Salinity has been identified as a major abiotic stressor, decreasing yield worldwide (Rouphael et al., 2016). About 400 million hectares of agricultural lands all over the globe have been affected by salinity (FAO, 2014). Moreover, based on the global climate changes, salinization is expected to have an increased negative impact, posing a major threat to crop productivity in the years to come (Munnis, 2002).

Excessive concentration of sodium chloride (NaCl) in soil and water induces osmotic (i.e., water stress) and ionic (i.e., Na⁺ and Cl⁻) stresses, leading to several physiological, anatomical, molecular, and metabolic changes (Munnis, 2005; Ntäsi et al., 2017). Several greenhouse studies carried out on vegetable species demonstrated that salinity stress retards growth thereby reducing fresh and dry biomass accumulation (Colla et al., 2013b; Mori et al., 2008, 2011), induces impairment of membrane integrity (Colla et al., 2013a), hampers the nutrient uptake and translocation (Grattan and Grieve, 1999), reduces chlorophyll synthesis (Lucini et al., 2015; Rouphael et al., 2017b), and limits the photosynthetic CO₂ fixation (Rouphael et al., 2017a). Moreover, anatomical changes in vegetable leaves such as increase in palisade, spongy parenchyma thickness, and the volume of intercellular spaces were also observed under saline conditions (Rouphael et al., 2017a). Although saline water generally reduces crop productivity, in many cases it can enhance nutritive quality as demonstrated with several vegetable crops grown under greenhouse conditions (Colla et al., 2006a, 2006b, 2006c, 2008, 2012; Rouphael et al., 2006, 2012c). However, the quality response of vegetables to salinity may change in relation to several interacting parameters such as the growing environment, species or cultivars as well as the salt concentration, source, and the time of exposure of the stress (Rouphael et al., 2012a, 2017a).

Leaves (Lactuca sativa L.) is a major salad crop from the Asteraceae family, which is widely grown and consumed in Italy and in several other European countries (Rouphael et al., 2017c). It has garnered a crucial role in the human diet as it combines generally pleasing organoleptic properties with a rich content of bioactive compounds (minerals, vitamins, phenolic acids, and flavonoids; Kim et al., 2016b). On the other hand, lettuce plants are noted for their high nitrate accumulation, leading to a high intake of nitrate in the human diet (Amr and Hadidi, 2001). Nitrate per se is not toxic; however, its reaction metabolites (i.e., nitrite and nitric oxide) could have detrimental effects on human health in some target population groups (vegetarians, infants, and the elderly) with a higher probability of developing cancer (EFSA, 2008). Therefore, the European Commission regulation No. 1881/2006 established a safe threshold for nitrates in lettuce [3000–5000 mg·kg⁻¹ fresh weight (FW)].

Most of the leafy vegetable crops including lettuce are salt-sensitive species which grow poorly in salinized soils (Mass and Hoffman, 1977). In the scientific literature, the agronomical, physiological, and structural responses of lettuce were investigated only at low (0.9–1.5 dS·m⁻¹) or very high (>15 dS·m⁻¹) saline conditions (Garrido et al., 2014; Kim et al., 2016a; Mahmoudi et al., 2011), whereas no published data are available concerning the effects of moderate salt stress (1.8–7.2 dS·m⁻¹) typical of coastal areas where leafy vegetables are grown under protected cultivation.

The aim of this 2-year study was to evaluate the effects of different concentrations of saline water on growth, yield, leaf gas exchange and water potential, antioxidant activity, ascorbic acid, and nitrate content of lettuce grown under greenhouse conditions.

Materials and Methods

Growth conditions, plant material, and experimental design. Two experiments were conducted in two different years (Expts. 1 and 2) in a polyethylene shelter situated at the experimental station of the University of Naples “Federico II” located in Portici (Naples), south Italy (lat. 40°49’N; long. 14°20’E). In both growing seasons, Lactuca sativa L. cv. Cambria (Vilmorin, Paris, France) was used.

A completely randomized block design with three replicates was used to compare five NaCl concentrations in the irrigation water having the following electrical conductivities (ECₗw): 0.7 (nonsalinized control), 0.9, 1.8, 3.6, or 7.2 dS·m⁻¹. Lettuce were transplanted at the two true-leaf stage on 10 Nov. (Expt. 1) and 7 Dec. (Expt. 2) at a plant density of 8 plants/m². Lettuce was grown in reinforced fiber glass with a diameter of 70 cm and a depth of 65 cm. A layer of coarse sand and gravel, 10-cm thick, was overlain by a repacked soil profile of 50 cm. At the bottom of the lysimeter, a pipe serving as a drainage outlet connected the lysimeter to...
a drainage reservoir. The lysimeters were filled with a loamy sandy soil (83% sand, 12% silt, and 5% clay), with a pH of 7.5, EC of 0.9 dS m\(^{-1}\), organic matter of 0.72% (w/w), total N at 0.56%, available P at 6.7 mg kg\(^{-1}\), and exchangeable K at 107 mg kg\(^{-1}\). In both growing years, preplant fertilizers were broadcast (kg ha\(^{-1}\)): 130 P\(_2\)O\(_5\) and 150 K\(_2\)O and incorporated into the soil. Additional top-dressing fertilizer (100 kg ha\(^{-1}\) N) was applied three times (at transplanting, 35 and 60 d after planting) at equal rates using NH\(_4\) NO\(_3\) as a source of N. All plots were irrigated at 100% irrigation level based on the Hargreaves formula. Weeds were controlled with hand hoeing, and no pesticide applications were required to control pathogens and pests.

**Crop growth and yield measurements.** In both experiments, the number of leaves per plant, total leaf area, and specific leaf weight were measured during three phenological growing stages (rosette, early-heading, and midheading), whereas the head diameter and fresh weight were recorded four times during the growing cycles at the following phenological stages: rosette, early-heading, mid-heading, and mature head (i.e., harvest) (Subbarao, 1998). The leaf area was measured with an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). The specific leaf weight was calculated as the ratio of leaf dry weight per unit area and expressed as mg cm\(^{-2}\).

In both experiments, lettuce plants were harvested on 5 Feb. (Expt. 1) and 9 Mar. (Expt. 2) and the marketable yields were determined on all plants per experimental unit.

**Analysis of nitrate, total ascorbic acid content, and hydrophilic and lipophilic antioxidant activities.** Nitrate nitrogen (N-NO\(_3\)) content was determined during both experiments, the number of leaves per plant, in three plants per treatment. The leaf gas exchange measurements were performed 50 days after transplanting, on fully expanded leaves (excluding the most external leaf layer), on two leaves per plant, in three plants per treatment. The leaf gas exchange measurements were carried out within 2 h across solar noon (i.e., between 1100 and 1300 hr). The net CO\(_2\) assimilation rate (\(A\)), gs, and transpiration rate (E) were measured with a portable infra-red gas analyzer WALZ HCM 1000 (Walz, Effeltrich, Germany). During the measurements, temperature inside the leaf chamber was 13.2 ± 1.5 °C (mean ± standard deviation), and the light intensity recorded at the leaf level was 586 ± 64.5 µmol m\(^{-2}\) s\(^{-1}\) (mean ± standard deviation). Instantaneous and intrinsic water use efficiency (WUE\(_i\) and WUE\(_{int}\)) were calculated as A/E and A/\(g_s\), respectively.

At the same date, LWP (\(\Psi_L\)) at midday (1200 hr local time) was measured using the pressure chamber (model 3005; Soil Moisture Equipment Corp., Santa Barbara, CA) technique (Scholander et al., 1965) and taking the precautions proposed by Turner and Long (1980).

**Statistical analysis.** Analysis of variance (ANOVA) of the experimental data was performed using the SPSS software package (SPSS 13 for Windows, 2001). Combined ANOVA over two growing seasons was performed for crop growth parameters, marketable yield, and nitrate content. In both years, orthogonal contrasts were used to compare concentration of saline water effects (Gomez and Gomez, 1983) on selected crop parameters.

### Results

**Plant growth, marketable yield, and nitrate content**

In both years, the leaf number and leaf area decreased linearly in response to an increase in water salinity at the three phenological stages (Table 1). Moreover, except for the second phenological stage (i.e., early-heading), the lettuce head characteristics (head diameter and head fresh weight) were significantly affected by both growing years and salinity treatments (Table 2). The decrease in the head fresh weight was much more pronounced at midheading and mature-head stages (Table 2). In fact, the percentage of the head fresh weight reduction at mid-heading stage in comparison with nonsaline control was 14.9%, 21.4%, 35.4%, and 48.4% at 0.9, 1.8, 3.6, and 7.2 dS m\(^{-1}\), respectively (Table 2).

Similarly to leaf and head characteristics, the nitrate content decreased linearly during the four phenological stages with the increase in water salinity from 0.7 to 7.2 dS m\(^{-1}\), with the lowest values recorded under severe salt stress conditions at early–mid-heading as well as at mature head (Table 2). Finally, irrespective of water salinity treatments, the leaf number, leaf area, head diameter and fresh weight, and nitrate content were significantly higher in the first year in comparison with the second year, whereas an opposite trend was observed for the specific leaf weight (Tables 1 and 2).

| Table 1. Mean effects of growing year and irrigation with saline water on leaf characteristics of lettuce plants at three phenological growing stages. |
|-------------|-------------|-------------|-------------|-------------|
| Treatment   | Leaf number (no./plant) | Leaf area (cm\(^2\)/plant) | Specific leaf weight (mg DW/cm\(^2\)) |
| Year 1      | Rosette     | Early-heading | Mid-heading | Rosette     | Early-heading | Mid-heading | Rosette     | Early-heading | Mid-heading |
| Year 2      | 11.7 b      | 24.3 b       | 27.3 b      | 146.1 b     | 512.7 b      | 775.6 b     | 5.9 a       | 5.1 a       | 6.2 a       |
| EC (dS m\(^{-1}\)) | 0.7         | 14.6 a      | 25.5 b      | 33.6 a      | 444.0 a     | 788.9 a     | 1,436.5 a   | 4.1         | 3.8 b       | 5.5         |
| 0.9         | 12.8 b      | 29.3 a      | 31.8 a      | 371.8 b     | 777.2 ab    | 1,232.8 b   | 4.2         | 4.2 a       | 5.7         |
| 1.8         | 13.1 b      | 26.3 ab     | 31.8 a      | 371.7 b     | 769.2 ab    | 1,169.0 b   | 4.3         | 4.1 ab      | 5.6         |
| 3.6         | 11.3 c      | 24.1 b      | 27.8 b      | 312.1 c     | 640.6 bc    | 1,055.3 bc  | 4.5         | 4.4 a       | 5.6         |
| 7.2         | 12.1 bc     | 25.9 ab     | 28.9 b      | 297.2 c     | 597.5 c     | 876.4 c     | 4.5         | 4.4 a       | 5.7         |

**Significance**

*Year (Y)*  
*Significance (S)*  
Y × S

L** = linear; *, **, *** Nonsignificant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.
When averaged over water salinity treatments, the highest marketable yield was recorded during the first experiment compared with the second (Fig. 1). Moreover, irrespective of the growing year, increasing the EC of the irrigation water from 0.7 to 7.2 dS·m⁻¹ decreased the marketable yield linearly. The percentage of marketable yield reduction in comparison with nonsaline control treatment was 22.7%, 36.4%, 45.4%, and 63.6% at 0.9, 1.8, 3.6, and 7.2 dS·m⁻¹, respectively (Fig. 1).

**Antioxidant activity and vitamin C contents**

In Expt. 2, the HAA, LAA, and AA ranged from 10.9 to 12.6 mmol ascorbic acid/100 g dry weight (DW) and from 21.0 to 26.3 mmol Trolox/100 g DW and from 10.9 to 11.3 mg/100 g FW, respectively (Table 3). The highest values of HAA were recorded in the nonsalinated treatment, whereas the lowest values of LAA and AA were observed under severe salt stress conditions (7.2 dS·m⁻¹; Table 3).

### Leaf gas exchange and water potential.

In Expt. 2, the net CO₂ assimilation, gₛ, E, and midday leaf potential decreased linearly as the EC of the irrigation water increased from 0.7 to 7.2 dS·m⁻¹, whereas an opposite trend was recorded for the WUE (Table 4). The lowest values of net CO₂ assimilation, gₛ, and E were recorded with the 3.6 and 7.2 dS·m⁻¹ treatments, whereas no significant difference in comparison with the control was observed between the 0.9 and 1.8 dS·m⁻¹ treatments. Finally, the lowest and highest WUE values were observed with the 1.8 and 3.6 dS·m⁻¹ treatments, respectively (Table 4).

### Discussion

The inhibition of the photosynthesizing leaf area, stunted growth, and yield reduction are general responses of vegetables to excessive NaCl in irrigation water; and the decreasing in crop productivity may change in relation to several factors such as species/cultivars, cultural environment, NaCl concentration, as well as the time of exposure to salt stress conditions (Munns, 2002). In the current experiment, irrigation with saline water had negative effect on plant growth parameters (i.e., leaf number, total leaf area, and head diameter) as well as head fresh weight, especially at 1.8, 3.6, and 7.2 dS·m⁻¹, confirming that lettuce is a salt-sensitive crop (Lucini et al., 2015; Rouphael et al., 2016). Furthermore, the marketable yield reduction was much more pronounced under salt stress conditions ranging from 23% to 64% (at 0.9 and 7.2 dS·m⁻¹, respectively). Reduced shoot fresh weight of lettuce plants under saline treatments could be associated with NaCl increasing Wₛ (osmotic stress) and to salt-specific effects (ionic stress) inside the plant because of excessive Na⁺ and Cl⁻ uptake, leading to nutritional disorders and to the production of extreme ratios of Na⁺/K⁺, Na⁺/Ca²⁺, and Cl⁻/NO₃⁻ (Grattan and Grieve, 1999; Munns, 2005). The significant depression in plant growth parameters and yield with increasing salinity has been demonstrated in other greenhouse experiments on tomato (Savvas et al., 2011), cucumber (Rouphael et al., 2012c), mini-watermelon (Colla et al., 2006b), melon (Colla et al., 2006c), pepper (Colla et al., 2006a), zucchini squash (Colla et al., 2008) as well as lettuce (Kim et al., 2016a; Lucini et al., 2015). In the present study, the marketable lettuce fresh yield started to decline at 0.7 dS·m⁻¹. Our findings are in agreement with those of Mass and Grattan (1999) and Kim et al. (2016a). By contrast, other authors have reported that the threshold for salt tolerance of lettuce ranged between 1.1 and 2.0 dS·m⁻¹ (Andriolo et al., 2005; Unliķara et al., 2008). Explanations of this disagreement could be the different environmental conditions in which the plants were grown, and variations between lettuce cultivars or varieties (Mahmoudi et al., 2011). For instance, Pasternak et al. (1986) demonstrated that Butterhead and Crisphead lettuce types are generally less salt tolerant than Cos (i.e., Romaine) types. It is generally accepted that moderate salinity reduces the vegetable productivity, but in many cases, improves the composition and concentration of phytochemicals (Colla et al., 2013a; Rouphael et al., 2006, 2012a, 2012b). In fact, plants exposed to abiotic stress (i.e., salinity) will activate several physiological mechanisms to adapt to the suboptimal environment. Among these mechanisms, the synthesis of secondary metabolites (i.e., ascorbate and carotenoids) able to ensure plant growth even under harsh conditions (Kyriacou and Rouphael, 2017; Orsini et al., 2016). Interestingly, these secondary metabolites are considered an added value to basic nutritional characteristics of vegetables because of their health-promoting effects (Colonna et al., 2016). This was not the case in the present study as no significant differences between the non-salt control and the saline treatments (up to 3.6 dS·m⁻¹) were recorded for the LAA and total ascorbic acid contents, whereas a significant decrease in these quality parameters was observed under severe salt stress treatment (i.e., 7.2 dS·m⁻¹). The lowest content of total ascorbic acid observed at 7.2 dS·m⁻¹ could be attributed to the fact that under high NaCl concentration, the antioxidant system did not sufficiently support reactive oxygen species scavenging in relation to salinity, as demonstrated by the sharp decrease in photosynthesis activity, plant growth, and marketable fresh yield.

It is also interesting to note that significant reduction in the accumulation of undesired compounds such as nitrate, most notably present in leafy vegetables, could be feasible under salt stress conditions (Kyriacou and Rouphael, 2017). In the present study, the percentage of nitrate content reduction at harvest (i.e., mature head) in comparison with nonsaline control was 16.9%, 24.9%, 45.2%, and 61.1% at 0.9, 1.8, 3.6, and 7.2 dS·m⁻¹, respectively. Moreover the nitrate content found were below the maximum limit of 4000 mg·kg⁻¹ FW imposed by the European.
The present study reveals that plant growth parameters and marketable fresh yield of greenhouse lettuce decreased linearly with increasing salinity of irrigation water especially at 3.6 and 7.2 dS m⁻¹. The reduction in yield could be partly compensated by a decrease in nitrate content of salt-treated lettuce. Our results also demonstrated that the reduction of crop productivity under saline conditions (>1.8 dS m⁻¹) depends on the reduced uptake of water as well as on the decrease of net CO₂ assimilation.

**Conclusion**

Community for lettuce grown under cover irrespective of the season (European Commission, 2011). The linear reduction in nitrate content with increasing NaCl concentration in the irrigation water is associated to the antagonism effect between nitrate and chloride. Rubinigg et al. (2003) and Borgognone et al. (2016) showed that in the presence of high chloride levels, root-to-shoot translocation of nitrate could be reduced at the site of entrance into xylem parenchyma cells through competition for the same channel.

High level of NaCl in irrigation water or the growing medium hampers plant physiological processes such as photosynthesis as well as water relations (Munns, 2005). This was the case in the current experiment because the significant differences between the water irrigation treatments on the agronomical traits were also observed at the physiological level. Net CO₂ assimilation rate declined with increasing NaCl concentration in the irrigation water. The reduction in photosynthetic capacity in response to salinity could be correlated to the accumulation of toxic elements Na or Cl in leaf tissues as observed previously in several vegetable crops (Colla et al., 2012; Martinez-Ballesta et al., 2004; Rouphael et al., 2012c). Furthermore, the inhibition of photosynthesis induced premature senescence, inhibition of chlorophylls and carotenoids biosynthesis as well as compromising the translocation of assimilates to photosynthetic sinks leading to a significant reduction in plant growth (Colla et al., 2012; Stepien and Klobus, 2006).

It is well established that the salt-induced limitation of photosynthesis could be due to stomatal or non-stomatal factors, the latter are not fully elucidated (Debez et al., 2008). The concomitant reduction in CO₂ assimilation, which was linearly correlated to gₑ ($R^2 = 0.82$, data not shown) indicate the occurrence of stomatal limitation of photosynthesis (Mahmoudi et al., 2011; Neocleous et al., 2014; Parida and Das, 2005). In fact the salt-stressed lettuce plants especially at 3.6 and 7.2 dS m⁻¹ reacted by the closure of stomata to minimize water loss by E, which was reduced by 50% in comparison with the 0.7, 0.9, and 1.8 dS m⁻¹ treatments. The intrinsic water use efficiency (A/gₑ) remained quite high (ranging 0.118–0.092) and did not strongly decreased under most stressful conditions, therefore, indicating the non-stomatal components of photosynthetic limitations had not developed yet (Flexas et al., 2004). Moreover, the WUE calculated by the ratio A to E, increased at 3.6 dS m⁻¹, indicating that there were water restrictions rather than inhibition of net photosynthesis (Flexas et al., 2004). The concept of high WUE is an important aspect for salt tolerance as high WUE may suppress the uptake and translocation of toxic ions and mitigate water deficiency induced by salt stress conditions (Karaba et al., 2007; Moya et al., 1999; Neocleous et al., 2014).

Similarly to leaf gas exchange measurements, salinity stress caused a marked decrease in LWP (more negative), especially at 3.6 and 7.2 dS m⁻¹. The salt-induced reduction in LWP has been observed earlier on several vegetable crops (Shahbaz et al., 2012; Singh et al., 2010; Youssif et al., 2010). The decrease in LWP under severe salt stress conditions has been attributed to several mechanisms including the excessive accumulation of toxic ions (e.g., Na⁺ and Cl⁻) in the shoot tissues and also to decreased accessibility of water to the root system because of the reduction of water conductivity, which leads to decreased evapotranspiration and crop productivity (Maksimovic and Ilin, 2012; Solan et al., 1999).

**Table 4. Effect of irrigation with saline water on net CO₂ assimilation rate (A), stomatal conductance to water vapour (gₑ), transpiration rate (E), instantaneous water use efficiency (WUEᵢ) and leaf water potential at midday (Ψₑ) of lettuce plants grown in the second growing year.**

| ECₑ (dS·m⁻¹) | A (µmol CO₂/m²·s) | gₑ (mmol H₂O/m²·s) | E (mmol H₂O/m²·s) | WUEᵢ (µmol CO₂/mol H₂O) | Ψₑ (MPa) |
|-------------|------------------|--------------------|-------------------|--------------------------|---------|
| 0.7         | 7.1 a             | 60.1 a             | 1.0 a             | 8.0 ab                   | -0.24 c |
| 0.9         | 7.5 a             | 60.2 a             | 1.1 a             | 7.3 ab                   | -0.50 bc|
| 1.8         | 7.1 a             | 60.5 a             | 1.1 a             | 6.6 b                    | -0.62 b |
| 3.6         | 5.7 ab            | 46.9 b             | 0.5 b             | 10.6 a                   | -0.71 ab|
| 7.2         | 4.4 b             | 47.8 b             | 0.4 b             | 9.9 ab                   | -0.96 a |

**Significance**: L* = linear; ns, *, ** Nonsignificant or significant at P ≤ 0.05 or 0.01, respectively.

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