Tetragonia tetragonioides (Pallas) Kuntz. as promising salt-tolerant crop in a saline agricultural context

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Abstract

The lack of natural resources, especially good-quality cropland and renewable water resources is threatening food production potential in marginal agricultural ecosystems, which are already negatively affected by climate change. Since the world's major crops are proving inadequate to supply the calories and nutrients for people in these areas, new crops are sought that can withstand harsh ecological environmental conditions. In the current trial, we assessed the growth and productivity of *Tetragonia tetragonioides* (Pallas) Kuntz**.** in a floating hydroponic system supplied with different seawater proportions (i.e. 15% and 30% seawater, EC=9.8 and 18.0 dS m^{-1}). Moreover, the effects of different salinity elements and their respective accumulations, and the production of osmotic solutes and secondary metabolites were determined, along with the salt removal capacity of the crop. The results indicated that plant growth was not affected by either of the seawater treatments used in this study. The increased leaf succulence and the reduction of both leaf area and specific leaf area with increasing salinity might represent an essential feature of this salt-tolerant species in association with to the plant's need to limit transpiration. Low seawater treated plants showed a significantly higher biomass yield per liter of (sea)water used (117%) than the control. Under these conditions plants accumulated the highest amount of Mg (+31% and 48% in medium and high seawater treated plants compared with the control) and Cu (+14% and 30%, respectively) along with increasing proline and decreasing nitrate concentrations. By contrast, we found that seawater supply resulted in a Na-enriched leaf biomass that may represent an issue for human health. We concluded that *Tetragonia tetragonioides* can be grown in saline agriculture up to a salinity level characterized by an EC of 18 dS m^{-1} but further investigation is required to address Na accumulation in leaves.

Keywords

New Zealand spinach, saline agriculture, hydroponics, seawater irrigation, salt-tolerant crop, salt removing crop, seawater footprint

1 Introduction

Increasing population likely will result in an increase of the global food demand for at least another 40 years (Godfray et al., 2010). Lack of natural resources, especially high-quality cropland and renewable water resources, will reduce the food production potential in several regions (FAO, 2013). Moreover, the effects of climate change represent a further threat (Godfray et al., 2010), especially in marginal, already-stressed agroecosystems (Cheeseman, 2016). Today more than 34 MHa are salt-affected (FAO, 2011), either because they are coastal or because inappropriate irrigation practices have degraded soil and depleted or salinized groundwater (Cheeseman, 2016). Although significant advances have been made in the last 25 years in reducing hunger worldwide (FAO, 2013), the situation seems to be less optimistic in areas affected by both drought and salinity (Cheeseman, 2016). Given that the world's major crops have proven inadequate to supply people in these areas with sufficient amount of calories, proteins, fats and nutrients, new crops are needed that can specifically withstand such harsh ecological conditions (Cheeseman, 2016). New crops tolerant to saline conditions are likely to be found among edible halophytes. Halophytes are plants that can grow at salinity levels higher than 200 mM NaCl (Flowers and Colmer, 2008), roughly corresponding to half-strength seawater. Several morphological, physiological, and biochemical adaptations are adopted by halophytes to withstand or even to benefit from saline environments (Panta et al., 2014). Furthermore, favorable effects on yield and its quality can even be related to saline conditions (Flowers and Muscolo, 2015; Shannon and Grieve, 1998). The idea of growing salt-tolerant plants in agricultural systems irrigated with brackish and saline water is not new (Glenn et al., 1999; Rozema and Flowers, 2008; Rozema and Schat, 2013). However, advances in this direction have been slow, and in only a few cases has there been the goal of developing new crops (Cheeseman, 2016). According to Cheeseman (2016), this is due to the fact that there is little urgency for plant biologists, crop scientists, and politicians of the developed world. In the context of saline agriculture, the water requirements of salt-tolerant crops are met through brackish water and/or seawater, thus relieving pressure on fresh water resources. However, large-scale, sustainable agriculture involving pure seawater irrigation seems to be impractical for reasons mainly connected to the deterioration of soil structure (Breckle, 2009). Irrigating with seawater on fertile and well-structured soils would lead to a salt contamination through Ca^{2+}/Na^{+} exchange and resulting clay dispersion (Ventura et al., 2015), with

additional significant impacts on soil microbial properties (Chaudhary et al., 2016). On the other hand, there is growing interest in the possibility of recovering lost coastal soils while minimizing inputs, i.e. freshwater (Fedoroff et al., 2010); an ecologically-acceptable compromise to the using of saline waters for food production and the preservation of soil is represented by soilless cultivation (Atzori et al., 2019b).

Another benefit of complementary seawater irrigation relies on the fact that moderate saline stress has been often associated with an increase in plant-based compounds that demonstrate healthy properties for humans (Di Baccio et al., 2004; Sgherri et al., 2008). Plants cope with salinity by means of several strategies including selective accumulation or exclusion of ions, synthesis of osmotic solutes, induction of antioxidant compounds (Parida and Das 2005) and secondary metabolite production (Ramakrishna and Ravishankar, 2011), most of which show positive effects on human health. Thus, halophytes under salinity condition could also become sources of biochemical compounds with the potential of additional nutritive value (Flowers and Muscolo 2015). *Tetragonia tetragonioides* (Pallas) Kuntze, Aizoaceae, Caryophyllales -,the common New Zealand spinach, and hereafter referred to as simply Tetragonia–is an annual herbaceous plant native to cool sandy and rocky seacoasts, notably in New Zealand, Japan, Argentina and Chile, now widely distributed throughout the world (Taylor, 1994). It is used as a vegetable, an ornamental ground and for medicinal purposes due to its anti-ulcerogenic and anti-inflammatory characteristics (Yousif et al., 2010a). Tetragonia is a salt-tolerant plant and several trials have shown that it may withstand an electrical conductivity (EC) of the growing medium as high as 10 dS m^{-1} (Neves et al., 2008; Wilson et al., 2000). One study identified a salt-induced growth response at salinity levels of 50-100 mM NaCl (EC 5-10 dS m^{-1}) (Yousif et al., 2010b), though this salt-stimulated growth appeared to depend greatly on the age of the plant, which was further able to tolerate up to 17.4 dS m^{-1} in late-salinization treatments (Wilson et al., 2000). Similarly, in hydroponics conditions, Ahmed and Johnson (2000) set a salinity tolerance threshold for this species at an EC value around 12.5 dS m^{-1}. Literature data on salinity tolerance refer solely to saline irrigation using NaCl solutions, whereas no information is available on the salinity tolerance of *Tetragonia tetragonioides* using seawater. Interestingly, for most species, salt stress tolerance seems to be higher when treated with seawater than with NaCl solutions treatments with the same EC (Boyko and Boyko,

1966). Further research is still needed to confirm such a statement, yet Sakamoto et al. (2014) suggest a similar assumption. In addition, this plant has been proposed as a salt-removing species, because of its high Na^+ and Cl^- uptake (Neves et al., 2014). Salt-removing species include grasses, shrubs and trees that can extract salts from contaminated soils. In contrast to costly desalination technologies such as thermal (distillation) processes, membrane-based processes, electro dialysis and reverse osmosis (Islam et al., 2019), phytodesalination is a cost-effective green technology for the remediation of salt-impacted sites (Hasanuzzaman et al., 2014). The same principle can also be tested in hydroponics, to assess a salt-removing species- capability of desalinating saline water (Islam et al., 2019). However, the salt removal potential of this plant has not been assessed in seawater-fed hydroponic systems. The current study thus had the aims of *i)* evaluating the effects of seawater irrigation on growth productivity of *Tetragonia tetragonioides* in hydroponic culture, *ii)* assessing the accumulation of ions and the production of osmotic solutes along with secondary metabolites related to physiological adaptation and to the nutritive value of the crop in response to different salinity levels, and *iii)* assessing the salt removal capability at increasing seawater concentrations in hydroponic conditions.

2 Materials and methods

2.1 Experimental design, plant material and growth conditions

The trial was carried out in 2018 at the greenhouse facilities of the Department of Agricultural, Food, Environmental and Forestry Sciences and Technologies (DAGRI) at the University of Florence, Italy. A hydroponic system was set up with 18 plastic containers (4 L volume) that were continuously aerated. Seeds of *Tetragonia tetragonioides* (Pallas) Kuntze were obtained from the Tuttosemi company (www.tuttosemi.com) and germinated in a dark chamber at 18.5°C starting from the 27th of July. Two months later, young plantlets were transplanted into 5 cm mesh pots filled with expanded clay and transferred to a polystyrene layer (one plant per container) that was used as a support in the hydroponic floating system. Half-strength Hoagland solution (Hoagland, 1938) was used as the growing medium for an additional 10 days. Throughout the trial, plants were maintained at a relative humidity ranging from 40 to 55%, natural light with the light intensity reaching 700 µmol m^{-2} s^{-1} during sunny days and 28°C/18°C

day/night air temperature. Plants were grown under three different EC levels: control (half-strength Hoagland solution, EC = 1.5 dS m^{-1}); medium (15% seawater and 85% half-strength Hoagland solution, EC = 9.8 dS m^{-1}); and high (30% seawater and 70% half-strength Hoagland solution, EC = 18.0 dS m^{-1}) seawater share, with a total of 6 plants randomly assigned per treatment. The seawater used in this experiment was collected at Marina di Pisa (Italy) one week before the beginning of the experiment and stored at 4˚C. Seawater chemical and physical characteristics are reported in Table 1. Starting from October 8th for 2 weeks, plants were gradually acclimatized to salinity by increasing the seawater concentration by 5% every 2-3 days until reaching the final concentration on October 22nd, which represents the starting day of the experiment.

Samples from the nutrient solution were collected twice a week, and pH and EC were measured by a laboratory pH meter (pH meter PHM 210 Meter Lab, Radiometer Analytical). The nutrient solutions were replaced every two weeks. The trial lasted 9 weeks and was designed to cover one complete crop cycle (60 days approx.).

2.2 Growth, biomass yield and morphological parameters

The biomass increase of the crop was determined by weighing all plants along with the pot on a weekly basis. After the final sampling the entire plant's weight was obtained. Whole plant fresh weights during the trial are reported to show plant growth over time. The dry weight of plants collected at the final destructive harvest were instead used to calculate the relative growth rate, as follows:

$$RGR = (\ln DW_f - \ln DW_i)/\Delta t \qquad \text{(Equation 1)}$$

where $\ln DW_f$ is the natural logarithm of the plant's dry weight at the end of the trial, $\ln DW_i$ is the natural logarithm of the plant's dry weight at the beginning of the trial, and Δt is the number of days between the beginning and the end of the trial (Pérez-Harguindeguy et al., 2016). At harvest, fresh leaf samples from 6 replicates per treatment were collected, frozen in liquid nitrogen and stored at -80˚C for further analyses. Subsequently, plants were divided into leaves, stems and roots, and weighed separately. Pictures of all leaves from 6 plants per treatment were obtained to calculate the leaf area (LA) using ImageJ software.

Afterwards, all samples were oven-dried (70˚C to constant weight) and dry leaf, stem and root biomass was determined. Moreover, the specific leaf area (SLA), leaf succulence, leaf dry matter content (LDMC) and leaf water content (LWC) were determined on 6 replicates per treatment to investigate possible morphological responses to salinity, as follows:

$$SLA = L_A/L_{DW} \quad \text{(Equation 2)}$$

where L_A is the leaf area (cm^2) and L_{DW} the leaf dry weight (g), according to Hunt et al. (2002)

$$\text{Leaf succulence} = L_{FW}/L_A \quad \text{(Equation 3)}$$

where L_{FW} is the leaf fresh weight (g) and L_A the leaf area (cm^2) (Agarie et al., 2007; Jennings, 1976)

$$LDMC = L_{DW}/L_{FW} \quad \text{(Equation 4)}$$

where L_{DW} is the leaf dry weight (g) and L_{FW} the leaf fresh weight (g) (Garnier et al., 2001)

$$LWC = (L_{FW} - L_{DW})/L_{FW} \quad \text{(Equation 5)}$$

where L_{FW} is the leaf fresh weight (g) and L_{DW} the leaf dry weight (g)

2.3 Water use efficiency, water productivity and water footprint

Crop evapotranspiration (ET) was recorded biweekly by measuring the volume of solutions for each treatment before replacing the nutrient solution. Water use efficiency (WUE) was calculated as the ratio between the whole plant dry biomass and total ET throughout the crop cycle, as follows:

$$WUE = DW_{\text{whole plant}} / ET \quad \text{(Equation 6)}$$

where $DW_{whole\ plant}$ is the whole plant dry weight (g), ET is the crop evapotranspiration (L)

Water productivity (WP) was used to better correlate the biomass production and ET, as the fresh shoot is the edible part of the species under consideration. This parameter was calculated as the ratio between the fresh marketable biomass and total ET throughout the crop cycle, as follows:

$$WP = FW_{leaves} / ET \qquad \text{(Equation 7)}$$

FW_{leaves} is the fresh weight of the edible and marketable leaves (g), ET is the crop evapotranspiration (L), according to Atzori et al. (2016).

The crop water footprint (WF) under different treatments was calculated as the ratio between total ET and the fresh marketable biomass, as follows:

$$WF = ET / FW_{leaves} \qquad \text{(Equation 8)}$$

where ET is the cumulative crop evapotranspiration (L), FW_{leaves} is the fresh weight of the edible and marketable leaves (g) at harvest, according to Atzori et al. (2019a).

2.4 Leaf gas-exchange parameters

Leaf gas-exchange parameters were determined using the open gas-exchange system Li-6400 XT (Li-Cor, Lincoln, NE, USA) weekly on 6 plants per treatment. Net photosynthetic rate (A_n) and stomatal conductance (g_s) were measured on the youngest fully expanded leaves from the apex at ambient relative humidity, reference CO_2 concentration of 400 µmol mol^{-1}, flow rate of 400 µmol s^{-1}, chamber temperature of 25˚C and photosynthetically active radiation (PAR) of 700 µmol m^{-2} s^{-1}.

At the end of the trial, total pigment concentration was determined by reading the absorbance at 665, 652 and 470 nm of methanol extracts obtained from randomly selected fully-expanded leaves from 6 replicates per treatment. Chlorophyll a (Cha), chlorophyll b (Chb) and carotenoid (Car) concentrations were determined according to Wellburn (1994) using a Tecan Infinite 200 spectrophotometer (Männedorf, Switzerland).

2.5 Root respiration

Root respiration was measured on root samples (6 replicates per treatment) cut just prior to the measurement. An oxygen electrode (Rank Brothers, Ltd, Cambridge, England), prepared and calibrated according to the manufacturer instructions, was used to assess the root respiration rates. Roots samples of 1 cm from the tip (0.1 g) were cut from plants of all treatments, weighed and placed in the electrode chamber with 2 mL of fresh incubation solution (BSM). The amount of oxygen (nmol ml^{-1} O_2) consumed after 15 min in the dark (respiration rate) was recorded. After normalizing the respiration rate on the weight of the root sample used, linear regression curves were obtained and the relative slopes were compared in order to assess significant differences among treatments.

2.6 Concentration of mineral elements in plant tissues

Oven-dried leaf, stem and root ground samples (0.1 g, 6 replicates per treatment) were mineralized into Teflon vessels using a CEM microwave Mars Xpress with 10 ml of HNO_3. The microwave settings were: power 1600 W applied at 100%; ramp of 15:00 minutes to reach 200°C; held for 15:00 minutes. At the end of this process, the final volume of the solution was obtained by adding 25 ml of water 18 MΩ and diluted extracts were analyzed for Na, K, Ca, Fe, Mg, Cu, Mn, P and Zn concentrations determined by means of ICP OES (Inductively Coupled Plasma - Optical Emission Spectrometer) Thermofisher Iris Intrepid II, based on Atomic Emission Spectroscopy.

2.7 Sodium localization through confocal microscopy

Sodium identification and localization were performed through confocal imaging on leaf samples using an upright Leica Laser Scanning Confocal Microscope SP5 (Leica Microsystems, Germany) equipped with a 63x oil immersion objective. Tetragonia leaves were infiltrated with a 10 µM CoroNa-Green (Molecular Probes,

USA) solution. After 2 h of incubation, small sections of the infiltrated leaves were mounted in a water solution on a slide and observed. The excitation wavelength was set at 488 nm, and the emission was detected at 510 − 520 nm, according to Cuin et al., 2011).

2.8 Phenolics, nitrates and proline concentration in edible leaves

The total phenolic concentration was determined using the Folin-Ciocalteu method. Leaf tissue of 6 replicates per treatment was mechanically ruptured using the TissueLyser II system (QIAGEN, cat. no. 85,300) for 30 s at 20 Hz. 1 mL of ice-cold MetOH (95%, v/v) was added to each sample, and then incubated at room temperature for 1 h in the dark. The extract was used to measure the total phenolic concentration as described by Ainsworth and Gillespie (2007). The absorbance of samples and standard curve were measured at the wavelength of 765 nm with a microplate reader (Tecan, Infinite 200). The calibration curve ranged from 20 to 500 mg/ml (R^2 = 0.997). The reported values are expressed as µg/g, gallic acid equivalents (GAE). Nitrate concentration in leaves was determined after shaking dry samples in water for 2 h (5 replicates per treatment). Filtrated samples were left to react with sulfosalicylic acid and sodium hydroxide, cooled and read at 410 nm in a UV-visible spectrophotometer (Bio-Rad SmartSpec^{TM}Plus), using a standard curve for KNO_3 as in Cataldo et al. (1975). The values of the calibration curve ranged from 0.2 to 1 mg/ml of KNO_3 (R^2 = 0.987). Proline concentration in leaves was determined according to Bates et al. (1973) on ground, frozen leaf samples using sulfosalicylic acid, acid-ninhydrin and acetic acid. The sample absorbance was read at 520nm in a UV-visible spectrophotometer (Bio-Rad SmartSpec^{TM}Plus), using a standard curve for L-proline as a standard. The values of the calibration curve ranged from 0 to 0.312 mM L-proline (R^2 = 0.998).

2.9 Relative phytodesalination rate

The relative phytodesalination rate (RPR) of the tested species was determined on 4 replicates per treatment, according to (Rabhi et al., 2015), and expressed as the measure of shoots aptitude to accumulate sodium ions per unit of biomass per unit of time, as follows:

$$RPR\ (mg\ Na^+\ g^{-1}\ DW\ day^{-1}) = RGR * (Na_f^+ - Na_i^+) / (DW_f - DW_i) \quad \text{(Equation 9)}$$

where RGR is the relative growth rate, Na_f^+ is the concentration of sodium in leaves at the end of the experiment, Na_i^+ is the concentration of sodium in leaves at the beginning of the experiment, DW_f is the dry weight of leaves at the end of the experiment, DW_i is the dry weight of leaves at the beginning of the experiment.

2.10 Statistical analyses

The experimental set-up followed a complete randomized design to uniform experimental conditions. All collected data were analyzed through one-way ANOVA using GraphPad Prism 5 for Windows (GraphPad software Inc, California, USA). Posthoc comparisons (Tukey HSD) were made to contrast the levels of the independent variables, and differences were deemed significant when $p \leq 0.05$.

3 Results

3.1 Growth

As reported in Figure 1, no significant differences in growth were assessed throughout the trial between salt-treated plants and the control, even if a decreasing trend was observable for high seawater treated plants during the last three weeks of the experiment. Similar results were found for the RGR, where control plants showed a rate of 1.4 ± 0.4 g g^{-1} day^{-1} and medium and high seawater treated plants a rate of 1.2 ± 0.1 and 1.2 ± 0.2 g g^{-1} day^{-1} respectively. No significant differences among treated and control plants were observed. Regarding the morphological screening of leaves, both leaf water content and leaf dry matter content were not affected by salinity (Table 2). By contrast, leaf succulence did significantly increase compared with the control with increasing salinity, whereas the leaf area and the specific leaf area decreased in high seawater treated plants compared with both the medium salinity treatment and the control.

3.2 Water consumptions, WUE, WP, WF

Figure 2 shows the water-related parameters. Both seawater treatments showed a significant decrease in terms of plant water use compared with the control (Fig. 2A), with a decrease in medium and high seawater treated plants of 30% and 31%, respectively, compared with the control. By contrast, seawater increased the WUE in both medium and high seawater-treated plants compared with the control (Fig. 2B).

Similarly, WP increased in both seawater treatments, even if significantly only in medium seawater treated plants (Fig. 2C). Lastly, the WF (Fig. 2D) significantly dropped for both seawater treatments (76% in medium seawater and 71% in high seawater treatment) compared with the control.

3.3 Leaf gas-exchange parameters

Figure 3 shows the results of the photosynthetic rate (Fig. 3A) and stomatal conductance rate (Fig. 3B) measurements under different growing conditions. At the very beginning of the trial both seawater treatments negatively affected the net assimilation rate (A_n). In particular, the medium seawater treatment initially lead to a decrease of the A_n, but began to recover starting from the 4th measurement such that by the end of the trial it had reached the level of the control. On the other hand, in the high seawater treatment A_n decreased starting from the 3rd measurement onwards. The stomatal conductance rate showed a decreasing trend compared with the control in both treatments starting from week 4 (for both seawater treatments) and onwards for the high salinity treatment.

Medium seawater treatment did not negatively affect the Cha and carotenoid concentrations but decreased the Chb concentration in leaves (Table 3). By contrast, high seawater treatment reduced the concentration of all pigments compared with the control.

3.4 Root respiration

As reported in Table 4, the slopes of the root respirations curves of plants did not present any significant differences among the three treatments.

3.5 Concentration of mineral elements

Table 5 reports the concentration of mineral elements accumulated in leaves, stems and roots. Seawater treatments led to a significantly higher accumulation of Mg (31% and 48% in medium and high seawater treatments compared with the control), Cu (14% and 30%, respectively) and Na (79% and 82%, respectively) in the three tissues, with roots also accumulating higher amounts of P and Zn compared with control plants. However, seawater led to a significant decrease in P, K, Ca and Fe in leaves; of K and Ca in stems; of Ca in roots.

Figure 4 reports absence and presence of sodium in the bladders cells of Tetragonia in control (A) and saline (B) conditions, respectively. Images assessed a qualitative increase of sodium in seawater treated leaves compared with control ones. In particular, sodium accumulation occurred in the bladder cells located on the leaves surface.

3.7 Nutritional characterization of edible leaves

Total phenolics (Fig. 5A) and nitrates (Fig. 5B) did significantly decrease in seawater treated plants compared with the control. The concentration of proline instead increased accordingly with increasing salinity (Fig. 5C) of 43% and 61% in medium and high seawater treated plants compared with control conditions, respectively.

3.8 Phytodesalination capacity

As reported in Fig. 6, the relative phytodesalination rate was significantly higher in seawater treated plants compared with the control. However, despite the difference in EC of the two seawater treatments (i.e. 9.8 and 18.04 dS m^{-1}, respectively), no significant differences in the salt-removing capacity were assessed between the two groups of plants grown with seawater.

4 Discussion

4.1 Growth and morphological responses to increased salinity

The current trial shows that plant growth was not negatively affected by any seawater treatments even if a decreasing trend is observable in the last weeks of the experiment in 30% seawater treated plants. The results obtained in medium seawater treatment (EC 9.8 dS m^{-1}) are in agreement with those found by other scientists Neves et al. (2008) and Wilson et al. (2000), who reported a salinity tolerance for Tetragonia at EC approx. 10 dS m^{-1}. Similarly, Ahmed and Johnson (2000) found in hydroponic conditions a salinity tolerance threshold at EC = 12.5 dS m^{-1}. By contrast, in the current trial, the results for high seawater treatments (18.0 dS m^{-1}) suggest a remarkably higher tolerance threshold, comparable only to the results obtained by Wilson et al. (2000) on well-developed plants. Nevertheless, even if not significantly, high seawater treated plants showed a growth reduction in the last two weeks of the trial. This could be due to the significant reduction in the net assimilation rate at the end the experiment. However, this drop occurred at the very

end of the crop cycle and the final biomass did not suffer a significant reduction. Regarding the morphological adaptations, the increasing in leaf succulence with increasing salinity represents a common response to salt stress. Halophytes are known for maintaining their growth rate in saline conditions through osmotic adjustment (Flowers and Yeo, 1986). The increase in leaf succulence, (i.e. the water content per unit area) is one of the mechanisms plants use to respond to a low external water potential induced by salinity (Flowers and Colmer 2008). Moreover, in accordance with dicotyledonous halophyte behaviors, such morphological changes allow high carbon assimilation rates per unit area, ensuring high growth rates despite decreased SLA, (Atzori et al., 2017; Ayala and O'Leary, 1995; de Vos et al., 2013, 2010; Geissler et al., 2009; Rozema et al., 2015) which is another strategy used by plants to reduce transpiration water loss (Flowers and Flowers, 2005).

4.2 Water saving

The decrease of water use observed in this trial with increasing salinity can be explained, to a certain extent, by the limited water uptake and translocation in salt stressed plants due to decreased transpiration rates (expressed by the stomatal conductance) under saline conditions. In addition, decreasing LA, SLA and increasing leaf succulence in seawater treated plants also limited the transpiration. The higher WUE of seawater-treated plants led to biomass yields comparable to the control: this was particularly true for medium seawater-treated plants, where the biomass produced per liter of (sea)water used was significantly higher than the control. Similar results have been observed on other species. For instance, *Plantago coronopus* L. grown at different levels of salinity showed an increase in WUE with increasing salt concentrations (Koyro, 2006). By contrast, salt-sensitive species are generally characterized by a decrease of WUE in saline conditions (Katerji et al., 2003). The increased WP, observed in medium seawater treatment, sets the optimum salinity for the tested crop, even if further studies should be made on the salinity range between 15% and 30% seawater. In line with other parameters, WF for both seawater-treated plants was significantly lower compared with the control. Interestingly, both seawater treatments showed the same WF values, thereby suggesting that the medium seawater treatment, reducing crop water use and increasing water productivity, is likely the most justified.

4.3 Leaf and root physiological adaptations

Both chlorophyll a (in high seawater treatment) and b (in both seawater treatments) decreased with increasing salinity of the growing medium. Such a decrease, however, seemed to affect the plants' photosynthetic apparatus only by the end of the cycle, suggesting that also pigments reduction occurred at the same time. These findings are consistent with other studies on halophytes showing a decrease in chlorophyll pigments under saline conditions (Aghaleh et al., 2009; Ayala and O'Leary, 1995; Koyro et al., 2013; Parida et al., 2002). The accumulation of mineral elements in shoots and roots represents another crucial physiological adaptation to salinity. In our trial, plants exposed to seawater showed higher Mg, Cu and Na concentration in both shoot and root tissues, and a decrease of P, K, Ca and Fe in leaves and of Ca in roots compared with control. The high amount of Na represents one of the most common responses of halophytes to salinity. It has been shown that Tetragonia, as many salt-tolerant includer species (Neves et al., 2008; Yousif et al., 2010b), may accumulate sodium in its vacuoles and use it as an osmoticum (Glenn et al., 1999). The different accumulation patterns of the other elements in tissues might also play a role in osmotic adjustment if they were efficiently compartmentalized at the cell level (Ghoulam et al., 2002). Root respiration rates did not change among treatments. A study on the grey mangrove, *Avicennia marina (Forssk.) Vierh.*, *1907*, found that a concentration of 25% seawater led to an increased respiration compared with both control and higher salinity conditions, following the same pattern of the growth responses of the plant (Burchett et al., 1984). Moreover, another trial found a rather small increase in root respiration for *S. physophora* Pall. that was correlated with its high salt tolerance capacity (Liangpeng et al., 2007). Our results might be explained by the fact that the seawater treatments used in the present trial neither increased nor reduced the plants growth compared with the control.

4.4 Nutritional properties of Tetragonia with increased salinity

Since leaves are the edible parts of Tetragonia, the accumulation of Mg, and Cu following seawater exposure might represent an interesting improvement of nutritional value achievable in salinity conditions. Magnesium and copper are in fact among the mineral elements most frequently lacking in human diets (White and Broadley, 2009), with deficiencies common in both developed and developing countries. It is noteworthy that agricultural products are the primary source of all nutrients. Agricultural systems cannot

fail in providing enough products containing adequate quantities of nutrients, otherwise dysfunctional food systems would result in not supporting healthy lives (Welch and Graham, 2004). To address this issue, agronomic approaches to increase the concentrations of mineral elements in agricultural products (i.e. biofortification) are of interest (Lynch, 2007). Seawater irrigation seems to be a feasible option for the biofortification of crops. Interestingly, species from families within the Caryophyllales tend to accumulate very high Mg and Zn amounts in leaves (White and Broadley 2009; Broadley et al. 2004; White 2001). In contrast, the increase of Na concentration in leaves could represent a severe concern for the healthy characterization of the crop. In fact, sodium excesses in the human diet are related to cardiovascular disease risk (O'Donnell et al., 2015). Nevertheless, the New Zealand spinach is a species requiring cooking before consumption. In a recent study on the common spinach, Caparrotta et al. (2019) assessed a significant reduction in the sodium content of leaves after processing by boiling and steaming.

The total phenolics decrease under saline conditions suggests that Tetragonia enhanced the production of other compounds to act as compatible solutes for osmotic adjustment, i.e. proline, in accordance to previous studies (Yousif et al., 2010a). Proline is known to have a positive effect on enzyme and membrane integrity and to show adaptive roles in mediating osmotic adjustment in plants exposed to abiotic stress (Ashraf and Foolad, 2007). Interestingly, proline plays an essential role in protein synthesis and structure, metabolism and nutrition: therefore, physiological needs for proline are particularly high during animal and human life cycles (Wu et al., 2011). Likewise, the decrease in nitrates in seawater-treated plants represents another important achievement from a nutritional standpoint. Our results are in line with a study on another halophyte, *Portulaca oleracea* L., that showed a decrease of nitrate levels accordingly to salinity (Franco et al., 2011). Some authors relate this reduction to an increase in chloride concentrations within the plant (Roussos et al. 2007). However, this aspect needs further investigation on the tested crop.

4.5 Salt removal potential and prospective of Tetragonia crop in saline agriculture

Although a complete and holistic approach on plant, and associated rhizosphere microorganisms, impacts in the salt-affected soil system (or liquid nutritive solution) is not fully explored in the literature, the main mechanisms are well established (Jesus et al., 2015). In particular, there are two main mechanisms to

explain the role of halophyte plants in the remediation of salt-affected soils: the first one is pH reduction, which increases the dissolution of $CaCO_3$ and, therefore, the available Ca^{2+} for cation exchange with Na^{+} (Walker et al., 2014). The second mechanism is plant uptake of dissolved salts in general, sodium in particular (Rabhi et al., 2015). Our results confirmed this latter strategy in accordance with previous studies assessing this species as the best salt removing crop among many others (Neves et al., 2008). Although none of the tested salt concentrations has resulted in biomass loss, focusing on the concerns raised on water use and the nutritional value of the edible parts, the best salinity conditions for the Tetragonia seem to be between the 15% and 30% seawater concentrations. The already appreciated taste of saline agriculture vegetables in different countries (Rozema and Schat 2013), and of the New Zealand spinach in particular, also encourage such a possibility. According to our results, only the Na concentration in the edible leaves could constitute a concern for the healthy characterization of the crop, yet the cooking processes can help in remarkably reducing its content.

5. Conclusions

This species' ability to achieve remarkable growth rates under saline conditions validates its potential in saline environments. The results of this study show that the production of the New Zealand spinach as a food can be obtained in hydroponic conditions characterized by salinity as high as 18 dS m^{-1}. Plant water use dropped in saline conditions, yet thanks to an increased WUE the biomass production was not negatively affected, again validating the seawater irrigation of this species up to the tested EC. Seawater introduction in the hydroponics solutions also led to the enhancement of nutritional value. Such characteristics along with the increased leaf succulence provide the edible leaves with a taste and consistency that could be particularly appreciated by consumers.

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FIGURE

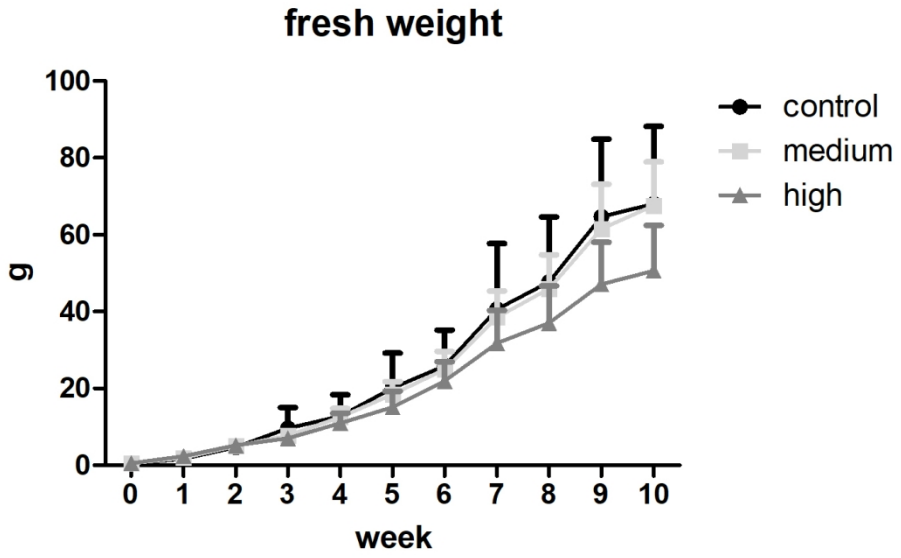

Figure1. The fresh weight of whole plants. Values are single plants weight means (*n* = 6) ± SEM expressed in grams. No significant differences were assessed at *P* < 0.05 (Tukey's Test)

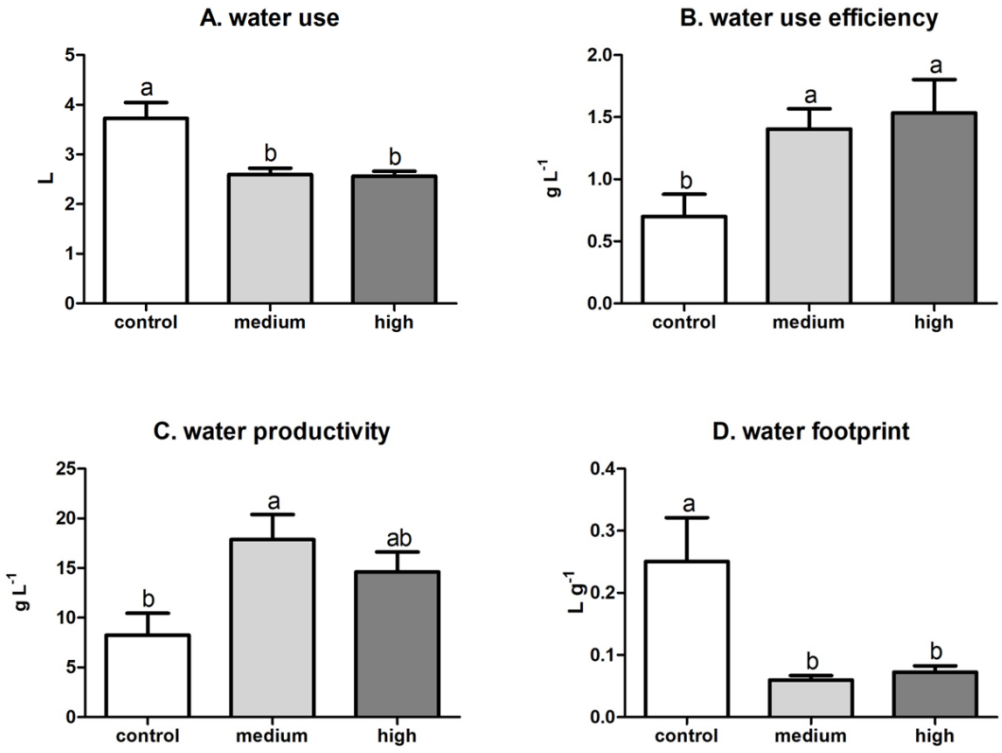

Figure 2. Plant water parameters at the end of the trial. A, Water use per plant (L); B, WUE per plant (g L^{-1}); C, WP per plant (g L^{-1}); D, WF per plant (L g^{-1}). Data are means (*n* = 6) ± SEM. Different letters in the same graph indicate significant differences among treatments at *P* < 0.05 (Tukey's Test)

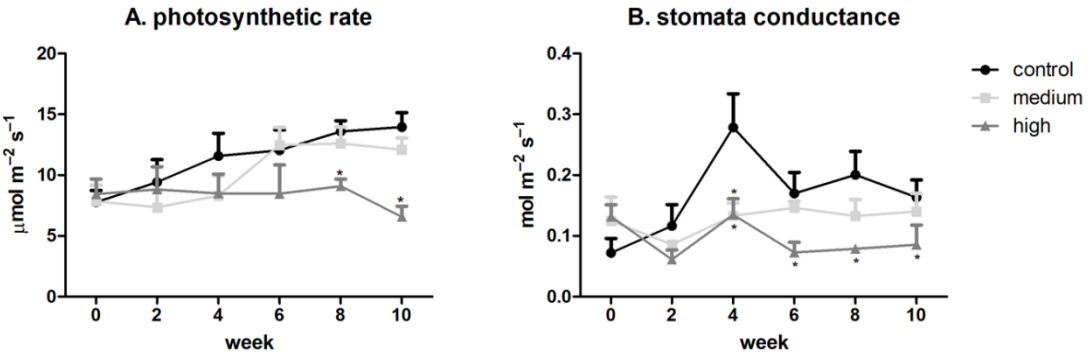

Figure 3. A, Photosynthetic rate A_n (A); stomata conductance g_s(B). Data are means (*n* = 6) ± SEM, asterisks represent significant differences compared to the control at *P* < 0.05 (Tukey's Test)

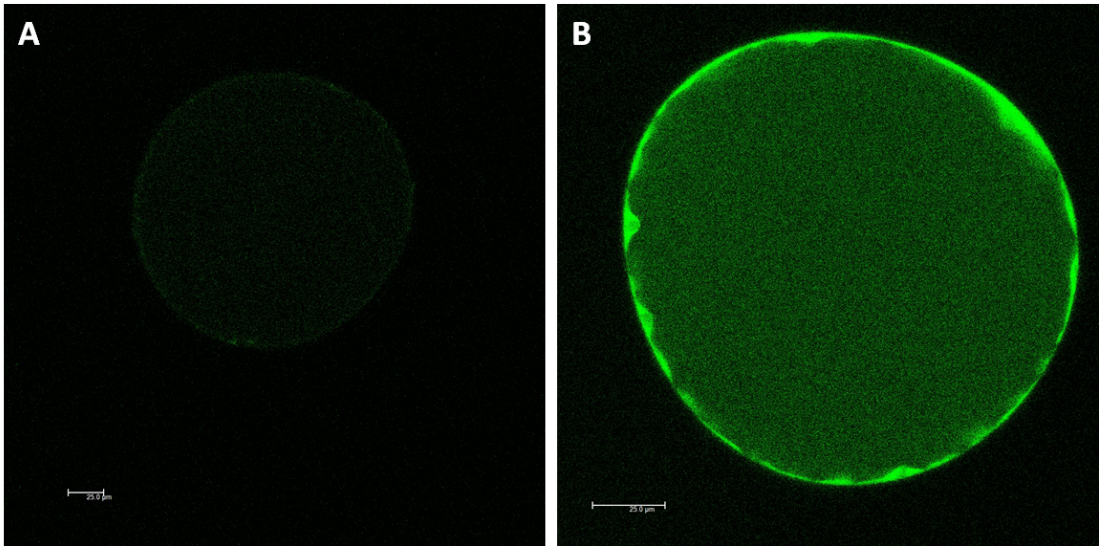

Figure 4. Na accumulation and intracellular distribution in control (A) and 30% seawater (B) treated leaves bladder cells visualized by the CoroNa Green fluorescent dye after 30 days of trial. One typical image for each treatment is shown. All images were taken using the same settings and exposure times to enable direct comparisons.

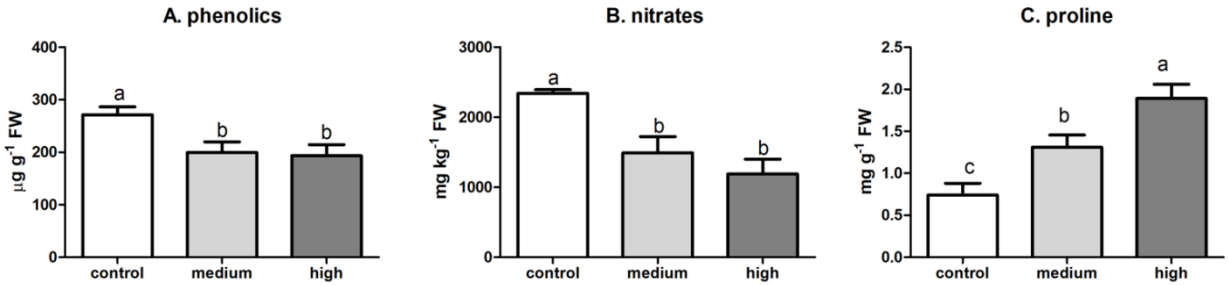

Figure 5. Phenolics (A); nitrates (B); proline (C) concentration in leaves. Values are means (*n* = 6). ± SEM. Different letters in the same graph indicate significant differences at *P* < 0.05 (Tukey's Test)

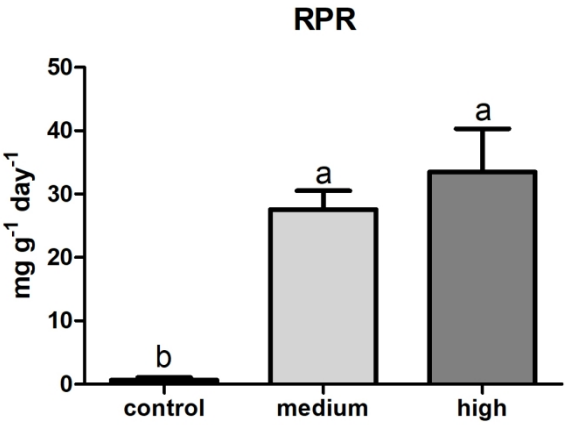

Figure 6. Relative phytodesalination rate. Data are means (*n* = 5) ± SEM expressed in mg g^{-1} day^{-1}, different letters indicate significant differences at *P* < 0.05 (Tukey's Test)

TABLE

Table 1: Seawater chemical and physical characteristics

Na	K	NO_2-N	Silicates	PO_4	NO_3-N	pH	EC
mg L^{-1}	mg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}		dS m^{-1}
11,300	400	0.013	0.048	0.01	0.383	7.74	54

Table 2. Morphological leaf traits of Tetragonia under different treatments

Treatment	LA (cm^2)	SLA ($cm^2\ g^{-1}$)	Leaf succulence ($g\ cm^{-2}$)	LWC	LDMC
Control	643.3 ± 69.3^a	344.3 ± 26.36^a	0.1 ± 0.001^c	0.9 ± 0.005	0.1 ± 0.005
Medium	606.8 ± 52.9^a	303.4 ± 6.14^a	0.1 ± 0.001^b	1.0 ± 0.001	0.05 ± 0.001
High	252.5 ± 82.0^b	210.3 ± 10.84^b	0.1 ± 0.002^a	0.9 ± 0.003	0.1 ± 0.003

LA is the leaf area expressed in cm^2; SLA is the specific leaf area expressed in $cm^2\ g^{-1}$; leaf succulence is expressed in g of DW on the leaf area; LWC is leaf water content; LDMC is leaf dry matter content. Values are means (n = 6) ± SEM. Different letters in the same column indicate significant differences at $P < 0.05$ (Tukey's Test)

Table 3. Pigments concentration in Tetragonia leaves under different treatments

Treatment	Cha µg g^{-1}	Chb µg g^{-1}	Car µg g^{-1}
Control	253.9 ± 23.8^{a}	71.3 ± 4.3^{a}	51.7 ± 0.02^{a}
Medium	203.2 ± 16.7^{ab}	57.9 ± 3.4^{b}	50.4 ± 2.7^{a}
High	145.2 ± 15.8^{b}	45.6 ± 2.1^{c}	37.0 ± 4.1^{b}

Values are means (*n* = 6). ± SEM. Different letters in the same column indicate significant differences at *P* < 0.05 (Tukey's Test)

Table 4. Slopes of the root respiration curves

Treatment	Root respiration's curve slope
Control	-0.00161 ± 0.00018
Medium	-0.00231 ± 0.00016
High	-0.00200 ± 0.00026

Values are means (*n* = 6) ± SEM. No significant differences were assessed at *P* < 0.05 (Tukey's Test)

Table 5. Mineral element concentration in Tetragonia leaves, stems and roots, under different treatments

		Element concentration (mg kg^{-1})								
Tissue	Treatment	P	K	Ca	Fe	Mg	Mn	Cu	Zn	Na
Leaf	Control	3513 ± 230^{a}	69213 ± 2409^{a}	11004 ± 1176^{a}	106.1 ± 8.9^{a}	6969 ± 231^{c}	167.3 ± 16.8	6.9 ± 0.9^{b}	68.1 ± 2.9	11680 ± 883^{c}
	Medium	3054 ± 90^{a}	29569 ± 1252^{b}	4908 ± 191^{b}	69.9 ± 2.6^{b}	10130 ± 372^{b}	202.4 ± 15.7	8 ± 0.4^{ab}	74.2 ± 3.1	56339 ± 2706^{b}
	High	2459 ± 92^{b}	24844 ± 1968^{b}	4399 ± 123^{b}	47.7 ± 2.7^{c}	13350 ± 456^{a}	161.1 ± 16	9.8 ± 0.6^{a}	68.4 ± 5.3	65176 ± 2665^{a}
Stem	Control	3710 ± 287	62788 ± 428^{a}	15750 ± 1699^{a}	74.1 ± 17.3	3917 ± 232^{c}	64.5 ± 5.4	5.4 ± 0.7^{b}	51.1 ± 4.6	9100 ± 321^{b}
	Medium	3393 ± 121	38648 ± 1348^{b}	4620 ± 344^{b}	58.2 ± 5.6	6876 ± 263^{b}	54 ± 8.6	6.1 ± 0.5^{b}	61.7 ± 5.2	48272 ± 2262^{a}
	High	3216 ± 86	26606 ± 2518^{c}	4307 ± 737^{b}	41.6 ± 5.4	10732 ± 912^{a}	58.1 ± 11.7	9.3 ± 0.9^{a}	71.2 ± 8.6	60844 ± 8367^{a}
Root	Control	4872 ± 1602^{b}	34819 ± 6110	7105 ± 214^{a}	3860 ± 1553^{ab}	14460 ± 1193^{b}	761.6 ± 24.9	19.2 ± 3.8^{b}	86.3 ± 17.7^{b}	1291 ± 163^{c}
	Medium	4733 ± 88^{a}	38919 ± 1784	2678 ± 164^{b}	2570 ± 148^{a}	14872 ± 283^{a}	204.6 ± 31.7	16.7 ± 0.4^{b}	147.3 ± 8.9^{b}	16531 ± 967^{b}
	High	4000 ± 61^{ab}	27875 ± 1358	2813 ± 303^{b}	1727 ± 245^{b}	16211± 471^{a}	300.4 ± 61.7	46.5 ± 8.2^{a}	242.7 ± 32.7^{a}	22369 ± 1270^{a}

Values are means (n = 6) ± SEM. Different letters on the same column denote a significant difference among the treatments at P < 0.05 (Tukey's Test)