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ORIGINAL ARTICLE

Physiological, biochemical, and antioxidant responses of oregano subspecies (*Origanum vulgare* L. ssp. *gracile* and ssp. *vulgare*) to NaCl stress

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Abstract

Salinity is an outstanding barrier against the production of agricultural crops, especially in arid and semi-arid regions. Oregano (*Origanum vulgare* L.), a valuable herb of the Lamiaceae family, contains various types of biologically active constituents such as essential oils, tannins, resins, sterols, flavonoids, and phenolic glycosides. The present research was carried out to investigate the influence of salinity stress on some physiological and biochemical attributes and antioxidant responses in two oregano subspecies (ssp. *vulgare* and ssp. *gracile*). Salt treatments were applied using irrigation with different sodium chloride concentrations (0, 25, 50, and 100 mM NaCl). The results revealed a remarkable decline in relative water content (RWC) and photosynthetic pigments in both subspecies under NaCl stress. Total soluble sugars (TSS) decreased in plants exposed to severe salt stress (100 mM NaCl), whereas H_2O_2 production, electrolyte leakage (EL), malondialdehyde (MDA), and leaf proline contents increased in these plants compared to control plants. A positive relationship was found between H_2O_2 production with EL and MDA. Furthermore, salinity improved phenolic content, antioxidant capacity, and activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes. The highest total flavonoid content (TFC) was achieved at 50 mM NaCl salinity, which increased by 19.33% compared to control plants. A positive relationship between the activity of phenylalanine ammonia-lyase (PAL) and TPC and TFC was observed. Analysis of phenolic compounds by HPLC showed that the amounts of gallic acid, caffeic acid, chlorogenic acid, and quercetin in ssp. *gracile* and caffeic acid, cinnamic acid, and quercetin in ssp. *vulgare* significantly increased with increasing salinity stress. In general, the findings of this study demonstrated that oregano subspecies ameliorate salt-induced osmotic and oxidative damages through increasing proline accumulation, antioxidant enzymes activity, and secondary metabolites production.

Keywords Antioxidant enzymes activity · *Origanum vulgare* · Osmolytes · Phenolic compounds · Phenylalanine ammonia-lyase · Salinity stress

Introduction

Oregano (*Origanum* sp.) is an aromatic and perennial herb belongs to the mint family (Lamiaceae) (Giuliani et al. 2013; Moradi et al. 2021). *Origanum vulgare* L., a valuable oregano species, have received considerable attention due to a high diversity in morphology, trichomes, chemotype, and chemical constituents of the essential oil (Verma et al. 2010; Giuliani et al. 2013; Lukas et al. 2015). Out of six *O. vulgare* subspecies, ssp. *vulgare*, ssp. *gracile,* and ssp. *virens* have been reported in Iran (Rechinger 1982). Several chemical constituents, namely, flavonoids, phenolic glycosides, sterols, tannins, and essential oil, have been isolated from the oregano (Liu et al. 2012; Lukas et al. 2015). Phenolic compounds, an extensive group of secondary metabolites with

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antioxidant activity, are plentiful in the mint family (Ben Taârit et al. 2012; Leyva-Lopez et al. 2017). Anti-cancer, antibacterial, cardioprotective, anti-inflammatory, immune-boosting, and UV-protective are among the pharmacological properties of these compounds (Meng et al. 2018). Rosmarinic, caffeic, vanillic, *p*-coumaric, protocatechuic acids, luteolin, and apigenin are the most important phenolic acids (Lukas et al. 2013) and flavonoids (Gutierrez-Grijalva et al. 2018) identified in oregano aerial parts. Recent pharmacological investigations have demonstrated that various oregano species have antimicrobial, antifungal, antiviral, and antioxidant activities due to their phenolic compounds. Furthermore, these species have commonly used for medicinal purposes such as carminative, diaphoretic, expectorant, sedative, stimulant, stomachic, diuretic, antineuralgic, antitussive, and antirheumatic (Kintzios 2002; Chishti et al. 2013; Leyva-Lopez et al. 2017).

Plants are often exposed to various environmental stresses that dramatically reduce their growth and production. Salinity, the main plant growth limitation, can seriously restrict agricultural production, particularly in arid and semi-arid areas (Acosta-Motos et al. 2017). High salinity often causes osmotic stress and ionic toxicity (Na^+ and Cl^-) or ion imbalance, which leads to water use deficiency, nutrient deficiencies, and eventually oxidative stress in plants (Parida and Das 2005; Shabala 2009; Demidchik 2015). Plants achieve osmotic regulation to overcome physiological drought induced by salinity and postponing cellular dehydration through the accumulation of low molecular weight and hydrophilic compounds such as proline, glycine betaine, and sugars (Kerepesi and Galiba 2000; Ashraf and Foolad 2007; Chen and Jiang 2010). The accumulation of compatible solutes reduces the cytoplasmic osmotic potential, so the main functions of these osmolytes are to protect cell structure and maintain osmotic balance through maintaining an influx of water (Chen and Jiang 2010; Puniran-Hartley et al. 2014).

Salinity-induced overproduction of reactive oxygen species (ROS) can cause oxidative stress which hamper plant growth through membrane lipid peroxidation, protein degradation, inactivation of enzymes, chlorophyll degradation, and suppression of photosynthesis (Verma and Mishra 2005; Ashraf 2009; Gill and Tuteja 2010; Demidchik 2015). The main product of membrane lipid peroxidation in plants exposed to salinity is malondialdehyde (MDA) which is considered as an indicator for assessing cell membrane damage (Liang et al. 2018). System survival under stress conditions depends on the equilibrium between production and elimination of ROS by different antioxidants. To minimize the destructive effects of ROS, plants have developed non-enzymatic (tocopherol, ascorbic acid, carotenoids, and phenolic compounds) and enzymatic (superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)) antioxidant protective systems (Baltruschat et al. 2008; Gengmao et al. 2015; Huang et al. 2019). Phenolic compounds (flavonoids and phenolic acids) play an essential role in ROS scavenging (Ksouri et al. 2007). The augmentation of phenolic compounds synthesis in retort to salt stress was observed in *Salvia mirzayanii* (Valifard et al. 2014), *Salvia miltiorrhiza* L. (Gengmao et al. 2014), *Thymus vulgaris* L. and *Thymus daenensis* Celak (Emami Bistgani et al. 2019), *Origanum onites* L. (Hancioglu et al. 2019), and *Amaranthus tricolor* (Sarker and Oba 2020).

The effect of salinity stress on growth and phytochemical aspects of different *Origanum* species has been previously studied, but details regarding the physiological responses and salinity tolerance mechanisms have not been investigated in this species. Baatour et al. (2010) investigated the effect of different NaCl concentrations on *O. majorana* and found that *O. majorana* could tolerate a moderate NaCl concentration of 50 mmol L^{-1}. In another study, evaluation of salt tolerance mechanisms in basil, thyme, sage, and oregano plants showed that oregano is less tolerant to salinity than thyme, basil, and sage due to a significant decrease in chlorophyll content, a significant increase in malondialdehyde production, and a large flow of Na into the shoots (Tanaka et al. 2018). Hancioglu et al. (2019) reported that increasing irrigation water salinity in *O. onites* enhances total phenolic and flavonoid contents, whereas a significant decrease was observed in total fresh and dry yield and total oil content. Based on the results of their study, *O. onites* was a very sensitive plant to salinity.

Due to the importance of soil and water salinization in Iran, shifting from current commercial crops to some medicinal plants, which are often more adaptable to adverse environmental conditions, is recommended. Nowadays, the cultivation of medicinal and aromatic plants has received much attention due to their high antioxidant and antimicrobial properties. Oregano has diverse applications in pharmaceutical and food industries, and therefore, its cultivated areas are constantly increasing in Iran. However, no comprehensive studies have been conducted on the cultivation of these valuable medicinal subspecies under adverse conditions such as salinity stress. Since the ability of medicinal plants to synthesize secondary metabolites may be affected by various environmental stresses, in this study the physiological, biochemical, and antioxidant responses of two *O. vulgare* L. subspecies (ssp. *gracile*, and ssp. *vulgare*) to different concentrations of NaCl in irrigation water were investigated. The possibility of improving the production of their biologically active compounds under salinity stress was also studied.

Materials and methods

Plant materials and growing conditions

The present study was carried out at the research greenhouse of Urmia University, West Azerbaijan province, Iran, as a

2×4 factorial experiment in a completely randomized design with three replications during 2019–2020. Experimental factors included two oregano subspecies (ssp. *vulgare* and ssp. *gracile*) and salinity stress imposed by sodium chloride at four levels (0 (control), 25, 50, and 100 mM). Seeds of the subspecies were obtained from the Department of Horticultural Science, Urmia University. The seeds were sown in plastic pots (25 cm diameter and 30 cm height) containing a mixture of soil and sand (3:2). Some of the soil characteristics used were as follows: soil texture (sandy loam), pH (8.02), EC (1.27 ds m^{-1}), organic material (0.62%), total nitrogen (0.12%), available P (9.45 ppm), and exchangeable K (0.46 meq/100g soil). After seed germination, the seedlings were thinned over several stages and finally kept 7 plants in each pot. The greenhouse temperature was in the range of 20 ± 2 to 28 ± 2°C with 50–60% relative humidity and natural sunlight.

Salt treatment

The plants were exposed to different salt stress levels (0, 25, 50, and 100 mM NaCl) one month after planting (eight-leaf stage). Salinity treatments were imposed through saline irrigation. To avoid sudden shock from salinity stress, salinity treatments gradually reached the final concentration during the three irrigation stages. Salinity treatments were continued until the flowering stage and leaves were harvested at 45 days after the onset of stress treatments, which is coincided with the flowering of about 80% of the plants.

Relative water content (RWC)

To determine leaf RWC, ten disks (8 mm in diameter) were prepared from the fully developed leaves. Immediately, their fresh weight (FW) was measured and incubated in a petri dish containing distilled water for 4 h in a refrigerator (4°C) in the dark. After removing the disks from distilled water, their turgid weight (TW) was determined and then transferred to an oven (70°C) for 48 h, and their dry weight (DW) was measured. Finally, the following formula was utilized to estimate RWC (Turner 1981).

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

Measurement of photosynthetic pigments

To measure chlorophyll *a*, *b*, and total chlorophyll and carotenoids, 0.1 g of fresh leaves (fully developed and healthy leaves) was homogenized with 5 mL of acetone (80% V/V). The extract was centrifuged at 2500 rpm for 10 min. The absorbance of samples was recorded by spectrophotometer (Dynamica Halo DB-20 model) at wavelengths of 662 nm, 645 nm, and 470 nm. The quantities of chlorophyll and carotenoid were reported in mg g^{-1} FW (Lichtenthaler 1987).

Hydrogen peroxide (H_2O_2) production

The determination of H_2O_2 content in oregano leaves was accomplished by the protocol of Velikova et al. (2000). The leaf samples (100 mg) were extracted by 5 mL of trichloroacetic acid (TCA) (0.1% w/v) in an ice bath. The extract was centrifuged at 12,000*g* for 15 min, and 500 µL of the supernatant was mixed with 500 µL of potassium phosphate buffer (10 mM, pH = 7.0) and 1 mL of potassium iodide (1 mM). The absorbance of samples was read at 390 nm. The H_2O_2 content was measured according to the standard calibration curve and stated as µmol g^{-1} FW.

Electrolyte leakage

Electrolyte leakage (EL) as an index for membrane stability in oregano leaf tissues was measured using the protocol described by Lutts et al. (1996). Leaf disks (8 mm in diameter) were prepared from the developed leaves and shaken in 10 mL of distilled water at 25°C for 24 h. The electrical conductivity of the solution (EC_1) was measured at the end of the incubation period. Then the same samples were placed in an autoclave (120°C for 20 min), and after cooling, electrical conductivity of solutions (EC_2) was recorded again. Finally, EL (%) was determined using the following formula:

$$\mathrm{EL}(\%) = \left(\mathrm{EC}_1/\mathrm{EC}_2\right) \times 100.$$

Lipid peroxidation

The method defined by Heath and Packer (1968) was used to measure the malondialdehyde (MDA) content. Accordingly, 0.1 g of fresh leaf sample was subjected to extraction by 2 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 min. The extract (500 mL) was combined with 2 mL of 20% TCA solution comprising 0.5% thiobarbituric acid. The samples were placed in a warm water bath (95°C) for 30 min and instantly cooled in ice water. The absorbance of solution was read using a spectrophotometer at 532 nm. The absorbable material at this wavelength is a red compound. The absorbance of the other non-specific (MDA-TBA) dyes was determined at 600 nm and subtracted from this value, and then MDA concentration was reported as µmol g^{-1} FW.

Assessment of proline and total soluble sugars (TSS)

To prepare extract, 500 mg of fresh leaf sample was crushed in 5 mL of 95% ethanol. The upper phase was separated

and the sediments were washed using 5 mL of 70% ethanol. Then, the resulting homogenate was centrifuged at 3500 rpm for 10 min. The obtained ethanolic extract was used to determine the proline and soluble sugars (Irigoyen et al. 1992).

The amount of free proline content was estimated according to the protocol defined by Paquin and Lechasseur (1979). For this purpose, 10 mL of distilled water, 5 mL of ninhydrin reagent, and 5 mL of glacial acetic acid were mixed to 1 mL of the ethanolic extract. The resulting solution was incubated in a warm water bath (100°C) for 45 min. After cooling, 10 mL of benzene was added to each sample and shaken powerfully. The absorbance of standard solutions and samples was estimated using a spectrophotometer at 515 nm and free proline content was reported as µmol g^{-1} FW.

To evaluate TSS content, the method proposed by Irigoyen et al. (1992) was used. Briefly, 0.1 mL of ethanolic extract was mixed with 3 mL of anthrone reagent and samples were kept at 100°C for 10 min. After cooling, their absorbance was read at 625 nm. The amount of TSS was calculated by the glucose standard curve and stated as mg g^{-1} FW.

Measurement of antioxidant enzymes activity

For this purpose, 500 mg of fresh leaf sample was extracted in 2 mL extraction buffer consisting of 0.5% HCl-Tris and 0.05% polyvinyl pyrrolidone (PVP), pH 8.0. Then, centrifugation was performed at 13,000 rpm for 15 min at 4°C, and the upper phase was separated to evaluate the antioxidant enzymes activity (Sudhakar et al. 2001).

Superoxide dismutase (SOD) assay

The measurement of SOD activity (EC 1.15.1.1) was performed by evaluating the ability of plant extract to suppress nitro blue tetrazolium chloride (NBT) reduction (Beauchamp and Fridovich 1971). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH = 7), 13 mM methionine, 75 µM NBT, 0.1 M EDTA, 2 µM riboflavin, and 50 µL enzyme extract. The tubes were placed under a fluorescent lamp for 10 min at a distance of 20 cm and the reaction was stopped by turning off the lamp. One unit of SOD was defined as the quantity of enzyme that inhibited the NBT reduction by 50% at 560 nm and SOD activity was stated as enzyme unit per g fresh weight (U g^{-1} FW).

Ascorbate peroxidase (APX) assay

Ascorbate peroxidase (EC 1.11.1.11) activity was measured by Nakano and Asada (1987) method with some alterations. The reaction mixture consisted of 15 µL of the enzymatic extract, 2 mL of phosphate buffer (50 mM, pH = 7), 20 µL of H_2O_2 solution (5 mM), and 10 µL of ascorbic acid (50 µM). The absorbance of prepared extract was read for 1 min at λmax = 290 nm using a spectrophotometer and the APX activity was stated as enzyme unit per g fresh weight (U g^{-1} FW).

Catalase (CAT) assay

The Aebi (1984) method was used to measure CAT (EC 1.11.1.6) activity. The reaction mixture consisted of 2.5 mL of phosphate buffer (50 mM, pH = 7), 20 µL H_2O_2 solution (3%), and 5 µL of extract. The CAT activity was recorded for 1 min at λmax = 240 nm using a spectrophotometer and reported as one unit of enzyme/ min/g FW (U min^{-1} g^{-1} FW).

Measurement of PAL enzyme activity

The enzymatic extract was prepared using 2 mL extraction buffer (50 mM Tris–HCl with pH, 8.5) containing 15 mM beta-mercaptoethanol and 100 mg of plant tissue. Then, the extracts were centrifuged at 4000 rpm for 10 min and the upper phase was used to measure PAL activity. To estimate the enzyme activity, the reaction mixture consisted of 500 µM of Tris–HCl (pH = 8), 6 µmol phenylalanine, and 20 µL of enzyme extract. The samples were placed at 40°C for 60 min. The reaction was stopped by the addition of 50 µL 5N HCl. The activity of the enzyme at 290 nm was reported according to the rate of cinnamic acid production in µmol g^{-1} FW (Beaudoin-Eagan et al. 1985).

Total polyphenol, total flavonoid content, and antioxidant activity

Five hundred mg of dried leaf samples was extracted with 20 mL of 80% methanol. The homogenates were placed in an ultrasonic bath (Elmasonic S30 H model) at 37°C for 30 min and then were filtered through Whatman filter paper. The plant extracts were used to determine the amount of total phenol, total flavonoid, and antioxidant activity (Weremczuk-Jeżyna et al. 2013).

Total phenolic content (TPC)

Total phenolic content was evaluated based on the protocol proposed by Singleton et al. (1999). In summary, 30 µL of methanolic extract was mixed with 180 µL of distilled water and 1.2 mL of 10% Folin-Ciocalteu reagents. After 10 min, sodium carbonate (Na_2CO_3, 7%) solution was added. The tubes were shaken and placed in the dark for 30 min and TPC was measured spectrophotometrically at 760 nm. Gallic acid (GAE) was utilized to prepare calibration curve and TPC of the extracts was stated as mg GAE g^{-1} DW.

Total flavonoids content (TFC)

To measure total flavonoid content, 40 µL of methanolic extract was added to the reaction mixture, including 150 µL of 5% sodium nitrite, 300 µL of aluminum chloride solution ($AlCl_3$) (10% w/v), and 1 mL of 1M potassium acetate. Then their absorption was read using a spectrophotometer at 380 nm. Quercetin (QE) was utilized to draw the standard curve and TFC was reported as mg QE g^{-1} DW (Chang et al. 2002).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The method of Hatano et al. (1988) was utilized to determine the antioxidant activity of methanolic leaf extract. Briefly, 2 mL of DPPH solution was mixed by different concentrations of plant extracts (5, 10, 15, and 20 µg mL^{-1}) and its absorption was read after 30 min using the spectrophotometer at 518 nm. In this method, the positive control was DPPH solution plus different concentrations of ascorbic acid (Vit. C). DPPH solution plus methanol were used as a negative control. The percentage of DPPH radical scavenging activity was estimated using the following equation:

$$\text{DPPH inhibition}(\%) = \left[\left(A_0 - A_s\right)/A_0\right] \times 100.$$

Here, A_0 was control adsorption (containing all reaction elements without sample) and A_s was sample absorbance. Then results were reported as IC_{50} (IC_{50} is a concentration (mg mL^{-1}) of antioxidant needed for inhibition of 50% of DPPH° free radicals). The lowest IC_{50} value is correlated with higher antioxidant activity of the plant extracts (Patro et al. 2005).

Analysis of phenolic compounds by HPLC

To quantify phenolic compounds, dried powder samples (500 mg) were homogenized by adding 5 mL of methanol solvent containing 1% acetic acid. The extraction process was carried out under ultrasonic waves for 20 min at 25°C. The resulted methanolic extract was centrifuged for 10 min at 2000 rpm and 4°C. The isolation, identification, and quantification of polyphenolic compounds was conducted using an Agilent Technologies 1100 series HPLC (Agilent Technologies, Wilmington, DE, USA), equipped with a 20 µL manual sample loop, degasser, quaternary pump, column oven, and diode array detector. Separation was performed using a ZORBAX Eclipse XDB column (4.6 mm × 250 mm, 5 µm particle size, Dr. Mainsch, Germany) at 25°C. The plant extracts were filtered (syringe filter, 0.22 µm) and then 20 µL was injected to the HPLC system. Acquisition and integration of obtained data were performed using Agilent ChemStation software. Separation and identification of phenolic compounds were performed according to the method described by Seal (2016). A gradient elution program was used for the separation of phenolic acids, by varying the proportion of acetonitrile (solvent A) to acetic acid (1.0% V/V in water) (solvent B). The initial mobile phase composition was changed from 10 to 25% A in a linear fashion for the duration of 5 min, from 25 to 65% A in 10 min, and remained in this condition for 5 min. The mobile phase composition back to the initial condition (solvent A/solvent B: 10:90) in 5 min and allowed to run for another 5 min, before the injection of another sample. The HPLC chromatograms were detected using a photodiode array detector at different wavelengths (250 nm for quercetin and chlorogenic acid, 272 nm for gallic acid, cinnamic acid, and apigenin, and 310 nm for caffeic acid, rutin, and *p*-coumaric acid). Identification of the phenolic components was made by comparison of its retention time and spectra with standard compounds under the same conditions. The quantification of each phenolic compound was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area of the compound against the concentration of the respective standard sample. Total analysis time per sample was 30 min.

Statistical analysis

Analysis of variance (ANOVA) of the data was carried out using SAS software version 9.2 and Duncan's multiple range test was used to compare treatment means ($p = 0.05$). The relationship between the measured parameters was estimated using the Pearson's correlation coefficient by R software. In addition, heat map was created using CIMMiner software.

Results

Relative water content (RWC)

Leaf RWC was significantly affected by salinity and subspecies. Relative water content decreased by increasing NaCl concentration from 0 to 100 mM. The highest (78.48%) and lowest (63.53%) amounts of RWC were observed in control and 100 mM of NaCl, respectively. On the other hand, ssp. *gracile* had higher RWC than ssp. *vulgare* (Table 1). A positive relationship was found between RWC and chlorophyll (a, b and total), carotenoid, TSS, and antioxidant activity (IC_{50}). In addition, RWC showed a negative correlation with EL, MDA, proline and H_2O_2 production, activity of SOD, CAT, and PAL enzymes, TPC, and TFC (Fig. 6).

Photosynthetic pigments

Chlorophyll content (Chl *a*, *b*, and total) was significantly affected by salinity, subspecies, and their interactions. The results revealed *a decline in* chlorophyll content by increasing salinity intensity (Fig. 1a–c). The highest salinity level reduced the total chlorophyll content by 27% and 48% in ssp. *vulgare* and ssp. *gracile* compared to the controls, respectively. Thus, the reduction of photosynthetic pigments in ssp. *gracile* was more than those of ssp. *vulgare*. The carotenoid content of oregano leaves was significantly decreased when salt stress intensified. A negative relationship between total chlorophyll and other parameters (except carotenoid, RWC, IC_{50}, and TSS) was also detected (Fig. 6).

H_2O_2 production, lipid peroxidation, and electrolyte leakage (EL)

The results showed that the effects of salinity treatments, subspecies, and their interaction were significant on H_2O_2 production, lipid peroxidation, and electrolyte leakage. In the present study, H_2O_2 production, EL (as an index for membrane stability), and MDA contents (as an index for lipid peroxidation) increased in both oregano subspecies in response to increasing NaCl concentration (Fig. 2a–c). The correlation coefficient analysis showed that H_2O_2 production has a positive correlation with MDA and EL. In addition, these parameters had a negative relationship with RWC, chlorophyll content, TSS, and IC_{50} (Fig. 6).

Proline content

The accumulation of proline was significantly affected by salinity treatments, subspecies, and their interaction. In both subspecies, leaf proline content was increased in response to increasing NaCl concentration in irrigation water (Fig. 3a). There was a negative correlation between proline contents, total chlorophyll, and RWC (Fig. 6).

Total soluble sugars (TSS) content

Salinity, subspecies and their interactions significantly affected TSS content. Leaf TSS content enhanced with increasing NaCl concentration (up to 50 mM) and declined at the highest salinity level (100 mM NaCl) in both subspecies (Fig. 3b).

Activity of antioxidant enzymes

Results revealed a significance influence of subspecies, salinity stress and their interactions on the activities of SOD and APX enzymes. An increment in SOD activity was achieved with increasing the intensity of salinity (Fig. 4a). The maximum SOD activity was recorded in 25 mM, followed by 100 mM and 50 mM NaCl salinity. The APX activity enhanced with an increment in salinity stress, and it was remarkably higher in *gracile* subspecies (Fig. 4b). The activity of CAT enzyme was significantly affected by salinity and subspecies (Table 1) (Fig. 5). CAT activity enhanced by increasing NaCl concentration and it was almost twice as much in ssp. *vulgare* as ssp. *gracile*. As shown in Fig. 6, a negative relationship was observed between SOD and CAT activity with RWC. Additionally, SOD and CAT activity had a positive correlation with H_2O_2, MDA, and EL.

Table 1 Effect of salinity stress on some physiological and biochemical characteristics of *O. vulgare* ssp. *vulgare* and ssp. *gracile*

Treatments	RWC (%)	Carotenoid (mg g^{-1} fw)	CAT (U min^{-1} g^{-1} fw)	PAL (µmol cinnamic acid g^{-1} fw)	TFC (mg QE g^{-1} dw)	DPPH scavenging activity IC_{50} (mg ml^{-1})
NaCl concentration						
0 mM	78.48 ± 1.71^{a}	0.35 ± 0.02^{a}	5.50 ± 0.35^{d}	3.63 ± 0.24^{b}	1.81 ± 0.046^{c}	3.04 ± 0.13^{a}
25 mM	74.54 ± 1.71^{a}	0.32 ± 0.01^{ab}	9.16 ± 0.36^{c}	4.83 ± 0.27^{a}	2.06 ± 0.048^{ab}	2.67 ± 0.13^{ab}
50 mM	68.28 ± 1.68^{b}	0.27 ± 0.03^{b}	10.71 ± 0.32^{b}	4.43 ± 0.25^{ab}	2.16 ± 0.040^{a}	2.60 ± 0.03^{b}
100 mM	63.53 ± 1.71^{b}	0.21 ± 0.02^{c}	12.64 ± 0.36^{a}	5.28 ± 0.27^{a}	1.98 ± 0.040^{b}	2.19 ± 0.02^{c}
O. vulgare ssp.						
vulgare	69.11 ± 1.21^{b}	0.25 ± 0.02^{b}	12.59 ± 0.27^{a}	4.60 ± 0.19^{a}	2.03 ± 0.031^{a}	2.54 ± 0.05^{a}
gracile	73.30 ± 1.21^{a}	0.32 ± 0.01^{a}	6.41 ± 0.27^{b}	4.49 ± 0.19^{a}	1.98 ± 0.032^{a}	2.72 ± 0.09^{a}
						3.59 ± 0.03 (Vit. C)

The means in each column followed by similar letter(s) are not significantly different using Duncan's multiple range test ($p < 0.05$). Values are means ± SE ($n = 3$)

RWC Relative water content, *CAT* Catalase, *PAL* Phenylalanine ammonia-lyase, *TFC* Total flavonoid content, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

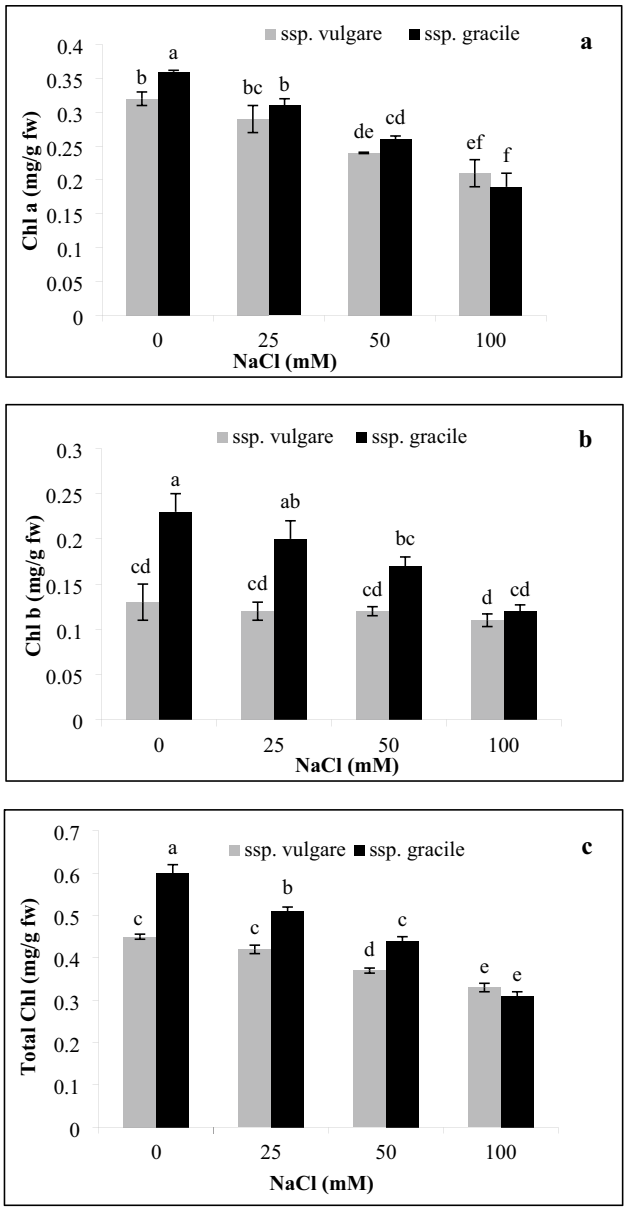

Fig.1 Chlorophyll *a* (**a**), chlorophyll *b* (**b**), and total chlorophyll (**c**) in the leaves of two *Origanum vulgare* subspecies grown under different NaCl concentrations (±SE, $n=3$). Means with different letters are significantly different according to Duncan's multiple range test ($p<0.05$)

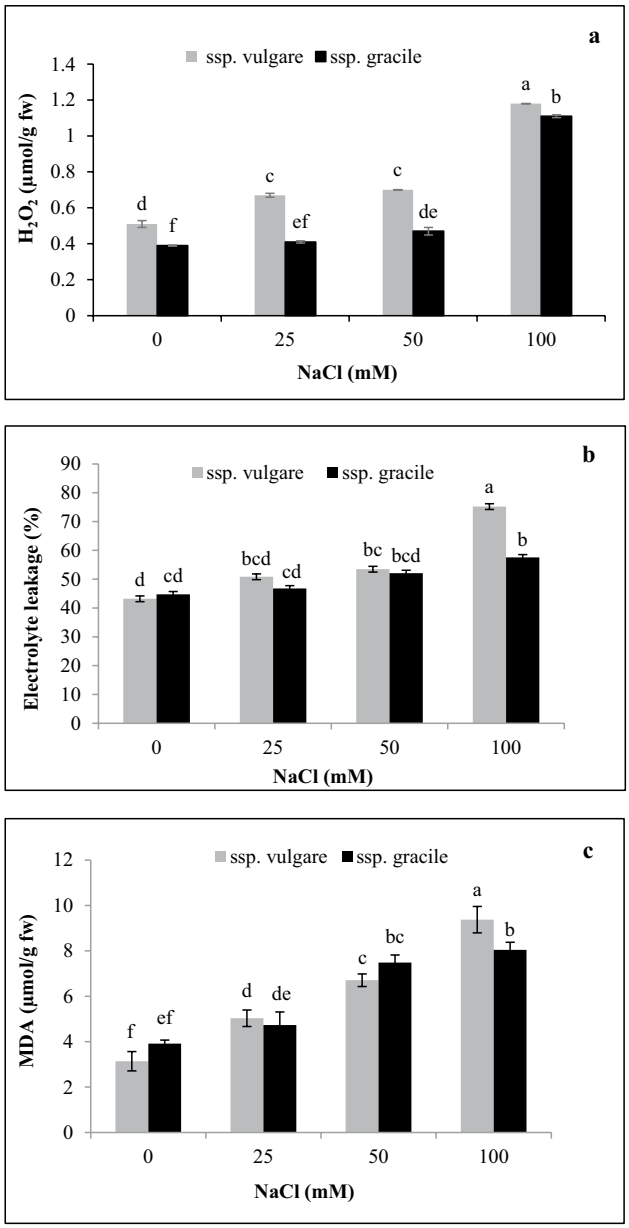

Fig. 2 H_2O_2 production (**a**), electrolyte leakage (**b**), and malondialdehyde contents (**c**) in two *Origanum vulgare* subspecies grown under different NaCl concentrations (±SE, $n=3$). Means with different letters are significantly different according to Duncan's multiple range test ($p<0.05$)

Phenylalanine ammonia-lyase (PAL) enzyme activity

The results indicated that the PAL enzyme activity was significantly affected by salinity treatments. The activity of this enzyme at 25, 50, and 100 mM NaCl salinity increased by 33.05%, 22.04%, and 45.45%, respectively, compared to the control (Table 1). Nevertheless, the difference between 25, 50, and 100 mM NaCl treatments was not significant. Also, PAL enzyme activity in ssp. *vulgare* was slightly higher than ssp. *gracile*. There was a positive relationship between the PAL activity and TPC and TFC (Fig. 6). In addition, a negative relationship between the PAL activity and IC_{50} was observed (Fig. 7).

Total phenolic and flavonoid contents

Salinity, subspecies, and their interactions significantly affected TPC. The results showed that increasing salinity was associated with an enhancement in TPC in both oregano

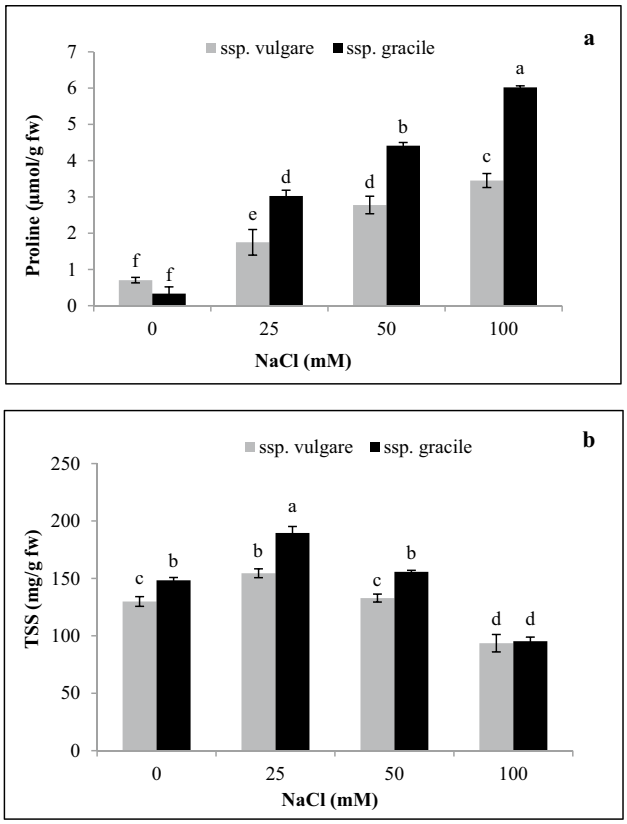

Fig. 3 Proline (**a**) and total soluble sugar (TSS) contents (**b**) in the leaves of two *Origanum vulgare* subspecies grown under different NaCl concentrations (±SE, $n=3$). Means with different letters are significantly different according to Duncan's multiple range test ($p<0.05$)

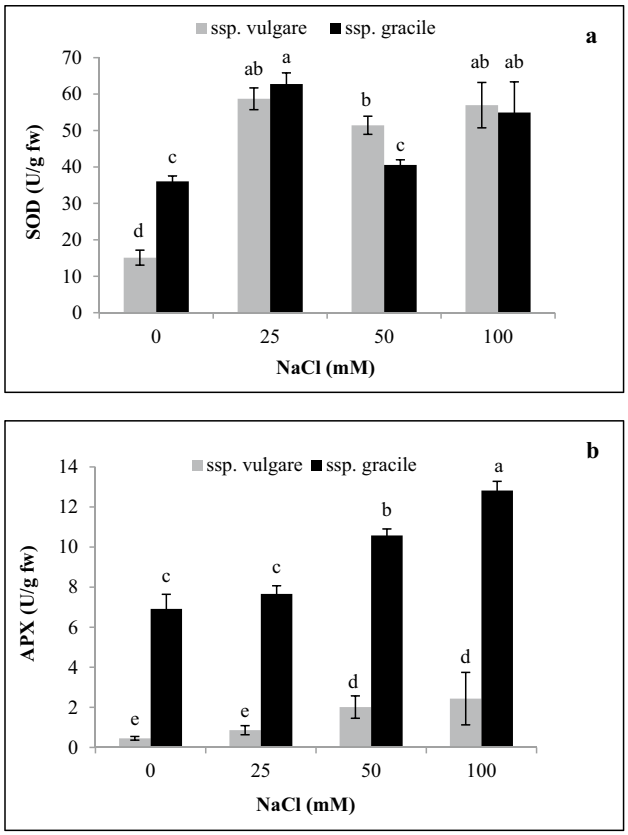

Fig. 4 Superoxide dismutase (SOD) (**a**) and ascorbate peroxidase (APX) (**b**) activity in two *Origanum vulgare* subspecies grown under different NaCl concentrations (±SE, $n=3$). Means with different letters are significantly different according to Duncan's multiple range test ($p<0.05$)

subspecies. However, ssp. *vulgare* had higher TPC in all salinity levels. Also, TFC was significantly affected by salinity treatments. The highest TFC (2.16 mg QE g^{-1} dw) was achieved at 50 mM NaCl that increased by 19.33% compared to the control plants (Table 1). In addition, there was no significant difference between the two subspecies for TFC. As shown in Fig. 6, TPC and TFC displayed a negative correlation with IC_{50}.

HPLC analysis for polyphenolic compounds

The quality and quantity of polyphenolic compounds in both oregano subspecies were determined by HPLC–DAD analysis. HPLC analysis revealed the presence of eight constituents, i.e., five phenolic acids (gallic, caffeic, chlorogenic, cinnamic, and *p*-coumaric acids) and three flavonoids (rutin, quercetin, and apigenin) (Table 2, Fig. 7). The results demonstrated that all eight identified compounds were significantly affected by the interaction of salinity and subspecies. As depicted in Table 2, application of 25 mM NaCl in ssp. *vulgare* reduced the amounts of gallic acid, caffeic acid,

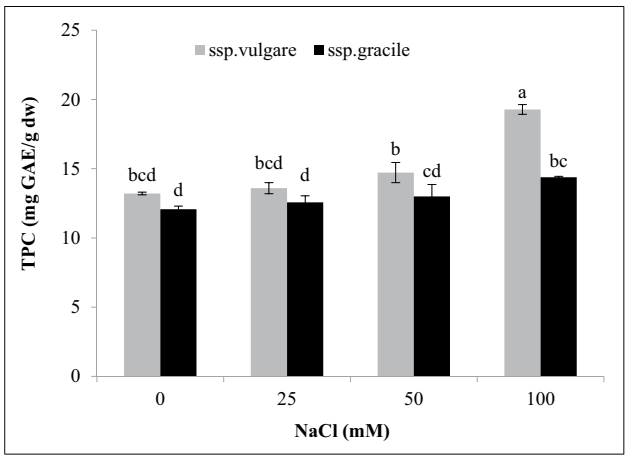

Fig. 5 Total phenol content (TPC) in the leaves of two *Origanum vulgare* subspecies grown under different NaCl concentrations (±SE, $n=3$). Means with different letters are significantly different according to Duncan's multiple range test ($p<0.05$)

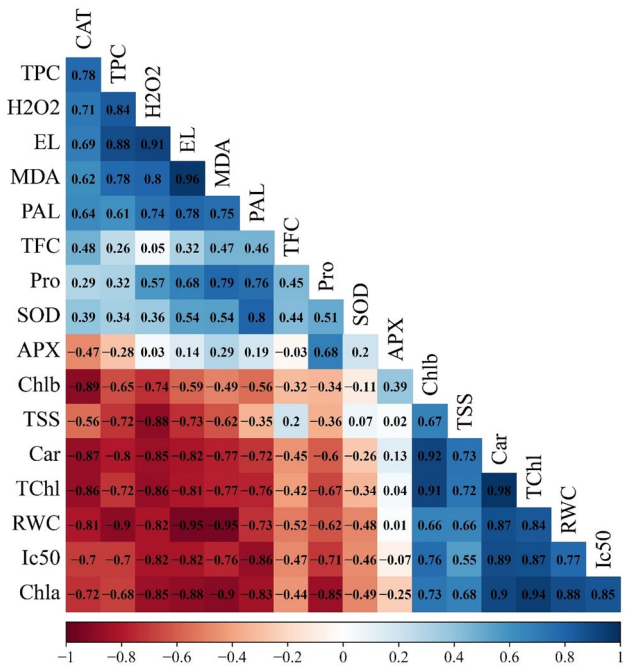

Fig. 6 Pearson's correlation coefficients between the studied traits in two *Origanum vulgare* subspecies grown under different NaCl concentrations. Positive and negative correlations are displayed in blue and red, respectively. RWC (Relative water content); Chl *a* (Chlorophyll *a*); Chl *b* (Chlorophyll *b*); TChl (Total chlorophyll); Car (Carotenoid); EL (Electrolyte leakage); MDA (Malondialdehyde); Pro (Proline); TSS (Total soluble sugars); SOD (Superoxide dismutase); APX (Ascorbate peroxidase); CAT (Catalase); PAL (Phenylalanine ammonia-lyase); TPC (Total phenolic content); TFC (Total flavonoid content)

cinnamic acid, and quercetin compared to the control plants, while at the concentrations of 50 and 100 mM, the amounts of these compounds increased. Conversely, chlorogenic acid content increased in 25 mM NaCl and declined at concentrations of 50 and 100 mM NaCl in ssp. *vulgare*. An increment in gallic acid, caffeic acid, and chlorogenic acid content in ssp. *gracile* was achieved in response to the increase of salinity concentration. The highest cinnamic acid content (3.71 µg g^{-1} dw) in ssp. *gracile* was observed in plants treated with 100 mM NaCl, which was not significantly different from the control plants. The amounts of rutin and apigenin decreased in response to salinity stress in ssp. *vulgare*. The changes of rutin, quercetin, and apigenin contents in ssp. *gracile* did not show a definite trend and the highest amounts of these compounds were recorded at 100 mM salinity. It is very interesting to note that among all phenolic compounds, the highest recorded values were related to *p*-coumaric acid (132.88 and 263.3 µg g^{-1} dw for ssp. *vulgare* and ssp. *gracile*, respectively) in the non-stressed plants.

Antioxidant activity

The antioxidant activity of oregano subspecies was assessed by determining the effect of leaf extract on the scavenging of DPPH free radical. The results indicated that the antioxidant activity of oregano leaf extracts was significantly affected by salinity treatments. Plants grown at 100 mM NaCl demonstrated the highest DPPH free radical quenching activity

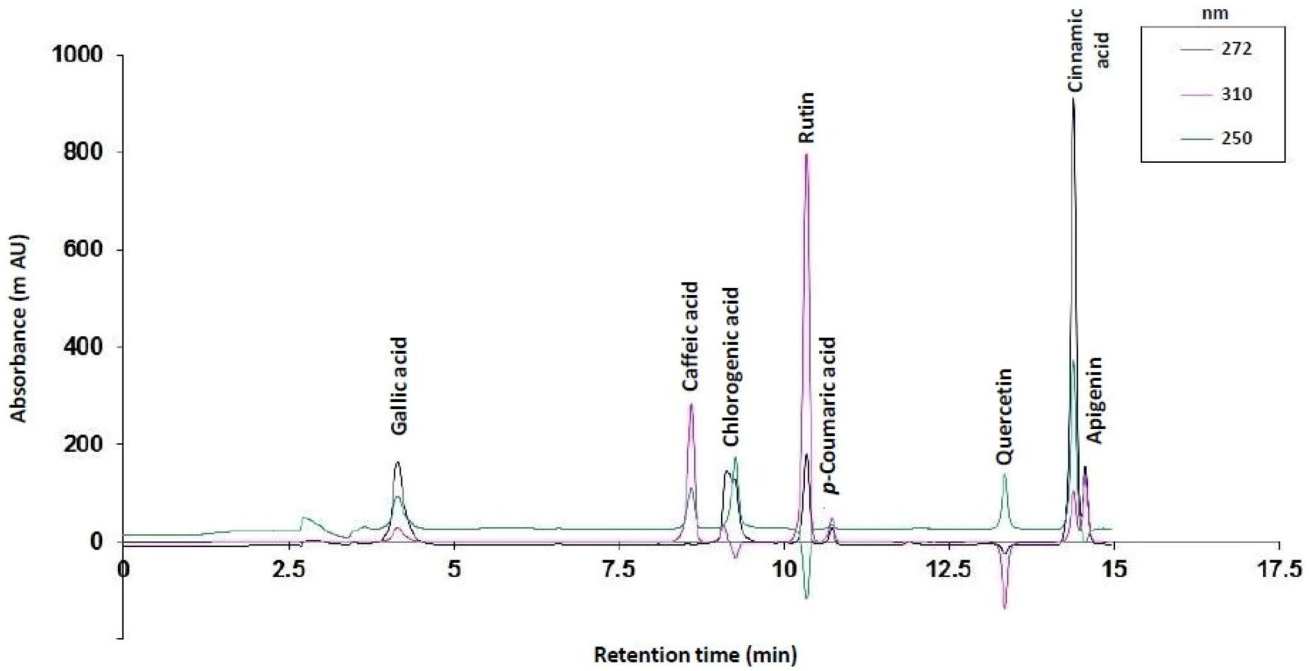

Fig. 7 HPLC chromatograms of standard peaks for phenolic compounds

Table 2 Polyphenolic compounds determined by HPLC in *O. vulgare* ssp. *vulgare* and ssp. *gracile* under salinity stress

Components (µg g^{-1} dw)	ssp. *vulgare*				ssp. *gracile*			
	Control	25 mM	50 mM	100 mM	Control	25 mM	50 mM	100 mM
Gallic acid	9.09 ± 0.00^{d}	7.83 ± 0.01^{g}	8.29 ± 0.01^{f}	10.42 ± 0.01^{c}	8.53 ± 0.01^{e}	10.30 ± 0.20^{c}	11.99 ± 0.01^{a}	11.63 ± 0.00^{b}
Caffeic acid	8.21 ± 0.01^{f}	7.95 ± 0.03^{g}	12.46 ± 0.03^{c}	10.26 ± 0.01^{d}	6.65 ± 0.01^{h}	9.01 ± 0.00^{e}	14.30 ± 0.01^{a}	12.88 ± 0.01^{b}
Chlorogenic acid	24.08 ± 0.05^{d}	28.32 ± 0.06^{b}	23.54 ± 0.05^{d}	11.47 ± 0.06^{f}	22.58 ± 0.06^{e}	26.00 ± 0.06^{c}	23.47 ± 0.06^{d}	32.65 ± 0.06^{a}
p-Coumaric acid	132.88 ± 0.01^{b}	7.89 ± 0.02^{f}	5.63 ± 0.01^{g}	5.13 ± 0.02 h	263.30 ± 0.02^{a}	19.49 ± 0.04^{e}	23.95 ± 0.02^{d}	33.48 ± 0.00^{c}
Cinnamic acid	1.51 ± 0.06^{d}	1.48 ± 0.06^{d}	2.37 ± 0.06^{c}	3.32 ± 0.30^{b}	3.10 ± 0.20^{ab}	1.43 ± 0.06^{d}	1.21 ± 0.06^{d}	3.71 ± 0.06^{a}
Rutin	0.40 ± 0.03^{d}	0.30 ± 0.01^{e}	0.13 ± 0.02^{f}	0.18 ± 0.02^{f}	1.03 ± 0.02^{b}	0.73 ± 0.02^{c}	1.03 ± 0.20^{b}	1.27 ± 0.02^{a}
Quercetin	1.70 ± 0.06^{f}	1.24 ± 0.02^{g}	17.81 ± 0.03^{b}	19.71 ± 0.06^{a}	5.50 ± 0.06^{d}	5.61 ± 0.06^{d}	3.88 ± 0.06^{e}	6.92 ± 0.06^{c}
Apigenin	26.61 ± 0.05^{b}	11.12 ± 0.06^{g}	15.72 ± 0.06^{d}	15.24 ± 0.05^{e}	24.75 ± 0.06^{c}	2.68 ± 0.06^{h}	13.36 ± 0.06^{f}	29.18 ± 0.06^{a}

The means in each row followed by similar letter(s) are not significantly different using Duncan's multiple range test ($p < 0.05$). Values are means ± SE ($n = 3$)

with the lowest IC_{50} value (2.19 mg mL^{-1}) compared with other treatments (Table 1).

Heat map for the phenolic compounds

Heat map was constructed to better imagine changes in phenolic compounds, PAL activity, and antioxidant activity (Fig. 8). TPC and quercetin were more pronounced at 100 mM NaCl in ssp. *vulgare*. Gallic and caffeic acids (at 50 mM NaCl), chlorogenic acid, and apigenin (at 100 mM NaCl) were more pronounced in ssp. *gracile*. Non-stress conditions were favored in relation to *p*-coumaric acid contents in both subspecies. Antioxidant activity and PAL enzyme activity peaked at 100 mM NaCl in both subspecies. Moreover, the measured phenolic parameters were divided into two main clusters, which show a positive relationship between these traits. Accordingly, PAL activity, TPC, TFC, quercetin, gallic acid, and caffeic acid showed a positive relationship. Also, there was a direct

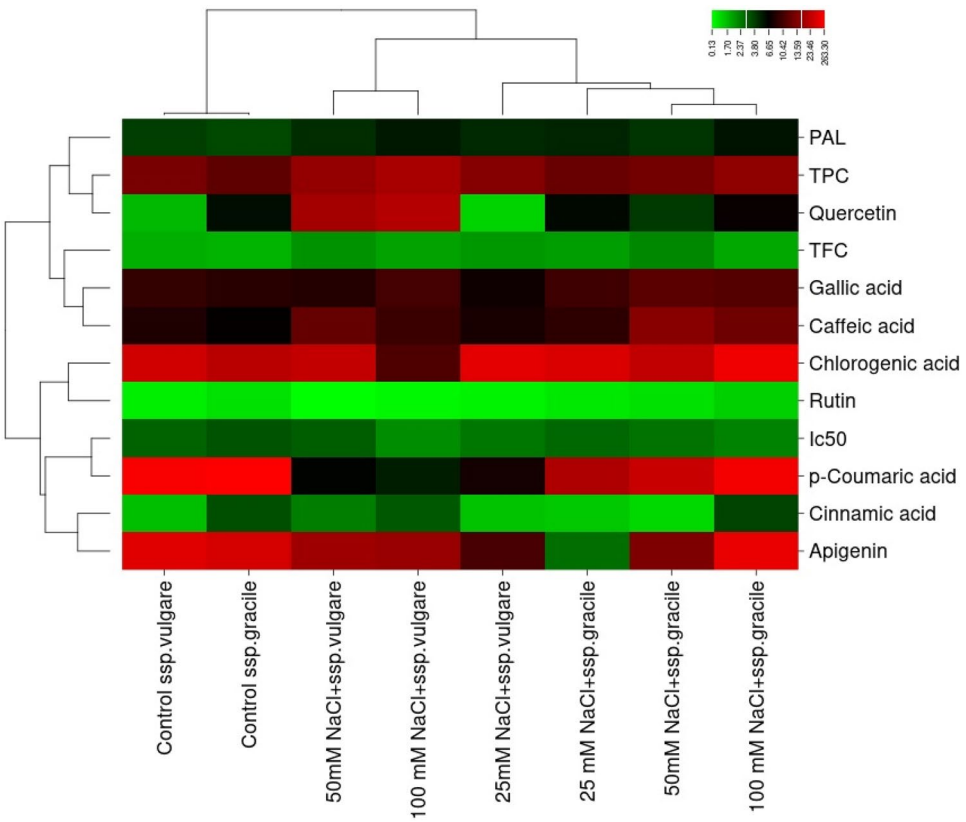

Fig. 8 Heat map for the phenolic compounds, PAL, and antioxidant activity in two *Origanum vulgare* subspecies grown under different NaCl concentrations. Both rows and columns are clustered using correlation distance and average linkage. The red color represents high levels and green color represents low levels. PAL (Phenylalanine ammonia-lyase); TPC (Total phenolic content); TFC (Total flavonoid content)

relationship between IC_{50}, chlorogenic acid, *p*-coumaric acid, cinnamic acid, rutin, and apigenin.

Discussion

The ionic and osmotic effects of NaCl salinity have complex consequences such as a decrease in the potential of soil solution, ion toxicities, and/or nutritional imbalance (Munns 2002), leading to reduced plant water content, stomatal closure, chlorophyll degradation, and reduced photosynthetic efficiency (Zhu 2001). Osmotic stress is the immediate impact of salinity due to hypertonic conditions which disrupts water absorption by roots and reduces the cellular turgor pressure due to a decline in cell water content (Munns 2002; Izadi-Darbandi and Mehdikhani 2018). Therefore, the change in water status is the primary response of plants exposed to salinity. In this study, both subspecies demonstrated a decline in RWC in retort to salt stress. The ssp. *gracile* showed a higher RWC than ssp. *vulgare* under salinity stress, which may be due to the regulation of higher osmotic potential and the accumulation of compatible solutes (proline and TSS) in this subspecies. Similar results have been reported in *Plantago* spp. (Izadi-Darbandi and Mehdikhani 2018), *Thymus vulgaris* and *T. daenensis* (Emami Bistgani et al. 2019), and *Pelargonium graveolens* (Hassanvand et al. 2019).

Chlorophyll, as an important component of photosynthesis apparatus, is one of the most important physiological indicators of salt tolerance. In this study chlorophyll content decreased with increasing salinity intensity. A decrement in chlorophyll and carotenoid contents under salinity stress has been reported in *Salvia miltiorrhiza* (Gengmao et al. 2014) *Thymus vulgaris* (Emami Bistgani et al. 2019), and *Pelargonium graveolens* (Hassanvand et al. 2019). The decrease in chlorophyll content in salt stress conditions may be due to a disturbance in chlorophyll synthesis along with its higher degradation by chlorophyllase activity (Emami Bistgani et al. 2019), oxidation by enhancing ROS production (Ilangumaran and Smith 2017), as well as the impaired absorption of ions participating in the chlorophyll structure (Ghorbani et al. 2018).

Abiotic stresses including salinity are major environmental factors that increase the production of ROS and induce oxidative stress which may damage cell membranes (Parida and Das 2005; Demidchik 2015; Farsaraei et al. 2020). The extent of this damage can be estimated by evaluating the cell membrane integrity. In this study, EL and MDA contents were evaluated as important indicators of oxidative damage to the cell membrane in oregano plants. The remarkable augmentation of H_2O_2 and MDA coincidently with an increment in the intensity of the salinity damaged cell membrane and increased EL in both subspecies. In accordance with our findings, Coban and Gukturk (2016), Hassanvand et al. (2019), Samaddar et al. (2019), and Sarker and Oba (2020) also found an increase in EL and MDA contents in retort to salinity stress. Increasing EL is a sign of stress-mediated damage, so maintaining the cellular membranes integrity under salinity stress is considered as one of the salt tolerance mechanisms (Khan et al. 2010). Accordingly, in *Brassica napus*, it was found that accumulation of H_2O_2 radicals and MDA production was lower in salt-tolerant cultivars compared to sensitive ones (El-Badri et al. 2021).

The accumulation of proline under a variety of environmental stresses, namely, salinity and drought, has been reported in different plant species (Chen and Jiang 2010). Osmotic adjustment, which is achieved through the accumulation of osmolytes such as proline, is one of the best known strategies used by plants to improve the water uptake and preserving cell turgor or osmotic balance (Ashraf and Foolad 2007; Chen and Jiang 2010). A dramatic increase in proline content along with a decrease in RWC indicates that proline accumulates for osmotic regulation of the cells and amelioration of the osmotic disturbances in both oregano subspecies. Furthermore, the scavenging of reactive oxygen radicals, protecting membrane integrity, and acting as a source of carbon and nitrogen are other key roles of proline that increase plant tolerance to stress (Hayat et al. 2012). In this study, a decrease in chlorophyll content was accompanied with an increase in proline accumulation. It has been found that in higher plants, aminolevulinic acid (ALA) is an important precursor in the biosynthesis pathway of chlorophyll (Wu et al. 2019). Given that glutamate is a common precursor for proline and ALA, it has been suggested that under salinity stress, the biosynthetic pathway of proline from glutamic acid surpasses the ALA synthesis, leading to increased proline accumulation to diminish the destructive effects of osmotic stress (Averina et al. 2010). In our investigation, an increment in NaCl concentration caused a remarkable enhancement in proline content in both subspecies, but this was more noticeable in the leaves of ssp. *gracile* compared to ssp. *vulgare*. Indeed, the high level of proline accumulation in ssp. *gracile* may explain the higher RWC in this subspecies.

One of the most common metabolic changes in retort to salt stress is the accumulation of amino acids, sugars, and other low molecular weight organic compounds that increase plant resistance to salt-induced osmotic stress (Chen and Jiang 2010). The accumulation of soluble sugars helps plants maintain osmotic regulation under salinity stress and acts as osmoprotectant to care for cell membranes from stress-induced damages (Chen et al. 2007; Sarabi et al. 2017). In the present research, TSS content decreased in response to severe stress in both subspecies. A decrease in TSS under severe stress conditions may be due to sugars consumption in the synthesis of metabolites like proline (Hellmann et al.

2000). The limitation of carbohydrate availability as a consequence of a decline in photosynthesis could be also another reason for decrease in TSS (Goicoechea et al. 2005). The increase in TSS was notable at low salinity levels in *gracile* subspecies. The higher leaf RWC in ssp. *gracile* can be attributed to the higher accumulation of compatible solutes like proline and TSS in this subspecies.

Oxidative stress is one of the consequences of salinity stress that disrupts normal cell metabolism through oxidizing proteins, lipids, DNA, and other cellular macromolecules (Gill and Tuteja 2010; Demidchik 2015). In plants subjected to salinity stress, the most important sources of ROS accumulation are chloroplasts and mitochondria (Acosta-Motos et al. 2017). To overcome the oxidative damages, plants use various defense systems such as osmoprotectants, non-enzymatic compounds, and antioxidant enzymes (Baltruschat et al. 2008; Gengmao et al. 2015; Huang et al. 2019). Superoxide dismutase is the first defensive barrier which transforms the superoxide radicals into H_2O_2 (Yazici et al. 2007; Mittler 2017), and eventually H_2O_2 is decomposed by CAT and APX. Catalase is the most efficient antioxidant enzyme for inhibiting oxidative injury, which is responsible for producing water and molecular oxygen from H_2O_2 in peroxisomes (Parida and Das 2005; Gill and Tuteja 2010). Catalase removes H_2O_2 produced during photorespiration in peroxisomes (Cantabella et al. 2017). Ascorbate peroxidase is another defense enzyme that uses H_2O_2 as a substrate to reduce oxidative damage, mainly in chloroplasts and to some extent in cytosols (Çoban and Gukturk 2016). Previous studies have shown that antioxidant enzymes activity increased under saline conditions in *Hyssopus officinalis* (Jahantigh et al. 2016), *Ocimum basilicum* (Jakovljevic et al. 2017), and *Brassica napus* (El-Badri et al. 2021). In this study, a negative relationship was found between SOD and CAT activity with RWC. Additionally, SOD and CAT had positive correlation with H_2O_2, MDA, and EL. Based on these evidences, it can be concluded that the enhancement in SOD, CAT, and APX activities in both oregano subspecies could alleviate the osmotic effects and oxidative damage induced by NaCl salinity. In other words, increasing the activity of antioxidant enzymes can be associated with salinity stress tolerance in the studied oregano subspecies. Sarker and Oba (2020) reported a higher SOD and APX activity as well as lower accumulation of ROS in a tolerant variety of *Amaranthus tricolor*. Similarly, in salt-tolerant cultivars of *Brassica napus*, the ROS and MDA production was diminished through the activation of SOD, POD, CAT, and APX enzymes under salinity stress (El-Badri et al. 2021).

Phenolic and flavonoid compounds are among the significant non-enzymatic antioxidants with vital roles in ROS detoxification in stressed plants (Huang et al. 2010; Sharma et al. 2019). Phenylalanine ammonia-lyase is a crucial enzyme in the phenylpropanoid route which produces a wide range of phenolic compounds with diverse functions in plants such as defense responses against environmental stresses (Huang et al. 2010). Therefore, PAL activity has been identified as a marker for assessment of the plant resistance against biotic and abiotic stresses (MacDonald and D'Cunha 2007). In this study, increased PAL activity in oregano plants under salinity stress could explain the increase in total phenolic and flavonoid contents. This biochemical reaction is likely considered as an important and compatible regulatory step against salinity stress. The existence of a positive relationship between the PAL activity and TPC and TFC under salinity conditions confirms this fact. In addition, the existence of a negative relationship between the PAL activity and IC_{50} displays the fundamental role of this enzyme in inducing the production of antioxidant compounds to ameliorate the destructive effects of salinity stress. An increment in the PAL activity and phenylpropanoid derivatives has also been reported in precedent researches (Valifard et al. 2014; Mohammadkhani et al. 2015). TPC and TFC displayed a negative correlation with IC_{50}, demonstrating their key role in antioxidant activity. The function of phenolic compounds and flavonoids in scavenging free radicals may be due to their electron donation properties (Huang et al. 2019). Our study revealed that TPC and TFC were augmented in both subspecies, whereas the increment was higher in ssp. *vulgare* compared to ssp. *gracile*. Similarly, Sarker and Oba (2020) reported that under salinity stress, the salt-tolerant variety of *Amaranthus tricolor* accumulated more phenols and flavonoids than the susceptible one. Accumulation of phenolic compounds in retort to salt stress has also been observed in *Salvia mirzayanii* (Valifard et al. 2014), *Mentha piperita* (Çoban and Gukturk 2016), *Sesamum indicum* (Khademian et al. 2019), and *Amaranthus tricolor* (Sarker and Oba 2020). Ksouri et al. (2007) stated that salinity stress disrupts the pathway of secondary metabolites and leads to an increase in phenolic compounds. However, the biosynthesis and accumulation of these compounds are sorely dependent on the plant species. In this study, gallic acid, caffeic acid, chlorogenic acid, cinnamic acid, rutin, and apigenin in ssp. *gracile* and quercetin in ssp. *vulgare* were significantly increased in moderate to severe salinity treatments. Also, the activation of antioxidant systems was probably due to the increment of polyphenols content in retort to salinity-induced oxidative stress. In other words, the biosynthesis of these compounds inhibits ROS and significantly reduces salinity-induced stress oxidative. Despite these statements, in this study, increasing stress intensity significantly decreased *p*-coumaric acid in both subspecies. Furthermore, plants grown at 100 mM NaCl displayed the highest DPPH free radical quenching activity with the lowest IC_{50} value compared with other treatments. These effects are probably

due to the higher TPC under 100 mM NaCl treatment. A negative relationship was found between TPC and TFC with antioxidant capacity (as IC_{50} value) might confirm this inference. Similarly, Emami Bistgani et al. (2019) demonstrated that in *Thymus* species exposed to salinity stress, the maximum amount of antioxidant activity was achieved in treatments with the highest amount of TPC. Phenolics and flavonoids have considerable redox activity and can counteract the overproduction of ROS (Sharma et al. 2012). Also, the antioxidant capacity in ssp. *vulgare* was slightly higher than ssp. *gracile*. An increment in antioxidant activity during salt stress has been previously reported in many plants like *Salvia sclarea* (Ben Taarit et al. 2012), *Salvia mirzayanii* (Valifard et al. 2014), *Mentha piperita* (Khalvandi et al. 2019), and *Sesamum indicum* (Khademian et al. 2019).

Conclusions

Based on the results obtained, the physiological and biochemical characteristics of oregano subspecies were affected by salinity treatment. Salt stress significantly reduced RWC and photosynthetic pigments in both subspecies, whereas H_2O_2 production, EL, and MDA contents increased in retort to raising NaCl concentration. To alleviate the salt-induced osmotic and oxidative damages, oregano subspecies increased TSS and proline accumulation, SOD, CAT, and APX activities and phenolics and flavonoids production. The increment in PAL enzyme activity was associated with an enhancement in TPC and antioxidant activity. Plants with higher phenolic compounds had higher ROS quenching activity. In conclusion, no significant difference was observed between the studied oregano subspecies in regards to salinity tolerance. The findings of this study support the idea that accumulation of secondary metabolite in the medicinal plants is firmly influenced by abiotic stresses such as salinity; hence, the cultivation of oregano plants under salinity stress conditions can be recommended as an appropriate approach to improve antioxidant activity and stimulate the production of biologically active constituents utilized in the pharmaceutical, food, and cosmetic industries.

Author contribution statement AH did supervision. ZA and AH designed and conducted the experiment. ZA, AH, BAM, and ES performed collection of data and laboratory analysis. ZA and BAM performed statistical analysis of the data. ZA and AH were involved in writing—review and editing of manuscript. All authors have read and approved the final version of manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this article.

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