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Potential and constraints of different seawater and freshwater blends as growing media for three vegetable crops

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ABSTRACT

Alternative water sources for irrigation are needed to be found, as agriculture is currently using the 70% of total freshwater. Seawater use for growing crops has long been studied; while an agriculture based on pure seawater is currently impossible, seawater hydroponics may be viable, not aggravating salinization problems in soils. This work aimed at assessing the possibility of growing lettuce, chard and chicory with 3 seawater and freshwater blends (i.e. 5%–10%–15% of seawater). We investigated: i) crops growth, water consumptions, water use efficiency (WUE), water productivity (WP); ii) photosynthetic parameters; iii) principal mineral elements, soluble sugars and phenolics concentration. Lettuce productivity was negatively affected by 10% and 15% of seawater, whereas chard and chicory's growth were not affected by any blend. Interestingly, water consumptions dropped and WUE significantly upturned in every tested crop accordingly with increased seawater concentrations. Leaf concentration of Na^+ and of some other ions increased. We concluded that certain amounts of seawater can be practically used in hydroponics, allowing freshwater saving and increasing certain mineral nutrients concentrations.

1. Introduction

Water scarcity is a major constraint to food production required to meet the quantitative and qualitative change of the global demand in the twenty first century (FAO, 2011). According to several international organizations, the current demographic growth rate will lead to a world population of 9.6 billion people by 2050 (FAO, 2009) and up to 10.9 billion people by the end of the century (UN, 2013). Thus, the human pressure on water resources will tremendously escalate. In particular, irrigation is crucial to food production and its role is expected to increase further, especially in developing countries (FAO, 2002). Nevertheless, water availability is already problematic in many regions, the agricultural sector at present using around 70% of all water from aquifers, streams and lakes. Therefore, it seems necessary to augment food production without a proportional increase of freshwater use.

An additional problem about food security is malnutrition, the diets of over two-thirds of the world population nowadays lacking one or more essential mineral elements (White and Broadley, 2009). In fact, humans require at least 49 nutrients to meet their metabolic needs, 22 of which are mineral elements, and the inadequate consumption of even one of those will result in adverse metabolic disturbances (Welch and Graham, 2004). At present, over three billion people are afflicted with mineral element malnutrition, and the numbers are increasing. Those deficiencies are linked not only to an inadequate quantity of food, but also to its quality: calcium, magnesium and copper deficiencies, for example, are common in both developed and developing countries (Frossard et al., 2000). Among the other important nutrients, vitamins and phytochemicals (such as ascorbic acid, carotenoids, polyphenols and fibers) have beneficial effects in protecting key biological constituents, such as proteins, phospholipids and DNA (Szeto et al., 2004). Since the primary source of nutrients for people comes from agricultural products, those considerations should be taken into account when increasing, or optimizing, food production.

Looking for freshwater alternatives, the most freely abundant source of water on the Earth is represented by seawater. This resource is increasingly emerging as a feasible option in the agricultural sector, either desalinized or blended with other water sources (Yermiyahu et al., 2007). In fact, seawater is rich in most plant

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nutrients (Eyster, 1968), which are often the same nutrients representing limitations in human diets. Furthermore, it is found where around 40% of the world population currently resides. Thus, seawater use in agriculture could represent a strategy to decrease the freshwater demand of the agricultural sector, exploiting, at the same time, the seawater nutrient content.

It is since the early sixties of the last century that seawater use in agriculture has been studied (Boyko, 1996). While a sustainable agriculture relying on pure seawater on a large scale is still utopian, in other cases (e.g. horticulture) small-scale seawater irrigation may be economically viable (Breckle, 2009). Overall, the substitution of a certain amount of freshwater with seawater can be an interesting option in soil-less growing systems, where there are no risks of creating or aggravating salinization problems in soils. Among the many soil-less growing systems, hydroponic culture is characterized by an expanding worldwide vegetable production of about 35000 ha (Hickman, 2011). Of course, the use of high salinity water might affect plants in many ways, as for example causing water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization and genotoxicity (Munns, 2002). Hence, this option should be carefully tested before being adopted.

Previous studies on many crops tested at different seawater concentrations generally concluded that the use of seawater in the growing media does not negatively affect the productivity up to certain concentrations (Sakamoto et al., 2014; Turhan et al., 2014; Sgherri et al., 2008, 2007), with maximum thresholds changing according to the species. In addition, specific studies have shown that saline-water treatments may enhance the production of secondary metabolites with high-nutritional value and acknowledged properties in the prevention of important human diseases (De Pascale et al., 2001), increasing also the organoleptic value of some crops (Mitchell et al., 1991). Thus, the possibility of administering certain salt concentrations to increase the content of useful components has been considered (Sgherri et al., 2008). For example, in tomato crop an augmentation of endogenous antioxidant (Sgherri et al., 2007) and carotenoid (De Pascale et al., 2001) concentration was observed under salt stress conditions. Similar results, at least up to certain seawater concentrations, were obtained also on species generally considered less tolerant, such as lettuce (Turhan et al., 2014). Nevertheless, regarding this latter species, controversial results reporting a salinity-induced increase (Unlukara et al., 2008) or reduction (Bartha et al., 2015; Turhan et al., 2014; Kim et al., 2008) of plants dry matter may indicate possible diversities even among cultivars.

All these results suggest that the use of seawater has the potential to achieve horticultural crop biofortification, meaning the endogenous nutrients fortification of food (Ding et al., 2016). In any case, despite scientific literature offers a variety of information on salt effects for over 130 crop species, there are still missing data about many others (Shannon and Grieve, 1998), especially considering their production of nutritional compounds as a response to salinity stress.

The present study aims at investigating the effect of different seawater and freshwater blends on three of the most cultivated horticultural crops: lettuce (Lactuca sativa L. var. Canasta), Swiss chard (Beta vulgaris L.) and chicory (Cichorium intybus L.). Lettuce was chosen because of its cultivar-dependent salinity response, thus the largely diffused variety Canasta is here studied for the first time; chard and chicory because they have scarcely been investigated, despite their spread cultivation and consumption area. In addition, the choice of those species, grown according to their particular seasonality, enabled the experiment to cover a 6-month period, thus keeping the closed-cycle hydroponic system active for a long productive phase. On the basis of these considerations, and to achieve information about the salinity effect on plant growth, water use efficiency (WUE) and the concentration of some important nutritional compounds under the same experimental conditions, the present work specifically explore the possibility of: i) growing lettuce, chard and chicory, using a share of seawater, and ii) assessing if seawater in the growing media affected photosynthesis and the concentration of some important nutritional compounds, particularly mineral elements, pigments, soluble sugars and phenolics.

2. Materials and methods

2.1. Experimental design, plant material and growth conditions

The experiment was carried out in 2014 at the greenhouse facilities of the Department of Agrifood Production and Environmental Sciences at the University of Florence, Italy. A closed-cycle NFT (Nutrient Film Technique) hydroponic system was set up allowing a pump to deliver a continuous flow of nutrients through the plant roots, thereby maximizing the irrigation efficiency. Three common commercial crops were selected for the current trial: lettuce (Lactuca sativa L. var. Canasta), Swiss chard (Beta vulgaris L.) and chicory (Cichorium intybus L.). Such crops were chosen according to their different seasonality (i.e. lettuce and chard are summer crops and chicory is an autumnal crop) with the aim of keeping the NFT hydroponic system active for a continuous productive period, thus covering with the selected crops a 6-month period. The crops were grown according to the following schedule: May 16th to June 19th (lettuce); June 27th to July 31st (Swiss chard) and September 17th to October 30th (chicory), respecting their cycle length as in traditional soil cultivation, thus planning the final harvest at the commercial maturity time.

For each crop, 150 ten-day-old seedlings were bought at a nursery and transplanted into 5 cm mesh pots filled with expanded clay. Plantlets were grown for an additional 10 days in hydroponics supplied with a nutrient solution made of tap water – analyzed, found constant in time and the values fell within the world average values of tap water WSSC (2014) – and liquid fertilizer Flora Series™ (General Hydroponics Europe Inc). Throughout the trial, plants were maintained in normal humidity (relative humidity ranged from 40 to 55%) and without artificial light, light intensity reaching 700 $\mu mol\ m^{-2}\ s^{-1}$ during sunny days, at 28 °C/18 °C day/night temperature for lettuce and chard and at 23 °C/13 °C day/night temperature for chicory cultivation. The experimental setup consisted of 12 independent hydroponic lines, bearing 9 plants each, for a total of 3 randomly distributed hydroponic lines (27 plants) for each treatment. The nutrient solution flow was regulated by a timer that switched the system on 15 min per hour throughout the whole crop cycle. Seawater used in this trial was collected at Marina di Pisa (Italy) one week before the beginning of each experiment and stored in 20 l sterile tanks at 4° C. Characteristics of the collected seawater are reported in Table 1: Na^+ and K^+ values were measured with Flame Photometer Digiflame2000 (Lab Services SAS, Rome, Italy); NO_2, silicates, PO_4, NO_3 were measured with an automatic analyzer AA3 (Bran-Luebbe) according to Grashoff et al. (1983), pH and EC were measured with a portable pH meter (Hanna Instruments™).

Four different growing media (treatments) were used in the NFT system, corresponding to increased seawater concentrations added to the nutrient solution showing the following electrical conductivity (EC) values: control (tap water and nutritive solution) EC = 0.3 $dS\ m^{-1}$; A: 5% seawater EC = 3.4 $dS\ m^{-1}$; B: 10% seawater EC = 6.1 $dS\ m^{-1}$; C: 15% seawater EC = 9.2 $dS\ m^{-1}$. In addition, pH and EC were measured twice a week by using a portable pH meter (Hanna Instruments™). The growing media were replaced every two weeks and their chemical and physical characteristics are reported in Table 2.

Table 1
Seawater chemical and physical characteristics.

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

Table 2
Growing media chemical and physical characteristics: average values of the three species measurements.

Treatments								
	Na^+ mg l^{-1}	K^+ mg l^{-1}	NO_2 μg l^{-1}	Silicates μg l^{-1}	PO_4 μg l^{-1}	NO_3 μg l^{-1}	pH	EC dS m^{-1}
Cont	20.4	3.09	0.011	3.53	8.33	12.38	6.94	0.33
A	516.7	34.3	0.003	2.91	7.49	18.29	6.74	3.43
B	1380	60	0.003	3.08	7.91	18.27	6.77	6.12
C	1950	72	0.004	2.57	7.35	17.89	6.8	9.19

2.2. Performed analysis

2.2.1. Growth, biomass yield, WUE and WP

Biomass growth of each crop was determined by weighting all plants weekly along with the pot. After plant sampling, empty pots and impermeable expanded clay weight was detracted from the previous weights, thus obtaining the entire plants weight. At harvest time, fresh leaves samples from 12 replicates per treatment of each crop were collected, frozen into liquid nitrogen and then stored at −80° C for further analysis of soluble sugar, chlorophyll, carotenoids concentration and phenolics content. Subsequently, plants were divided into shoots and roots and weighted separately. All samples were then oven-dried (70° C until constant weight) and dry biomass was determined.

Crop evapotraspiration (ET) was recorded by measuring the volume of solutions for each treatment on a weekly basis. The 12 tanks containing the recirculating growing media had liter graduations, thus allowing the recording of the plant water consumption (assuming zero water loss apart from plant evapotraspiration, being our experimental set up a closed-cycle hydroponic system).

Water use efficiency (WUE) was calculated as the ratio between the whole oven-dry biomass and total ET throughout the crop cycle, as follows:

$$WUE = wholeplantdrybiomass(g)/ET(L)$$

Water productivity (WP) was calculated as the ratio between the fresh marketable biomass (yield) and total ET throughout the crop cycle, as in the following formula:

$$WP = freshmarketablebiomass(g)/ET(L)$$

In particular, the second equation was used to better correlate the biomass production and ET, as the fresh shoot is the edible part of the considered species.

2.2.2. Leaf gas-exchange parameters

Leaf gas-exchange parameters were determined along with chlorophyll fluorescence measurements with the open gas-exchange system Li-6400 XT (Li-Cor, Lincoln, NE, USA), as in Bazihizina et al. (2015), using an integrated fluorescence chamber head (Li-6400-40; Li-Cor). These measurements were taken on a weekly basis on 12 plants per treatment in each crop. Net photosynthetic rate (A_n) and stomata conductance (g_s) were measured on the youngest fully expanded leaves at ambient relative humidity, reference CO_2 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 experimental period, total pigment concentration was calculated by reading the absorbance at 665, 652 and 470 nm of extracts obtained from randomly selected youngest fully expanded leaves from 12 replicates of each 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).

2.2.3. Concentration of mineral elements, soluble sugars and total phenolic content

The amount of 0.5 mg of oven-dried ground leaf samples (12 replicates per treatment) was mineralized in a Teflon becker containing 5 ml of HNO_3 (67%) and 5 ml of deionised water 18 MΩ. At the end of the mineralization, the final volume of the solution was obtained by adding 25 ml of water 18 MΩ and diluted extracts were analyzed for Na^+, K^+ and Ca^{2+} concentrations using a Flame Photometer Digiflame2000 (Lab Services SAS, Rome, Italy). Iron, Cu^{2+}, Mg^{2+} and Zn^{2+} were determined as in Pignattelli et al. (2012) by digesting 100 mg of oven-dried plant material (12 replicates per treatment) in a 5:2 (v/v) mixture of HNO_3 (Romil, 69%) and $HClO_4$ (Applichem, 70%) in 25 ml beakers at 120–200 °C, after which the volume was adjusted to 10 ml with milliQ-water. Elements concentration was determined with an atomic absorption spectrometer (Perkin-Elmer, Analyst 200).

The soluble sugar extraction of frozen-young fully expanded leaves (12 replicates per treatment) was performed twice in boiling 80% ethanol. The supernatant was then collected and used to measure total sugars with the anthrone reagent (Yemm and Willis, 1954): the concentration of total soluble sugars was determined by measuring the absorbance of extracted samples at 620 nm in a UV–vis spectrophotometer (Bio-Rad SmartSpec™Plus), using a standard curve for glucose. The reliability of this method was verified by determining the recovery of known amounts of glucose added to additional tissue samples immediately prior to extraction, and also added to ethanol only. The values of the calibration curve ranged from 0 to 100 mg/ml of glucose (R^2 = 0.995).

The total phenolic content was determined, according to Dewanto et al. (2002), using the Folin-Ciocalteu method. Samples were extracted overnight in a hydro-alcoholic solution (70% ethanol and 30% water). After that, 0.5 ml of deionised water and 125 ml of the Folin-Ciocalteu reagent were added to 125 ml of the suitably diluted sample extract. After 6 min, 1.25 ml of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 ml. After 90 min, the absorption was measured at 725 nm against water as a blank. The total phenolics concentration is expressed as gallic acid equivalents per gram of dry weigh (GAE, mg gallic acid/g sample) using a calibration curve with gallic acid. The calibration curve ranged from 20 to 500 mg/ml (R^2 = 0.997).

2.2.4. Statistical analyses

The experimental set-up was randomized in order to uniform the different treatments conditions. Statistical analyses were conducted using GraphPad Prism 5 for Windows. According to the different datasets, one-way ANOVA analysis of variance was used

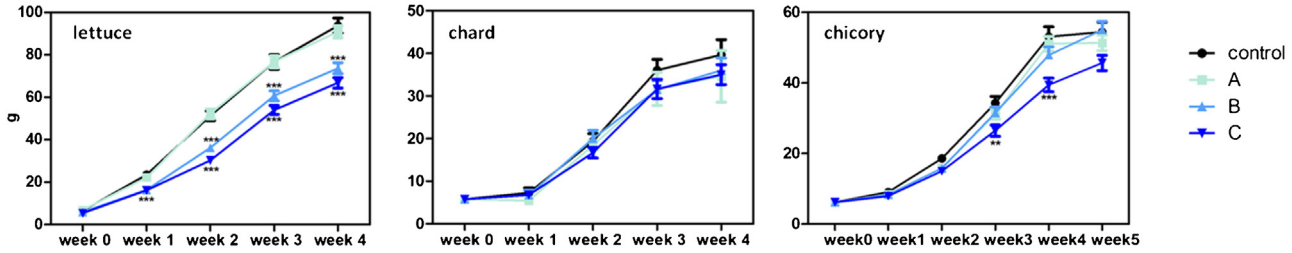

Fig. 1. Lettuce, chard and chicory biomass production: values are means of the entire plant fresh weight ± SEM. Asterisks indicate significant difference from control (* at $P < 0.05$; ** at $P < 0.01$, *** at $P < 0.0001$ Tukey's Test).

to assess significant differences between treatments. Significance level was $P \leq 0.05$ (unless differently stated).

3. Results and discussion

3.1. Growth, biomass yield, WUE and WP

In lettuce, A treatment did not significantly affect plant growth during the whole crop cycle. The two higher seawater concentrations (B and C treatments), on the other hand, significantly decreased plant growth starting from week 1 up to the end of the crop cycle, with the C treatment leading to the lowest weight (Fig. 1). In agreement with recent studies (Sakamoto et al., 2014; Turhan et al., 2014) at the end of the growing period, no significant differences were assessed between control and A treatment plants in the final weight of the plant marketable part (yield), that is the shoot fresh biomass (Table 3). On the contrary, a yield decrease of 23.8% and of 36.3% was observed in B and C treatments, respectively, compared to control plants. The whole plant dry mass followed the same trend (Table 3).

Chard biomass did not show any significant difference among the four treatments and during the whole crop cycle (Fig. 1). No significant differences were evaluated between treatments and control with respect to yield and to the entire plant dry weigh (Table 3). Other research indicated no decrease in chard biomass growth with diluted seawater (Zhang et al., 2008). The ability of this species to cope with salinity has been probably inherited from the wild sea beet (*Beta vulgaris* subsp. maritima), a common plant of the coastal environment of Europe and Western Asia, believed to be the ancestor of both leaf and root beets (Shannon and Grieve, 1998).

Similarly, chicory growth was generally not affected by seawater during the crop cycle: a significant biomass reduction was observed in C treatment plants compared to control in week 3 and 4, but at harvest time no differences were assessed among treatments (Fig. 1). Furthermore, no significant yield reduction was observed in treated plants in comparison to the control. On the other hand, dry weight showed a significant reduction in C treatment plants (Table 3). *Cichorium intybus* is native of the Mediterranean region (Shannon and Grieve, 1998), where it can be found quite commonly as weed in saline semi-arid waste places. The moderate salt tolerance of chicory crop (Boyd and Rogers, 2004) has been possibly retained from this salt resistant ancestor.

It is well known that, while the minerals contained in the seawater may stimulate growth (Sakamoto et al., 2014), the excessive concentration of salts (mostly sodium chloride) present in seawater is an important source of stress. Each crop growing in presence of seawater must balance between those two factors. In the perspective of cultivating with a reduced freshwater consumption, it is quite encouraging that the productive performances of chard and chicory were not significantly affected by seawater, while lettuce was the only one disturbed by the higher seawater treatments.

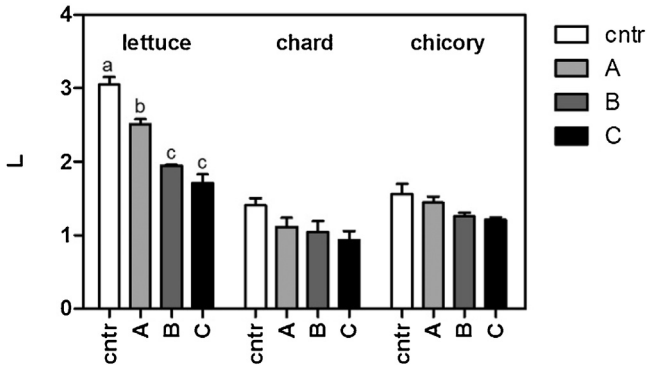

Fig. 2. Plant water consumption. Values are means ± SEM expressed in liters per plant. Different letters indicate a significant difference at $P < 0.05$ (Tukey's Test).

Interestingly, considering the entire growth period, all species resulted in reduced water consumptions, even if differences with respect to control were significant only in the case of lettuce (Fig. 2). To a certain extent, the decrease of water consumption can be due to the fact that stressed plants are no longer able to have a proper absorption and translocation of water. Despite this, due to a higher water use efficiency and to an increased water productivity (Fig. 3), the production obtained is comparable to control values in terms of yield (A treatment for lettuce and all treatments for chard and chicory).

Data available in literature show that tolerant crops have a relatively constant WUE at increasing levels of salinity, whereas sensitive crops exhibit a decrease of WUE (Katerji et al., 2003). On the contrary, our results demonstrate a significant increase of both WUE and WP for all the tested crops, mostly related to the significant drop of water consumptions at increasing salinity levels. In fact, the presence of seawater in the growing media has overall resulted in interesting performances on biomass production.

3.2. Leaf gas-exchange parameters

Lettuce photosynthetic rate (A_n) showed significant differences between treatments only one week after the beginning of the experiment in B and C treatment (Fig. 4). The values of stomata conductance (g_s) were significantly negatively affected by treatment C both one and two weeks after the beginning of the treatments (Fig. 5). Similarly, significant differences in A_n between control and treated plants were found in chard (Fig. 4), with A_n significantly reduced in every treatment compared to control at week 1 only. On the contrary, g_s in treated plant did not differ from control during the whole crop cycle (Fig. 5). In chicory, A_n and g_s differed among treatments only one week after seawater supply, being A_n lower in C treatment plants and g_s both in B and C treatments than in the control plants (Figs. 4 and 5).

In general, A_n seemed not to be negatively affected in the long term by seawater treatment suggesting a sort of adaptation of

Table 3
lettuce, chard and chicory yield and entire plant dry weight. Values are means ± s.e., different letters indicate a significant difference at P < 0.05 (Tukey's Test).

Treatments						
	Lettuce		Chard		Chicory	
	Fresh weight shoot (g)	Dry weight whole plant (g)	Fresh weight shoot (g)	Dry weight whole plant (g)	Fresh weight shoot (g)	Dry weight whole plant (g)
control	79.66 ± 2.96 [a]	4.57 ± 0.21 [a]	29.90 ± 2.99 [a]	2.27 ± 0.24 [a]	38.55 ± 2.11 [ab]	4.34 ± 0.21 [a]
A	72.6 ± 2.37 [a]	4.40 ± 0.14 [a]	22.88 ± 5.01 [a]	1.71 ± 0.29 [a]	39.61 ± 1.78 [ab]	3.97 ± 0.17 [ab]
B	57.69 ± 2.05 [b]	3.79 ± 0.13 [b]	26.54 ± 2.47 [a]	2.37 ± 0.27 [a]	43.44 ± 1.99 [a]	4.22 ± 0.22 [a]
C	50.73 ± 2.02 [b]	3.40 ± 0.11 [b]	26.09 ± 1.82 [a]	2.04 ± 0.21 [a]	33.18 ± 2.01 [b]	3.34 ± 0.22 [b]

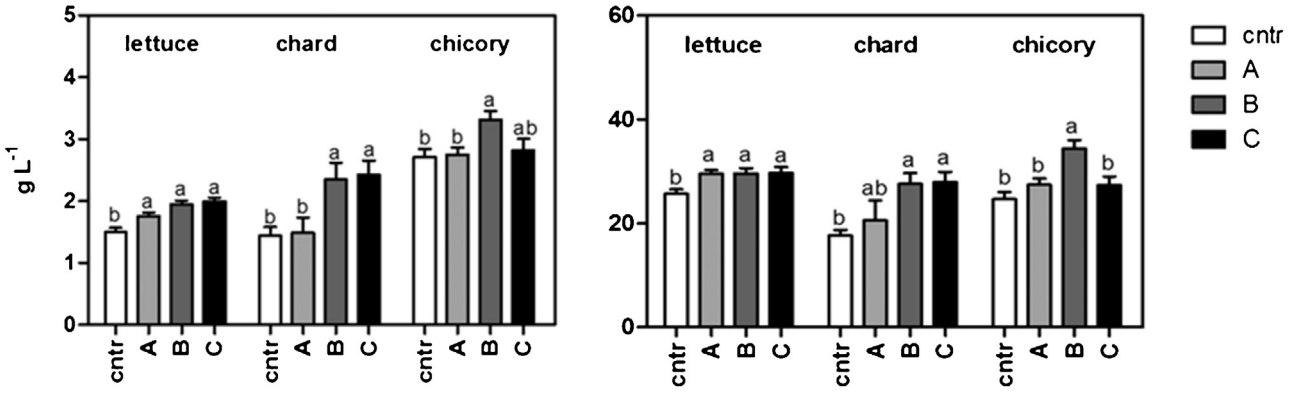

Fig. 3. WUE as a ratio between the plant total dry biomass and tot ET (WUE, graph 1); WP as a ratio between the fresh marketable biomass and tot ET (WP, graph 2). Values are means ± SEM expressed in gram per liter. Different letters indicate a significant difference at P < 0.05 (Tukey's Test).

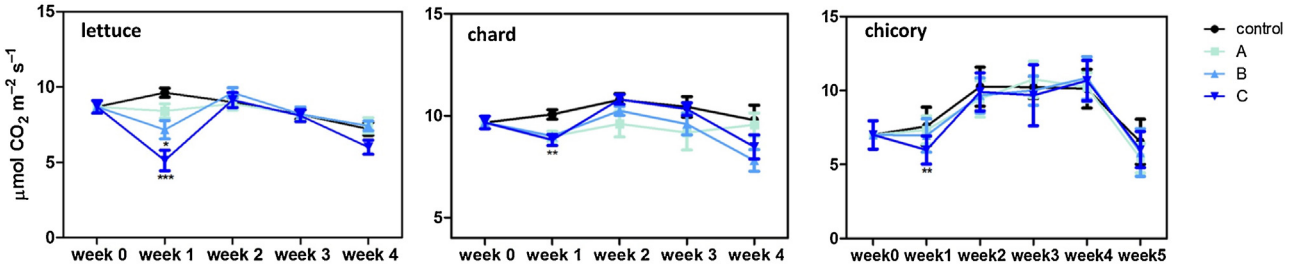

Fig. 4. Lettuce, chard and chicory A_n during the crop cycle: values are means ± SEM. Asterisks indicate significant difference from control (* at $P < 0.05$; ** at $P < 0.01$, *** at P < 0.0001 Tukey's Test).

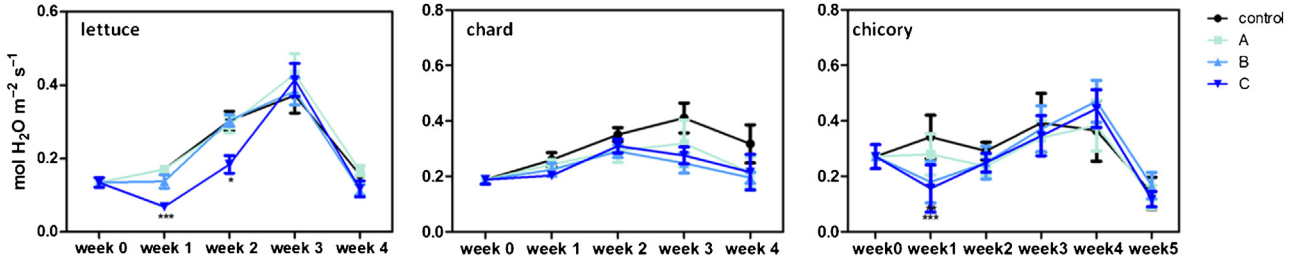

Fig. 5. Lettuce, chard and chicory g_s during the crop cycle: values are means ± SEM. Asterisks indicate significant difference from control (* at $P < 0.05$; ** at $P < 0.01$, *** at P < 0.0001 Tukey's Test).

the photosynthetic machinery to the presence of seawater. On the other side, except in chard, g_s resulted more affected by seawater treatment, even though such decrease did not restrict A_n. Present results are consistent with those described by Wilson et al. (2006) and Balibrea et al. (2000), implying that damages in the photosynthetic activity may not be the cause of plants limited growth under salinity conditions.

Generally, negligible differences were measured in pigments concentration at harvesting time (Table 4), in line with results reported by Santos (2004). Indeed, the absence of differences of A_n and g_s in proximity to the maturity time and the results on pigments concentration at the end of the crop cycle suggested that the pigment decrease, when present, was not strong enough to inhibit the plant photosynthetic apparatus, concurring to imply that sea-

Table 4
Pigment concentration in lettuce, chard and chicory leaves. Values are means ± s.e. expressed per gram of leaves fresh weight. Different letters indicate a significant difference at $P < 0.05$ (Tukey's Test).

Treatments	Lettuce			Chard			Chicory		
	Cha μg g^{-1}	Chb μg g^{-1}	Car μg g^{-1}	Cha μg g^{-1}	Chb μg g^{-1}	Car μg g^{-1}	Cha μg g^{-1}	Chb μg g^{-1}	Car μg g^{-1}
control	226.56 ± 16.52^{a}	72.77 ± 5.01^{a}	31.54 ± 2.08^{a}	434.52 ± 16.82^{a}	100.04 ± 15.09^{a}	52.38 ± 5.17^{a}	339.67 ± 11.50^{a}	122.04 ± 14.41^{a}	49.04 ± 3.39^{a}
A	252.31 ± 9.76^{a}	80.96 ± 5.20^{a}	34.57 ± 1.11^{a}	334.68 ± 62.11^{ab}	82.30 ± 12.53^{a}	41.71 ± 8.43^{ab}	337.21 ± 32.34^{a}	82.80 ± 6.68^{b}	50.93 ± 2.09^{a}
B	218.13 ± 13.87^{a}	71.55 ± 3.73^{a}	30.56 ± 1.76^{a}	297.25 ± 55.66^{ab}	72.52 ± 8.77^{a}	41.26 ± 5.34^{ab}	195.72 ± 22.39^{b}	77.84 ± 6.21^{b}	46.33 ± 3.07^{ab}
C	206.08 ± 15.15^{a}	66.20 ± 4.87^{a}	27.97 ± 2.20^{a}	184.58 ± 35.39^{b}	62.68 ± 6.00^{a}	25.52 ± 2.55^{b}	215.33 ± 32.43^{b}	78.23 ± 5.34^{b}	35.05 ± 4.00^{b}

water in the growing media could be used to grow the three studied crops, at least up to the tested concentrations.

3.3. Concentration of mineral elements, soluble sugars and total phenolics

Tables 5, 6 and 7 reports the element concentration in the leaves of the three crops at harvest. The concentrations found in the control plants were firstly compared with data from the literature and the outcome concurred in validating the closed-cycle NFT hydroponic system used in the experiment as a proper growing system. In fact, Na^+, K^+ and Ca^{2+}content in control plants is consistent with the literature (Bartha et al., 2015; Kawashima and Soares, 2003).

In lettuce, seawater increased Na^+, Cu^{2+}, and Mg^{2+} accumulation, reduced Fe^{2+} level and did not affect K^+, Ca^{2+} and Zn^{2+} concentration (data reported in Table 5). In chard, treatments did not lead to any difference with control regarding K^+, Fe^{2+} and Zn^{2+} concentration in leaves, whereas Na^+, Ca^{2+}, Cu^{2+} and Mg^{2+} levels significantly increased in treated plants (Table 6). In chicory, Na^+ concentration was significantly higher in treated plants. In addition, seawater led to a reduction of Fe^{2+} (only in treatment C) and Zn^{2+} concentration, whereas K^+, Ca^{2+}, Cu^{2+} and Mg^{2+} levels were not affected at all (Table 7).

As expected and in agreement with previous study (Bartha et al., 2015), Na^+ increased in every treatment compared to control. Moreover, while in Bartha et al. (2015) K^+ concentration significantly decreased in salt-treated plants, in the present study K^+ level was not affected by seawater treatments in none of the tested species. Similarly, Unlukara et al. (2008) showed no significant differences in K^+ accumulation between different salinity levels (up to 7.0 dS m^{-1}) in lettuce leaves. Given that Na^+ accumulation in mesophytes is reported not to be the result of competition between Na^+ and K^+ for one set of transporters, thus the K^+ transporters not failing in discriminating efficiently against Na^+ (Lazof and Cheeseman, 1988), our results on Na^+ and K^+ accumulation can be considered as expected. Moreover, Ca^{2+} accumulation was not affected in lettuce and chicory, while it increased in chard when treated with the higher seawater concentration, similarly to previous studies results (Unlukara et al., 2008; Lazof and Cheeseman, 1988). The variations of Ca^{2+} concentration in cytosol among cultivars, and among species too, can represent a possible reason for those differences (Bartha et al., 2015). With respect to seawater-induced changes in Mg^{2+} and micronutrients concentration, our results are confirmed by previous studies (Yousif et al., 2010; Unlukara et al., 2008; Al-Karaky, 2000; Lutts et al., 1996).

Among the three crops, lettuce, the most sensitive to seawater treatments, showed interestingly higher Cu^{2+} and Mg^{2+} concentrations, despite the same increase in Na^+ accumulation. On the other side, chard was the crop with the higher number of elements with increased concentration in treated leaves: Na^+, Ca^{2+}, Cu^{2+} and Mg^{2+}. Among them, only Na^+ could have a negative effect on human diet, and once assessed the higher acceptable amount, the augmentation of Ca^{2+}, Cu^{2+} and Mg^{2+} levels might represent an interesting case of biofortification, especially for chard, whose growth was not inhibited by any of the tested seawater concentrations. Besides, chicory is the crop showing the minor differences between treated and control leaf element accumulation, implying a lower effect of seawater on the mechanisms of ion acquisition and translocation in this species, thus suggesting a better salt tolerance compared to the two other tested species in terms of maintenance of the ionomic profile.

Soluble sugar concentration in lettuce was negatively affected by seawater treatment (Table 8), contrarily to what happened in chard and chicory. On the other hand, seawater did not lead to any significant effect with respect to the total polyphenols concentration at harvest time (Table 8).

The results on soluble sugar concentrations are partially similar to previous studies (Turhan et al., 2014; Reza Naeini et al., 2004; Gao et al., 1998). In particular, Reza Naeini et al. (2004) concluded that high osmotic pressure may inhibit activity of hydrocarbon-synthesizing enzymes and, as a result, decrease soluble sugars concentration. Thus, even if total soluble carbohydrates can be important solutes synthesized and accumulated under salt stress in cytosol (Nemati et al., 2011), literature shows remarkable differences concerning their accumulation in response to salinity, at both inter-specific or intra-specific levels, leaving the question about their role in plant adaptation to salt stress still open (Ashraf and Harris, 2004).

Similarly, the obtained results on total phenolic content, consistent with those of Kim et al. (2008), suggest that the phenolic compounds alteration due to salinity stress is critically dependent on the salt sensitivity of the plant. In fact, salt stress often creates both ionic as well as osmotic stress in plants, resulting in accumulation or decrease of specific secondary metabolites (Mahajan and Tuteja, 2005), and increases in polyphenols concentration in different tissues under increasing salinity have been reported in a number of plants (Parida and Das, 2005). Consequently, the seawater concentrations used in the present experiment proved to be unable to induce any change in phenolic content in the three crops, thus not showing biofortification effect in such compounds but at the same time suggesting the possibility of cultivation at the tested seawater concentrations.

In conclusion, our results suggest that the use of a certain level of seawater in the cultivation of lettuce, chard and chicory is a practical possibility to be explored in the direction of increasing both crop WUE and the concentration of some mineral nutrients. Besides, levels of soluble sugars and phenolics seemed not to be affected by seawater at the tested concentrations. Organoleptic tests, more studies on the physiological mechanisms of a moderate salt tolerance and Na^+ toxicity effects can be considered for further investigation.

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Table 5
Elements accumulation in lettuce leaves. Values are means ± s.e. expressed per gram of leaves dry weight. Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey's Test).

Treatments	Na mg g^{-1}	K mg g^{-1}	Ca mg g^{-1}	Cu μg g^{-1}	Fe μg g^{-1}	Mg μg g^{-1}	Zn μg g^{-1}
control	14.15 ± 1.97 ^{c}	53.19 ± 6.43 ^{a}	3.33 ± 0.19 ^{a}	5.29 ± 0.80 ^{b}	91.86 ± 13.59 ^{a}	5505.80 ± 259.78 ^{b}	65.88 ± 6.19 ^{a}
A	31.16 ± 0.75 ^{b}	68.53 ± 4.47 ^{a}	3.41 ± 0.08 ^{a}	5.44 ± 1.22 ^{b}	83.13 ± 11.42 ^{ab}	5895 ± 94.42 ^{ab}	37.40 ± 9.31 ^{a}
B	41.56 ± 2.09 ^{ab}	69.04 ± 5.22 ^{a}	3.77 ± 0.14 ^{a}	8.88 ± 0.94 ^{ab}	60.38 ± 5.37 ^{ab}	5855.70 ± 106.66 ^{ab}	53.97 ± 14.68 ^{a}
C	53.01 ± 4.27 ^{a}	57.33 ± 8.18 ^{a}	3.78 ± 0.12 ^{a}	10.14 ± 0.63 ^{a}	52.76 ± 7.85 ^{b}	6167.44 ± 116.42 ^{a}	37.29 ± 4.41 ^{a}

Table 6
Elements accumulation in chard leaves. Values are means ± s.e. expressed per gram of leaves dry weight. Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey's Test).

Treatments	Na mg g^{-1}	K mg g^{-1}	Ca mg g^{-1}	Cu μg g^{-1}	Fe μg g^{-1}	Mg μg g^{-1}	Zn μg g^{-1}
control	23.39 ± 1.20 ^{b}	39.65 ± 1.68 ^{a}	2.71 ± 0.23 ^{b}	4.18 ± 0.38 ^{b}	60.04 ± 8.77 ^{a}	8713.42 ± 507.10 ^{b}	47.37 ± 11.05 ^{a}
A	43.37 ± 5.65 ^{ab}	46.97 ± 5.15 ^{a}	4.10 ± 1.21 ^{ab}	6.53 ± 0.75 ^{ab}	60.87 ± 5.82 ^{a}	11764.04 ± 1066.91 ^{a}	53.47 ± 9.12 ^{a}
B	48.68 ± 2.02 ^{ab}	41.76 ± 1.46 ^{a}	3.44 ± 0.04 ^{ab}	8.91 ± 0.60 ^{a}	69.73 ± 6.34 ^{a}	11657.12 ± 905.84 ^{a}	49.36 ± 2.07 ^{a}
C	56.87 ± 7.24 ^{a}	37.86 ± 3.31 ^{a}	3.63 ± 0.16 ^{a}	8.27 ± 0.98 ^{a}	64.40 ± 2.01 ^{a}	10861.35 ± 355.22 ^{ab}	53.23 ± 6.05 ^{a}

Table 7
Elements accumulation in chicory leaves. Values are means ± s.e. expressed per gram of leaves dry weight. Different letters in the same column indicate a significant difference at $P < 0.05$ (Tukey's Test).

Treatments	Na mg g^{-1}	K mg g^{-1}	Ca mg g^{-1}	Cu μg g^{-1}	Fe μg g^{-1}	Mg μg g^{-1}	Zn μg g^{-1}
control	10.47 ± 3.25 ^{b}	41.33 ± 6.96 ^{a}	3.53 ± 0.70 ^{a}	5.47 ± 0.68 ^{a}	85.17 ± 15.30 ^{a}	6056.12 ± 723.55 ^{a}	66.57 ± 7.51 ^{a}
A	27.50 ± 2.58 ^{a}	46.77 ± 1.57 ^{a}	2.92 ± 0.14 ^{a}	3.30 ± 0.59 ^{a}	76.33 ± 11.88 ^{a}	4832.23 ± 219.03 ^{a}	33.75 ± 4.85 ^{b}
B	30.76 ± 2.05 ^{a}	54.23 ± 4.56 ^{a}	2.97 ± 0.06 ^{a}	3.52 ± 0.55 ^{a}	59.10 ± 2.96 ^{ab}	5264.68 ± 272.71 ^{a}	24.06 ± 4.53 ^{b}
C	33.08 ± 1.95 ^{a}	54.17 ± 4.66 ^{a}	3.26 ± 0.08 ^{a}	6.09 ± 0.96 ^{a}	27.60 ± 7.28 ^{b}	5185.29 ± 227.33 ^{a}	28.65 ± 7.15 ^{b}

Table 8
soluble sugar and total polyphenols concentration in leaves. Values are means ± s.e. expressed per gram of leaves fresh weight (soluble sugar) and as gallic acid equivalents (GAE, mg gallic acid/g sample). Different letters indicate a significant difference at $P < 0.05$ (Tukey's Test).

Treatments	lettuce		chard		chicory	
	soluble sugars mg g^{-1}	polyphenols mg g.a. g^{-1}	soluble sugars mg g^{-1}	polyphenols mg g.a. g^{-1}	soluble sugars mg g^{-1}	polyphenols mg g.a. g^{-1}
control	17.24 ± 1.45 ^{a}	0.33 ± 0.05 ^{a}	7.50 ± 0.63 ^{a}	0.65 ± 0.12 ^{a}	8.14 ± 0.78 ^{a}	0.28 ± 0.03 ^{a}
A	7.46 ± 0.99 ^{b}	0.29 ± 0.02 ^{a}	7.54 ± 1.59 ^{a}	0.71 ± 0.16 ^{a}	6.04 ± 0.89 ^{a}	0.27 ± 0.02 ^{a}
B	7.58 ± 0.66 ^{b}	0.22 ± 0.01 ^{a}	5.96 ± 0.79 ^{a}	0.64 ± 0.08 ^{a}	5.66 ± 0.80 ^{a}	0.32 ± 0.02 ^{a}
C	6.66 ± 0.51 ^{b}	0.28 ± 0.01 ^{a}	4.73 ± 0.60 ^{a}	0.42 ± 0.07 ^{a}	6.09 ± 0.71 ^{a}	0.37 ± 0.02 ^{a}

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