. [24], which registered no biomass loss. This is probably due to the shorter time the plants have been subjected to salt stress.

4.2 Gas exchange

Salinity reduced *Q. ilex* photosynthetic activity. This is probably due to a reduction of stomatal conductance and chlorophyll content of the leaf. A reduction in the photosynthetic activity was also registered by Guidi et al., who attribute it to stomatal closure which determines a strong dissipation of energy via the xanthophyll cycle [24]. Fusaro [23] evidenced a reduction in transpiration rates, as a strategy to limit Na absorption, which is a behavior put in practice by *Q. ilex* to resist to abiotic stress. This behavior was highlighted by Sultana [41]: reduction in photosynthesis in salinized rice plants is a consequence of reduction of available CO_2 by stomatal closure, but also of the cumulative effects of leaf water and osmotic potential, stomatal conductance, transpiration rate, relative leaf water content, and biochemical constituents such as photosynthetic pigments, soluble carbohydrates, and proteins.

4.3 Antioxidant, chlorophyll, and carotenoids distribution

The increase of TAA observed in the leaves at 75 and 150 mM NaCl indicates that *Q. ilex* plants act to protect themselves from salt stress. Similar behavior was observed in *Physalis peruviana* L. [42] where also an increase in free radical scavenging activity was seen. Similarly to TTA, also GA values increased in leaves at salt concentration increasing. The increase of phenolic content under salt stress was observed also in *Mentha pulegium* [43].

Our results showed a linear reduction in both chlorophyll a and b content due to the salinity increase, with a subsequent photochemical efficiency reduction and a slight increase in their rate (Chla/Chlb). Total chlorophyll content reduction was observed in *Q. ilex* at 75 mM NaCl by Fusaro et al. [23] while no reduction was observed by Guidi et al. [24]. Chlorophyll content reduction was also registered in *Eucaliptus camaldulensis* [37] and *Bruguiera parviflora* [44]. An increase in the chlorophyll a/b ratio is commonly seen as an indicator of an enhancement in the plant photochemical capacity [45]. Salt concentration induces a progressive reduction of carotenoids according to Fusaro et al. and Guidi et al. [23,24] who observed a decrease of β-caroten in 75 and 150 mM NaCl *Q. ilex*-stressed plants. The same behavior was observed in *Populus euphratica* [39].

4.4 Carbohydrates

The observed reduction in carbohydrate concentration in the different plant components is probably due to the progressive decrease in photosynthetic activities as a consequence of the NaCl content increase. A decrease in the carbohydrate content was observed by Sultana et al. [41] in rice.

4.5 Ion levels

In our experiment, sodium was accumulated in all plant parts and this accumulation occurred at the expense of potassium uptake. The increase of sodium was confirmed by the results obtained by Alaoui-Sosse et al. [36] in oaks when 40 mM NaCl was applied. Roots accumulate a major part of this element, as we observed in both years. In several *Eucalyptus* species, when plants were exposed to high salt stress, roots accumulated more sodium than leaves [46,47]. An opposite behavior was observed for the potassium content of the plants of our study. In fact, under a high concentration of salt, sodium was exchanged for potassium [48] and its accumulation was reduced in roots. Moreover, sodium could act as a substitute for potassium during the uptake but not for the important role it plays in different biochemical processes. Sodium replaces potassium and other cations on the soil exchange complex and can lead to nutrient deficiencies.

5 Conclusions

Our results confirm analytically what is observed in nature: *Q. ilex* natural forests extend until the sea in the species' natural habitat found in many Mediterranean areas. Here, the study evidenced how, until 200 mM NaCl the plant kept growing and did not show any visible damage on leaves. Only at 300 mM NaCl, the *Q. ilex* plants died. The study confirmed that oaks use several mechanisms, well-known in other species' physiology, to resist salt stress. As expected, at increasing NaCl concentrations, plant biomass decreased, with a reduction of leaf area and leaf number but without

partial desiccation or leaf damage. The Na ion content increased whereas the K ion content decreased in all plant parts as the excess of salt led to disturbance in ionic balance. As expected, photosynthetic activity and stomatal conductance decreased with the highest salt concentration influencing the carbohydrate content. In addition, the photosynthetic pigments decreased with an increase in salinity. TAA and GAE increase with salt concentration as an antioxidant response through preventing the oxidative damage.

Hence, based on the adaptive capacity of the plant confirmed by our results, the use of *Q. ilex* in urban areas represents a good strategy for improving vegetation presence in the cities. However, more studies should be conducted to understand the mechanism that *Q. ilex* plants use to resist high salt concentrations.

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Conflict of interest: Vittorio Farina, who is the co-author of this article, is a current Editorial Board member of Open Agriculture. This fact did not affect the peer-review process.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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